CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

208471Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date: July 20, 2016
From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 208471
Agency receipt date: July 27, 2015
Drug: ADLYXIN **(**Lixisenatide)
Sponsor: Sanofi Aventis

Indication: Adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The original evaluation of the nonclinical program was conducted under NDA 204961. Although the NDA was subsequently withdrawn, the pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data supported approval at that time. The current review team also concludes that the nonclinical program supports approval of lixisenatide for the indication listed above.

The recommended established pharmacologic class (EPC) for lixisenatide is a glucagon-like peptide-1 (GLP-1) receptor agonist. Exenatide (Byetta and Bydureon), liraglutide (Victoza), albiglutide (Tanzeum), and dulaglutide (Trulicity) are other GLP-1 agonists that have been previously approved in the US.

Lixisenatide is a synthetic 44-amino acid peptide that is injected subcutaneously once daily at doses up to 20 μg. It is resistant to degradation by dipeptidyl peptidase-4 (DPP4) which extends its half-life compared with endogenous GLP-1.

A complete general toxicology program was conducted in rats and dogs. The most prominent findings included reduced body weights and effects on male reproductive organs in rats and dogs. Based on the nonclinical findings, the sponsor conducted a 6-month clinical trial evaluating various endpoints related to sperm production; no drug-related effects were observed. Given large animal to human exposure margins (findings were observed at exposures that were greater than 4000-fold the maximum anticipated clinical exposure) and the lack of observed effects in the clinical trial, the risk of effects on spermatogenesis in males is considered to be low. In the chronic studies, mid- and high dose rats and dogs developed anti-drug antibodies (ADA).

Lixisenatide was negative in a battery of genetic toxicity studies. Carcinogenicity studies in rats and mice demonstrated a risk for thyroid C-cell hyperplasia and tumorigenesis, at exposures that were greater than 50-fold the clinical AUC. This finding is similar to those for other GLP-1 agonists. Although the human relevance of GLP-1 receptor agonist-induced C-cell tumorigenesis in rodents is unknown, human relevance to drug-induced C-cell tumors cannot be discounted. Since the tumor profile, half-life and receptor activation potency of lixisenatide more closely resemble that of short-acting GLP-1

agonists, the results of the carcinogenicity studies are to be discussed solely in section 13 of the label; a boxed warning is not warranted.

A complete battery of reproductive and developmental toxicity studies was conducted in rats and rabbits. Key findings included decreased/delayed fetal growth, visceral and skeletal malformations, and/or embryonic death. These findings occurred in the presence of maternal effects which may have contributed to some of the embryo-fetal observations; however, a direct drug-related effect cannot be excluded. In a peri-/post-natal rat study, observations included a slight increase in pup mortality, reduced male pup bodyweight, and skeletal malformations. The observed findings from the embryo-fetal development studies occurred at exposures that were 1- (rat) to 6-fold (rabbits) the maximum anticipated clinical exposure (AUC) while those from the peri-/post-natal study occurred at approximately 200 times the clinical dose based on body surface area comparisons. No effects on mating, fertility or early embryonic development were observed.

Discussions were held between the Division and Sponsor regarding the most appropriate approach to present animal to human exposure margins in the product label. Lixisenatide induces an ADA response in animals and humans that increases the amount of total lixisenatide in the blood; the increase is especially prominent in rats. Pharmacodynamic response remains despite the ADA response though there is some evidence of a decline in efficacy in patients with the highest ADA titers. The relative fraction of "active" lixisentatide in ADA positive humans and rats is unknown. Given the differential effects of ADA presence on exposure in rats and humans and the uncertainty regarding the relative "active" fraction of total lixisenatide exposure, the Division's more conservative approach to compare exposure data from ADA negative animals and ADA positive human subjects is reasonable.

Conclusion:
I agree with the division pharmacology/toxicology conclusion that lixisenatide can be approved from the pharmacology/toxicology perspective. The EPC is consistent with other GLP-1 receptor agonists. I have reviewed the proposed labeling and agree with the recommendations made by the division regarding the relevant nonclinical sections.

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/s/

TIMOTHY J MCGOVERN
07/20/2016

Memorandum

Pharmacology/Toxicology
Center for Drug Evaluation and Research
Division of Metabolic & Endocrine Products

NDA SECONDARY REVIEW, ADDENDUM

Date:	15 July 2016
NDA #	208471
Sponsor:	Sanofi Aventis
Drug:	Lixisenatide
Primary Reviewer:	Feleke Eshete/Tim Hummer
Secondary Reviewer:	Todd Bourcier

This addendum documents the Division's rationale for calculation of exposure margins found in sections 8 and 13 of the drug label.

Lixisenatide induces a high frequency anti-drug antibody response in animals and in human subjects. Pharmacodynamic activity remains evident despite the ADA response, although there is some evidence of a decline in efficacy in patients with the highest ADA titers, and an apparent decline exists in the percentage of 'biologically active' lixisenatide in ADA-positive blood samples (study TDR11215). Most prominently, the presence of ADA substantially increased the amount of total lixisenatide in the blood in animals and humans, which markedly confounded calculation of safety margins. For reproductive toxicology, the sponsor proposed comparison of [redacted] (b) (4) animal and human values for calculation of a margin; however, the more conservative comparison would be to exposure in ADA-positive [redacted] (b) (4) human samples. Approximately 70% of patients develop ADA to lixisenatide and such patients would likely have higher drug exposure due to ADA (≥4 fold) prior to experiencing a pregnancy (i.e., unlikely that lixisenatide would be initiated during a pregnancy). For carcinogenicity, the sponsor proposed [redacted] (b) (4) . A more conservative approach is comparing AUC in ADA-negative rodents (from initial AUC in DRF and 2yr study) to the AUC in ADA-positive humans (from study ACT6011, 4.4ng*h/ml). This approach is appropriately conservative, as it assumes only drug unbound to antibody is active in the rodent studies, and assumes that all drug in humans is active, both bound and unbound to antibody. Basing margins on body mass would effectively remove the confounding effect of ADA and yields values more similar to margins based on AUC from ADA-negative animals and humans; this approach, however, may overestimate safety margins. Therefore, pharm/tox proposes to base the margins in the label on plasma AUC from ADA-negative animals compared to ADA-positive humans.

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/s/

TODD M BOURCIER
07/18/2016
Addendum to secondary review; addresses basis for exposure margins in label

Memorandum

Pharmacology/Toxicology
Center for Drug Evaluation and Research
Division of Metabolic & Endocrine Products

NDA SECONDARY REVIEW

Date:	06 April 2016
NDA #	208471
Sponsor:	Sanofi Aventis
Drug:	Lixisenatide
Primary Reviewer:	Feleke Eshete/Tim Hummer
Secondary Reviewer:	Todd Bourcier

Sanofi is seeking marketing approval for lixisenatide as a treatment option for Type 2 diabetes. Lixisenatide is a synthetic peptide similar in sequence to exendin-4 and is similarly resistant to proteolytic degradation by DPP4. Lixisenatide acts as an analog of endogenous GLP1 peptide and achieves therapeutic efficacy by functioning as a GLP1 receptor agonist; several approved drugs in this pharmacological class are currently available as treatment options for diabetes. The established class name for lixisenatide is based on the drug's primary pharmacology and will be the same as for others in the class: 'XX is a glucagon-like peptide-1 (GLP-1) receptor agonist'.

The nonclinical program for lixisenatide was reviewed by Dr. Timothy Hummer under a prior NDA that was withdrawn by Sanofi before the action date. Both Dr. Hummer and Dr. Davis-Bruno (supervisory pharmacologist at that time) concluded that the nonclinical program supported NDA approval (22 Aug 2013 Review). The current nonclinical reviewer (Dr. Feleke Eshete) and I find no new nonclinical information submitted to NDA 208471 that would change that recommendation. Dr. Hummer's 2013 review serves as the primary nonclinical review for NDA 208471.

The reproductive toxicology studies supported a 'pregnancy category C' based on skeletal malformations and the occurrence of rare visceral closure defects in rats and rabbits. These effects occurred at doses that sharply reduced food intake and weight gain for the first few days of exposure, interpreted by Dr. Hummer as maternal toxicity. This brief interruption of nutritional status in the dams may indeed have resulted in the adverse skeletal findings, but it's contribution to the occurrence of 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 more prominent role of maternal factors in the adverse outcome, a direct adverse effect of lixisenatide on developing fetuses cannot be entirely excluded. The risk summary of section 8.1 will disclose that visceral and skeletal defects were observed in embryofetal development studies at doses that decreased nutritional intake and weight gain during gestation, (b) (4) This is consistent with the product

labeling in the EU (where lixisenatide is already approved, tradename Lyxumia), which recommends that insulin be used in place of lixisenatide during pregnancy.

The GLP1r agonist class is associated with thyroid C-cell neoplasms in 2yr rodent studies, with 'short-acting' agonists yielding a weak response at high doses and 'long-acting' agonists provoking a more robust tumor response at markedly lower doses. I concur with Dr. Hummer's analysis that the thyroid tumor profile of lixisenatide in rodents most closely resembles that of the short-acting exenatide IR, in terms of receptor potency, half-life, and exposure/response for tumor outcome in the rodent studies. As such, the results of the 2yr rodent bioassays will be restricted to a description of results in section 13 of the drug label.

Juvenile toxicology studies were conducted in rats and in dogs. The 8-month study in dogs was intended to address adverse but reversible findings in male reproductive organs in adult dogs (e.g., hypospermatogenesis, oligospermia). I concur with Dr. Hummer's assessment that the toxicology profile in juvenile rats and dogs is consistent with observations made in adult rats and dogs (b) (4)

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/s/

TODD M BOURCIER
04/08/2016
Secondary nonclinical review

DEPARTMENT OF HEALTH & HUMAN SERVICES **Food and Drug Administration**

Memorandum

Pharmacology/Toxicology
Center for Drug Evaluation and Research
Division of Metabolic & Endocrine Products

Date:	06 April 2016
NDA #	208471
Sponsor:	Sanofi Aventis
Drug:	Lixisenatide
Primary Reviewer:	Feleke Eshete/Tim Hummer
Secondary Reviewer:	Todd Bourcier

Attached below is the primary review for lixisenatide, authored by Dr. Timothy Hummer for a prior NDA that was withdrawn by Sanofi before the action date. Dr. Davis-Bruno, the supervising pharmacologist, concurred with Dr. Hummer's recommendations. Dr. Hummer's 2013 review serves as the primary nonclinical review for NDA 208471.

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	204961
Review number	1
Supporting document/s:	1
Applicant's letter date:	19 December 2012
CDER stamp date:	20 December 2012
Product:	Lixisenatide
Indication:	Type 2 diabetes
Applicant:	Sanofi Aventis
Review Division:	Metabolism and Endocrinology Products
Reviewer:	B. Timothy Hummer, PhD, DABT
Supervisor/Team Leader:	Karen Davis-Bruno, PhD
Division Director:	Mary Parks, MD
Project Manager:	Pooja Dharia, PharmD

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 204961 are owned by Sanofi Aventis or are data for which Sanofi Aventis has obtained a written right of reference. Any information or data necessary for approval of NDA 204961 that Sanofi Aventis does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 204961.

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1 Executive Summary

1.1 Introduction

Lixisenatide is intended for use as an adjunct to diet and exercise to improve glycemic control in the treatment of adults with type 2 diabetes mellitus. Lixisenatide will be injected subcutaneously once daily at doses up to 20 µg. Lixisenatide, a synthetic 44 amino acid peptide, is a GLP-1 receptor agonist with significant sequence similarity to exendin-4, a 39 amino acid peptide. 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.

1.2 Brief Discussion of Nonclinical Findings

Treatment with high subcutaneous doses of lixisenatide to rats and rabbits results in transient decreased activity and piloerection. In dogs, doses ≥300 µg/kg resulted in vomiting and diarrhea. As expected for the GLP-1 receptor agonist drug class, treatment results in body weight loss or decreased weight gain that correlates with decreased food and water consumption. Effects on food consumption and body weight tend to diminish as the treatment duration progresses.

The primary target organ identified after repeated dosing was the testes/epididymis. In a 6-month repeat-dose study in rats, an increased incidence and severity of microscopic findings in 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 (7,250X clinical exposure). These effects were treatment duration dependent and were mostly reversible after a 1-month recovery period. In dogs, microscopic findings were also observed in epididymis and testis, including moderate to severe hypospermatogenesis in seminiferous tubules and epididymal dilation, degeneration, oligospermia, or aspermia at ≥200 µg/mg BID after 12 months of treatment (≥4,062X clinical exposure). Effects on spermatogenesis in dogs could be observed after 13 weeks of treatment, with the incidence and severity increasing with duration of dosing. In an 8-month juvenile toxicology study in dogs at 5, 20, and 200 µg/kg BID (≥7X clinical exposure), similar dose-related findings were observed in the testis and epididymis, which were shown to be reversible after a 2-month treatment-free period.

Real-Time (RT) PCR data show that dogs have 21- to 4,147-fold higher GLP-1 receptor expression in the epididymis than in rat (depending on the epidiymal segment), which may explain the large difference in clinical safety margins between these two species. GLP-1 receptor expression in human epididymis was 10-fold lower (total tissue) or 4-fold lower (caput segment) than dog, whereas the expression in the corpus and cauda segments were approximately 11- and 8-fold higher than dog, respectively. In testis, GLP-1 receptor expression was at least 100-fold higher than in rats and 3- to 10-fold higher than in

humans. These data suggest that the increased GLP-1 receptor expression in testis and epididymis make dogs more sensitive to lixisenatide-induced inhibition of spermatogenesis. In support of this hypothesis, once daily doses of 20 µg (the maximum recommended clinical dose) for 6 months in obese men did not result in clinically significant effects on human spermatogenesis (total sperm count, motility, or morphology) or on reproductive hormones (clinical Study TDR11215). Therefore, based on the differences in GLP-1 receptor expression between dogs and humans, a large clinical exposure margin, and clinical trial results, the data indicate that human males are not at high risk for effects on spermatogenesis at the recommended clinical dose.

A slight increase in incidence and/or severity of injection site reactions (e.g., hemorrhage, fibrosis, abscess, inflammation) was observed in rats and dogs after chronic dosing. However, these types of reactions are easily monitorable in the clinic and can be minimized by using multiple injection sites to avoid repeated, continuous injury at the same injection site. Given the concern regarding the potential for GLP-1 receptor agonists to induce pancreatitis in humans, it is important to mention that there were no definitive, treatment-related adverse microscopic findings in the pancreas of mice or rats after treatment for up to 2 years or in dogs after treatment for up to 1 year.

Treatment of pregnant rats and rabbits with lixisenatide during fetal development resulted in decreased/delayed fetal growth, visceral and skeletal malformations, and/or embryonic death. In rats, single cases of fetuses with microphthalmia, anophthalmia, diaphragmatic hernia, and a few fetuses with multiple skeletal malformations were observed across all dose groups (≥2.5 µg/kg BID). Adverse effects on fetal development did occur in the presence of maternal toxicity, which was characterized by decreased motor activity, sleepiness, decreased reactivity, piloerection, reduced food consumption, and an initial dose-dependent decrease in body weight were observed at all dose levels. The NOAEL for maternal and developmental toxicity of AVE0010 was <2.5 µg/kg BID (<3X clinical exposure).

Treatment of pregnant rabbits also resulted in malformations. There were two fetuses each from the 2.5 and 25 µg/kg BID groups and one fetus from the 250 µg/kg BID group with multiple visceral and skeletal malformations, including thorocogastroschisis, amelia of forelimbs, absent bones, malformed bones, fused sternebrae, spina bifida, misshapen heart, malpositioned main arterial vessels, absence of organs, exencephaly, microphthalmia, and omphalocele. Aplasia of the gallbladder was seen in one and two fetuses from the 25 and 250 µg/kg BID dose groups, respectively, and one fetus from the 250 µg/kg BID dose group had a cardiac ventricular septum defect. A statistically significant increase in post-implantation loss occurred at 250 µg/kg BID. Maternal toxicity was noted at all dose levels and consisted of a dose-dependent decrease in mean body weight associated with reduced food consumption, decreased feces, reduced motor activity, and piloerection. The NOAEL for both maternal and developmental toxicity was considered to be <2.5 µg/kg BID (<22X clinical exposure).

A second study in rabbits using lower doses showed a slight increase in some developmental variations at ≥1.0 µg/kg BID, including skull (small hole, splitting of bone,

and additional suture of parietal bone), 13th rib (supernumerary – short or full), central caudal vertebrae (ossification of less than 15), and forepaw (hyperflexion). Signs of maternal toxicity were observed at ≥1 µg/kg BID and included decreased motor activity, piloerection, and decreased body weight that correlated with decreased food and water consumption. Because the skeletal variations observed at ≥1 µg/kg BID cannot be ruled out as being related to lixisenatide, the NOAEL for embryo-fetal development is considered to be 0.15 µg/kg BID (1X clinical exposure), which is also the NOAEL for maternal toxicity.

In a peri- and post-natal toxicity study in rats, lixisenatide caused a slight decrease in suckling in male pups at ≥20 µg/kg BID resulting in decreased body weight gain and a slight increase in pup mortality from PND 0 to 21 and abnormal findings on tails at 200 µg/kg BID. Two cases of multiple skeletal malformations of long bones and ribs were observed in two growth-delayed dead pups at 200 µg/kg BID. A slight, statistically significant decrease in the time to vaginal opening was observed at ≥20 µg/kg BID. Maternal effects included decreased motor activity and initial body weight loss that correlated with decreased food consumption at all dose levels. The NOAEL for F_0 maternal rats was considered to be <2 µg/kg BID based on the adverse effects on body weight. The NOAEL for F_1 pups was considered to be 20 µg/kg BID (~250X clinical exposure) based on treatment-related mortalities and skeletal and tail defects observed at the high dose.

In a 2-year carcinogenicity study in mice, a statistically significant increase in thyroid C-cell adenomas occurred at 1000 µg/kg BID for males only. Accordingly, the NOEL for thyroid C-cell adenomas was determined to be 200 µg/kg BID for males and 1,000 µg/kg BID for females. Exposures at the male and female NOAELs are approximately 272X and 5,000X higher than the anticipated clinical exposure at 20 µg/day. In a 2-year carcinogenicity study in rats, a statistically significant increase in thyroid C-cell adenomas was noted for all lixisenatide-treated groups. The NOEL for thyroid c-cell adenomas was not identified (<40 µg/kg BID). The clinical exposure margin based on the low dose used in this study is <1,028X. No other tumor types in mice or rats were determined to be related to lixisenatide treatment. Overall, the large clinical exposure margins for C-cell tumors indicate that the human risk for C-cell tumors is more similar to exenatide IR than to exenatide QW or liraglutide. Therefore, information in the label regarding C-cell tumor risk should be similar to other short-acting GLP-1 receptor agonists (i.e., without a boxed warning).

1.3 Recommendations

1.3.1 Approvability

On the basis of the nonclinical data reviewed in this marketing application, lixisenatide is recommended for approval.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling [Draft]

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

8.4 Pediatric (b) (4)

Safety and effectiveness of TRADENAME have not been established in pediatric patients below 18 years of age.

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

(b) (4) conducted in (b) (4) CD-1 mice (b) (4)

A statistically significant increase in thyroid C-cell adenomas was observed in male (b) (4) at

2000 µg/kg/day resulting in systemic exposures that are (b) (4) times the human exposure achieved at 20 µg/day, based on plasma area under the curve (AUC).

(b) (4)

Statistically significant increases in thyroid C-cell adenomas were seen at all doses (b) (4) systemic exposures that are ≥ (b) (4) times the human exposure achieved at a 20 µg/day, based on plasma AUC. A numerical increase in thyroid C-cell carcinomas was observed at ≥400 µg/kg/day, resulting in systemic exposures that are (b) (4) times the human exposure achieved at 20 µg/day, based on plasma AUC.

Mutagenesis

Lixisenatide was not mutagenic or clastogenic in a standard battery of gen (b) (4) toxic (b) (4) tests (bacterial mutagenicity (Ames), human lymphocyte chromosome aberration, mouse bone marrow micronucleus).

Impairment of Fertility

Studies in which male and female rats (b) (4) doses prior to pairing through gestation day 6 did not indicate any adverse effect on male or female fertility in rats up to the highest dose tested, (b) (4) approximately (b) (4) times the clinical (b) (4) at 20 µg/day, based on (b) (4) .

(b) (4)

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 320367-13-3

Proprietary Name: (b) (4)

Generic Name: Lixisenatide

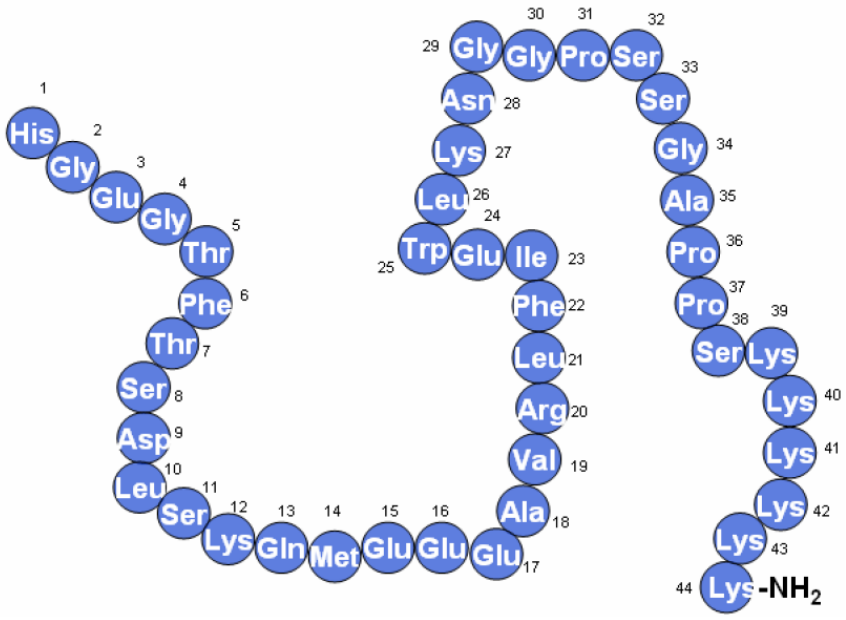
Code Name: AVE0010, ZS42-0010, ZP10

Chemical Name: 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: (b) (4) 4858.5 average

Molecular Weight: $C_{215}H_{347}N_{61}O_{65}S$

Structure: (Structurally similar to exendin-4)



Pharmacologic Class: Glucagon-like peptide-1 (GLP-1) receptor agonist

2.2 Relevant INDs and NDAs

Exendin-4 analogs

NDA 022200 / IND 67,092 - exenatide, Amylin, once weekly formulation
NDA 021773 / IND 57,725 - exenatide, BYETTA, Amylin
NDA 021919 / IND 57,725 - exenatide, BYETTA, Amylin, monotherapy
IND 62,724 - exendin-4 analog, Sanofi-Aventis

(b) (4)

GLP-1 analogs

NDA 22-341 / IND 61,040 - GLP-1 analog, liraglutide, Novo Nordisk

(b) (4)

2.3 Drug Formulation

Table 1 - Composition of lixisenatide solutions for injection

Components [a]	Composition						Function	Reference to standards [b]
	Percentage [%]		per mL [mg]		Per unit (3 mL cartridge) [mg]			
Dosage strengths [mg/mL]	**0.05**	**0.1**	**0.05**	**0.1**	**0.05**	**0.1**		
Lixisenatide	0.005	0.01	0.05	0.1	0.15	0.3	(b) (4)	In-house
Glycerol (85 per cent)	1.8		18.0		54.0			Ph. Eur.
Sodium acetate trihydrate [Sodium acetate]	0.35		3.5		10.5			Ph. Eur., USP
Methionine	0.3		3.0		9.0			Ph. Eur., USP
Metacresol [c]	0.27		2.7		8.1			Ph. Eur., USP
Hydrochloric acid (b) (4) [Hydrochloric acid]	---		q.s. pH (b) (4)		q.s. pH (b) (4)			Ph. Eur., NF
Sodium hydroxide	---		q.s. pH (b) (4)		q.s. pH (b) (4)			Ph. Eur., NF
Water for injection	(b) (4)							Ph. Eur., USP
							(b) (4)	Ph. Eur., NF

a Components are listed according to their pharmacopoeial names. 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 the dossier.

2.4 Comments on Novel Excipients

There are no novel excipients proposed for the clinical formulation.

2.5 Comments on Impurities/Degradants of Concern

The CMC reviewer identified some impurities/degradation products and inquired as to whether they have been qualified in the nonclinical program. As shown in Table 2, the impurities in question were tested in toxicology studies of 3 months' duration or greater in at least one species. Two toxicology studies of 2 weeks' duration were also conducted in rats with forced degradation products (Studies TSA1242 and TSA1331). The tested levels are approximately (b) (4)-fold higher than the maximum daily level for each impurity at the 20 µg clinical dose, based on the shelf life criteria. Therefore, these impurities/degradation products have been adequately qualified.

The sponsor has proposed a level of (b) (4)% for (b) (4) in the drug substance specifications. The certificates of analyses for the chronic toxicology and carcinogenicity studies reported that the content of (b) (4) was below the limit of quantitation ((b) (4)%), so it is uncertain how much (b) (4) was tested, if any, in those toxicology studies. The primary concern for high amounts of (b) (4) would be irritation or tissue damage at the injection site. However, even if a level of (b) (4)% was achieved, the maximum amount of (b) (4) in a clinical dose of 20 µg would only be (b) (4) ng; such a low concentration of (b) (4) is not expected to result in any adverse effects.

The CMC reviewer also had a question regarding the applicant's statement regarding genotoxic impurities: "among the raw materials, related impurities, solvents, and reagents investigated, only (b) (4) and (b) (4) were reported as genotoxic impurities. Due to the (b) (4), the likelihood that these potential impurities are present in the drug substance is low. This was confirmed on nine development batches tested for (b) (4) and (b) (4). Results obtained for each impurity were below (b) (4) ppm, which is far below the TTC [Threshold for Toxicological Concern] level." I am in agreement with the sponsor's conclusion that any residual amounts of these two genotoxic substances would be well below the TTC, and therefore, would not constitute a safety hazard for patients.

Table 2. Qualification of Drug Substance Impurities and Degradation Products

AVE0010 Batch Number	Area %							Tested in:
	Total Impurities	(b) (4)					(b) (4)	
1022/3330	(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		-	-	-	-	-	-	Mouse & rat carc
B002		(b) (4)	-	-	-	-	-	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)

2.6 Proposed Clinical Population and Dosing Regimen

Lixisenatide will be intended for use as an adjunct to diet and exercise to improve glycemic control in the treatment of adults with type 2 diabetes mellitus. Lixisenatide will be injected subcutaneously once daily at doses up to 20 µg.

In Phase 3 studies, the development of anti-drug antibodies increased over time, with approximately 70% of subjects testing positive after 24 weeks and approximately 72% testing positive after 76 weeks of treatment. Anti-lixisenatide antibodies were not shown to cross-react with endogenous GLP-1 or glucagon. The presence of antibodies resulted in increased drug exposure; in healthy individuals, mean AUC and C_{max} values increased by 4.4x and 3.1x, respectively after 182 days compared with antibody-negative subjects. After 28 days of treatment in patients with type 2 diabetes, AUC and C_{max} values were increased by approximately 5.6x and 3.2x, respectively (Study ACT6011). After 13 weeks of treatment in patients with type 2 diabetes, AUC and C_{max} values were increased by approximately 3.2x and 5.0x, respectively (Study DRI6012).

The mean clinical AUC_{tau} values for purposes of calculating nonclinical safety margins were 1.22 ng•h/mL for antibody negative patients and 7.25 ng•h/mL for antibody positive subjects (clinical study ACT6011).

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

- Binding affinity of AVE0010 to the GLP-1 receptor (Study mvt0010)
- Binding affinity of ZS42-0010 to the GLP-1 receptor (Study mvt0010ext)
- Receptor binding profile (Study mvt0011)
- Receptor binding profile for ZP10A (Study mvt0011exta)
- Receptor binding profile for SAR213916 (Study mvt0011extb)
- Receptor binding profile for SAR213916 and SAR274136 (Study mvt0011extc)
- Effect of AVE0010 on N-type calcium channels (Study nvt0222)
- Effect of AVE0010 on glucose-stimulated insulin secretion (Study mvt0012)
- Effect of AVE0010 on blood glucose in diabetic mice (Study mvv0002)
- Duration of action of AVE0010 on glucose tolerance in db/db mice (Study mvv0013)
- Effect of AVE0010 on oral glucose tolerance in Zucker diabetic fatty rats (Study mvv0008)
- Effect of AVE0010 on oral glucose tolerance in male dogs (Study mvv0009)
- Effect of lixisenatide and liraglutide on oral glucose tolerance in dogs (Study divv0006)
- Effect of AVE0010 on glucose tolerance in db/db mice (Study mvv0007)
- Insulinotropic effect of AVE0010 after 42 days of dosing in db/db mice (Study mvv0006)
- Effect of AVE0010 on diabetic progression in db/db mice (Study mvv0003)
- Effect of chronic AVE0010 on glycemic control in Zucker diabetic fatty rats (Study mvv0010)
- Effect of AVE0010 on glucose-stimulated insulin secretion - ex vivo (Study mvt0013)

Secondary Pharmacology

- Cardioprotective effect of lixisenatide on ischemia/reperfusion-induced injury (Study divt0002)
- Cardioprotective effect of lixisenatide on ischemia/reperfusion-induced injury (Study divv0030)
- Effect of chronic treatment on atherosclerotic plaque formation (Study divv0007)
- Profiling of VIP, PACAP, and glucagon family receptors in rat, dog, and human epididymis and testis by RT-PCR (Study div1283)

Safety Pharmacology

- Effect of AZ42-0010 on general activity and behavior in rats (Study 2004-1177)
- Effect of AVE0010 on general behavior in male mice - Irwin study (Study 2004-1224)
- Exploratory hERG channel affinity assay (Study 2003-1521)
- Electrophysiology assay on isolated male rabbit purkinje fibers (Study 2005-0098)
- Cardiovascular effects of ZS42-0010 in conscious rats (Study div1154)
- Cariovascular and respiratory effects in anesthetized dogs (Study 2004-1182)
- Cardiovascular effects of insulin glargine plus AVE0010 in dogs (Study cvr0345)
- Effect of AVE0010 and exendin-4 on the rate of gastric emptying (Study mvv0005)

Absorption, Distribution, Metabolism, and Excretion (ADME)

Analytical methods

- ELISA validation for measuring AVE0010 in rat EDTA plasma (Study f2004kin0107)
- ELISA validation for measuring AVE0010 in rat EDTA plasma after sample processing (Study f2005kin0248)
- ELISA validation for measuring AVE0010 in mouse EDTA plasma (Study f2004kin0145)
- ELISA validation for measuring AVE0010 in mouse EDTA plasma after sample processing (Study f2005kin0284)
- ELISA validation for measuring AVE0010 in dog EDTA plasma (Study f2004kin0152)
- ELISA validation for measuring AVE0010 in dog EDTA plasma after sample processing (Study f2005kin0308)
- ELISA validation for measuring AVE0010 in rabbit EDTA plasma (Study f2005kin0019)
- Method validation for measuring ZS42-0010 in rat, dog, and pig blood (Study 00-107)
- ELISA validation for measuring anti-AVE0010 antibodies in rat EDTA plasma (Study f2004kin0265)
- Influence of the presence of anti-AVE0010 antibodies on the measurement of AVE-0010 in rat EDTA plasma (Study f2004kin0246)
- ELISA validation for measuring anti-AVE0010 antibodies in mouse EDTA plasma (Study f2006kin0014)
- (b) (4) method validation for detecting anti-AVE0010 antibodies in dog EDTA plasma (Study dos1493)
- Validation of cell-based assay to detect AVE0010 activity in rat plasma (Study dos1043)

Absorption

- Single SC, IV, or IP dose PK study in db/db mice (Study 00-077)
- Single SC, IV, or IP dose PK study in nmri mice (Study 00-085)
- Single SC or IV dose PK study in Sprague-Dawley rats (Study 00-062)
- Single SC or IV dose PK study in Sprague-Dawley rats (Study 00-074)

- Single IP dose PK study in Sprague-Dawley rats (Study 01-012)
- Single SC dose PK study in anesthetized rabbits (Study 00-032)
- Single IV dose PK study in anesthetized rabbits (Study 00-044)
- Single SC dose PK study in conscious rabbits (Study 00-139)
- Single SC dose PK study in conscious rabbits (Study 02-139)
- 3-day SC infusion PK study in conscious rabbits (Study 03-013)
- Single SC or IV dose PK study in dogs (Study 00-141)
- Single SC or IV dose PK study in anesthetized pigs (Study 00-045)
- Single SC or IV dose PK study in pigs (Study 00-083)

Distribution

- Tissue distribution after SC or IV dose in Long Evans rat (Study dis0474)
- Tissue distribution and label biostability after SC or IV dose in Long Evans rat (Study dis0531 and amendment 1)
- Tissue distribution in pregnant rats and fetuses at GD12 and GD17 (Study plt0236)
- Tissue distribution in pregnant rabbits and fetuses at GD12 and GD18 (Study plt0236)
- In vitro protein binding in rat and dog plasma (Study Ipr1021)

Metabolism

- In vitro metabolite profiling in S9 fraction from liver and kidney of mouse, rat, rabbit, dog, and human (Study f2006kin0001)
- In vitro stability in heparin stabilized plasma from mouse, rat, rabbit, dog, pig, and human (Study 00-039)

Excretion

- Milk excretion of labeled drug after a single SC dose in lactating non-pigmented female rats (Study 00-039)

Toxicology

General toxicology

- Single SC dose study in mice (Study 2004-1181)
- Single IV dose study in mice (Study 2004-1180)
- day QD and BID SC study in mice with 5-day recovery (Study div1333)
- Single SC dose study in rats (Study 2004-1179)
- day QD and BID SC study in rats with 5-day recovery (Study div1332)
- Single SC dose study in rats with 14-day observation period (Study 2004-1186)
- Single IV dose study in rats (Study 2004-1178)
- Single IV dose study in rats with 14-day observation period (Study 2004-1184)
- Single SC dose study in dogs with 14-day observation period (Study 2004-1187)
- Single IV dose study in dogs with 14-day observation period (Study 2004-1185)

- 14-day SC study in mice (Study 2003-1952)
- 14-day SC bid study in mice (Study tsa1242)
- Exploratory 1-month SC infusion study in mice (Study ddo1171)
- 13-week SC bid dosing study in mice (Studies 2004-0062 and 2005-0443)

- 5-day SC study in rats (Study 2003-1276)
- 5-day SC study in rats (Study 2003-1986)
- 5-day SC study in rats (Study 2003-1987)
- 2-week SC study in rats with 2-week recovery (Studies 2003-1925, f2004kin0120, f2005kin0160, f2004kin0241, f2005kin0002, and f2005kin0161)
- 14-day SC study in rats (Study tsa1331)
- 4-week IV study in rats (Studies 2003-2059, f2005kin0227, f2004kin0249, and div1156)
- 13-week SC bid dosing study in rats with 4-week recovery (Study 2004-0063 and amendment 1)
- 6-month SC study in rats with 4-week recovery (Study 2005-0085)
- Exploratory bid SC dose escalation study in dogs (Study 2005-0233)
- 4-week SC study in dogs (Studies 2003-2060 and div1157)
- 13-week SC study in dogs with 4-week recovery (Study 2003-1926 and amendment 1)
- 12-month SC bid dosing study in dogs (Study 2004-0064 and amendment 1)

Genetic toxicology

- Bacterial reverse mutation assay (Study 2004-1183)
- Bacterial reverse mutation assay (Study 2004-1342)
- Bacterial reverse mutation assay (Study 2005-0234)
- In vitro mammalian chromosome aberration assay (Study maf0100)
- In vitro mammalian chromosome aberration assay (Study 2004-1343)
- In vitro mammalian chromosome aberration assay (Study 2005-0386)
- Mouse micronucleus assay (Study mut0212)

Carcinogenicity

- 2-year SC carcinogenicity study in mice (Study car0085 and amendment 1)
- 2-year SC carcinogenicity study in rats (Study car0084 and amendment 1)

Developmental, reproductive, and juvenile toxicology

- Fertility and early embryonic development study in rats (Study 2004-0550 and amendment 1)
- SC RF study in pregnant rats (Study 2003-1923)
- Embryo-fetal toxicity study in rats (Studies 2004-0551, f2005kin0022, f2005kin0162, and f2004kin0309)
- Range-finding study in pregnant rabbits (Study 2003-1924)
- Embryo-fetal toxicity study in rabbits (Studies 2004-0552, f2005kin0024, f2005kin0163, and f2004kin0250)
- Embryo-fetal toxicity study in rabbits (Study 2005-1086)
- Pre- and post-natal development RF in rats (Study dpp0025)
- Pre- and post-natal development RF in rats (Study dpn0327)
- 14-day juvenile RF toxicity study in rats (Study jup0012)
- 5-week SC bid dosing study in juvenile rats (Study juv0026)
- 2-week SC bid PK study in juvenile dogs (Study div1328)
- 8-month SC bid toxicity study juvenile males with 2 month recovery (Study txc1462)

Local tolerance

- Local IV, intra-arterial, paravenous, SC, and IM tolerance study in rabbits (Study 2005-0519)
- Local SC, IM, and paravenous tolerance study in rabbits (Study 2005-0771 and amendment 1)
- Local IV, intra-arterial, paravenous, SC, and IM tolerance study in rabbits (Study 2005-0327)

Mechanistic studies to evaluate thyroid C-cell proliferation

- GLP-1 receptor expression profiling in rat thyroid tissue (Study div1353)
- GLP-1 receptor expression profiling in human thyroid FFPE tissue (Study div1391)
- GLP-1 receptor expression profiling in normal human thyroid tissue (Study div1416)
- GLP-1 receptor expression profiling in normal human tissue (Study div1422)
- GLP-1 receptor expression profiling in normal human tissue (Study div1487)
- GLP-1 receptor expression profiling in normal human tissue (Study div1498)
- GLP-1 receptor expression profiling in human tissue with C-cell pathology (Study div1478)
- GLP-1, insulin, and IGF receptor expression profiling in normal human tissue compared with human tumor samples (Study div1404)
- Functional activity of lixisenatide in thyroid c-cells of rat and human origin (Study divt0007)
- Exploratory 14-day SC bid study in GLP-1R (-/-) KO and wild-type mice (Study tsa1481)
- 3-month SC study in mice (Study txc1491)
- 12-week SC infusion study in mice (Study txc1492)
- 3-month SC infusion study in mice (Study txc1505) - submitted to IND 62,724 (SD#391)

Other toxicology studies

- 3-month SC study in rats to qualify impurities (Study txc1482)
- B-cell response to SC and oral administration in mice (Study div1155)
- T-cell response to parenteral administration in mice (Study div1158 and amendment 1)
- Skin sensitization and irritation test [redacted] (b) (4) (Study ist0273)
- Skin sensitization and irritation test with [redacted] (b) (4) (Study ist0274)

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Some toxicology studies were previously reviewed by Dr. Dalin Yao, where indicated. Dr. Yao's reviews can be found in DARRTS under IND 62724.

4 Pharmacology

4.1 Primary Pharmacology

Binding Affinity of AVE0010 to the GLP-1 Receptor (Study MVT 0010 and MVT 0010ext)

Study Design: (sponsor-generated summary)

Test: Radioligand binding assay with human GLP-1 receptor vs. 0.03 nM ^{125}I GLP1(7-36) amide	**Test Article (batch):** AVE0010 (b) (4), human GLP-1 (b) (4)	**Location in CTD:** in module 4
Test system: CHO-K1 cells transfected with human GLP-1 receptor	**Vehicle:** 0.4% DMSO	**Study No.:** 99-034, (b) (4)
No. of Preparations / Group: AVE0010 n=2, human GLP-1, n=2	**Administration:** in vitro in buffer medium	**Report No.:** (b) (4) MVT0010
Study Period: 27.09.-27.10.1999	**Concentration:** 0.1 nM – 10 µM	**GLP Compliance: Not required**

Results: AVE0010 had a binding affinity (IC_{50}) for the human GLP-1 receptor of 1.43 ± 0.239 nM (K_i = 1.33 ± 0.222 nM). Human GLP-1 had a binding affinity for the human GLP-1 receptor of 5.48 ± 1.28 nM (K_i = 5.09 ± 1.19 nM).

Conclusions: The results of this study indicate that AVE0010 has a strong binding affinity to the human GLP-1 receptor that is approximately 4 times greater than that of human GLP-1.

Effect of AVE0010 on Glucose-Stimulated Insulin Secretion in Isolated Pancreas of Male Wistar Rats in vitro (Study MVT0012)

Study Design: (sponsor-generated summary)

Test: Acute effect of AVE0010 on GSIS in male Wistar rats of the isolated perfused pancreas	**Test Article (batch):** AVE0010 (b) (4)	**Location in CTD:**
Species/Strain: rats / male Wistar HsdCpd:WU (b) (4)	**Vehicle:** Krebs-Henseleit-Buffer (KRB)	**Study No.:** MVT0012
Gender/No. per Group: male / n = 6-8	**Treatment Schedule:** continuously perfusion	**Report No.:** (b) (4) MVT0012
Weight/Age: 200-220g / 6-7 weeks	**Administration:** perfusion of the isolated pancreas	**Study Period:** November 2005; February 2006
	Doses: 10 nmol/L	**GLP Compliance:** Not required

Results: The acute effect of AVE0010 on glucose-stimulated insulin secretion from isolated perfused pancreas of male Wistar rats was determined in response to high glucose perfusion (16.5 mmol/L). Total insulin secretion over 50 minutes was collected and calculated as AUC (Sponsor-generated table).

Insulin secretion $AUC_{(10\text{-}60min)}$ (min*µg/L)

	median	25 / 75% quartiles	n	p-value vs. CTRL
CTRL	775.5 (100%)	234.5 / 863.5	8	
AVE 10nmol/L	3784 (488%) *	2888 / 5034 [1)]	7	0,0002
GLP-1 10nmol/L	2391.5 (308%) *	1296 / 2817	6	0,0188

1) upper quartile for the AVE group is influenced by AUC values underestimated by overflow concentrations

Conclusions: AVE0010 significantly increased insulin secretion from perfused pancreas isolated from male Wistar rats compared with control levels.

Effects of Single Intraperitoneal Doses of AVE0010 on Blood Glucose Levels in Diabetic Mice after an Oral Glucose Tolerance Test (Study MVV0002)

Study Design: (sponsor-generated summary)

Test: Acute effects of i.p. AVE0010 on oral glucose tolerance (1g glucose/kg) in db/db mice	**Test Article (batch):** AVE0010 (b) (4)	**Location in CTD:** in module 4
Species/Strain: Mice / db/db BKS.Cg-m+/+ Lepr^{db} (b) (4)	**Vehicle:** isotonic saline	**Study No.:** MVV0002 [# 99-045 (Zealand)]
Gender/No. per Group: Male / n = 7-8	**Treatment Schedule:** single dose	**Report No.:** (b) (4) MVV0002
Weight/Age: 41g – 53 g / 8 weeks	**Administration:** i.p.	**Study Period:** 05.10.1999
	Doses: 0 - 0.17 - 1.7 - 17 and 170 nmol/kg	**GLP Compliance:** Not required

Results: When given 15 minutes before an oral glucose load, AVE0010 provided an anti-hyperglycemic effect in db/db mice. The effects were measured as glucose AUC that occurred within the first 120 minutes of the glucose challenge compared with control.

Conclusions: The results of this study showed that AVE0010 effectively and dose dependently improved oral glucose tolerance in diabetic animals with an ED_{50} of 0.256 nmol/kg i.p.

Long-Lasting Duration of Action of a Maximal Intraperitoneal Dose of AVE0010 on Glucose Tolerance in db/db Mice Subjected to an Oral Glucose Tolerance Test (Study MVV0013)

Study Design: (sponsor-generated summary)

Test: Oral glucose tolerance test (1g glucose/kg) in db/db mice with ip AVE0010 given at 20 different time points prior to the oral glucose	**Test Article (batch):** AVE0010 (b) (4)	**Location in CTD:** in module 4
Species/Strain: 33 mice / db/db BKS.Cg-m+/+ (b) (4)	**Vehicle:** PBS with 0.1% albumin, ph 7.4	**Study No.:** MVV0013 [# 00-050 (Zealand)]
Gender/No. per Group: Male / n = (b) (4)	**Treatment Schedule:** single dose, but same animals were tested repeatedly	**Report No.:** (b) (4) MVV0013
Weight/Age: 37±1g –39±1 g / 11-18 weeks	**Administration:** i.p.	**Study Period:** 09.06.-07.07.2000
	Doses: (b) (4)	**GLP Compliance:** Not required

Results: The duration of action of AVE0010 was evaluated through an oral glucose tolerance test in db/db mice. Statistically significant differences of $AUC_{0\text{-}240min}$ for AVE0010 compared with vehicle were shown for the time period between 15 and 720 minutes. At 1080 minutes, a non-statistically significant decrease in AUC was still observed for the AVE0010-treated group.

Conclusions: The anti-diabetic effect of 100 nmol/kg AVE0010 (i.p.) was statistically significant for 12 hours in db/db mice.

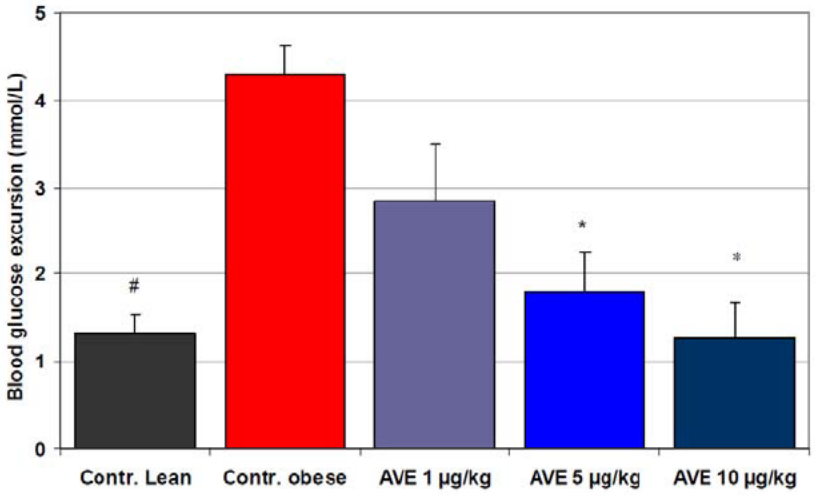
Acute Effect of Subcutaneous AVE0010 on Oral Glucose Tolerance in Zucker Diabetic Fatty Rats (Study MVV0008)

Study Design: (sponsor-generated summary)

Test: Acute effect of sc AVE0010 on oral glucose tolerance (2g glucosoe/kg) in ZDF rats	**Test Article (batch):** AVE0010 (b) (4)	**Location in CTD:** in module 4
Species/Strain: Rats/obese (ZDF/Gmi-fa/fa) and lean (ZDF/Gmi-Fa/?) (b) (4)	**Vehicle:** phosphate-buffered saline, pH 4.5	**Study No.:** R030903ZDF
Gender/No. per Group: Male/ n = 8	**Treatment Schedule:** single dose	**Report No.:** MVV008
Weight/Age: 332–408g (obese) and 287–319 g (lean)/11 weeks	**Administration:** s.c.	**Study Period:** 03-Sep-2003 – 05-Sep-2003
	Doses: 1, 5 and 10 µg/kg AVE0010	**GLP Compliance:** Not required

Results: Following oral glucose challenge, blood glucose increased by 4.30 nmol/L in insulin-resistant, obese ZDF control rats; however, in insulin-sensitive, lean controls, the mean blood glucose excursion was 1.32 mmol/L. The blood glucose excursions in obese ZDF rats treated with 1, 5, and 10 µg/kg AVE0010 (s.c.) were 2.84, 1.81, and 1.27 mmol/L, respectively (Sponsor-generated Figure 2). The mean blood glucose excursions in the 5 and 10 µg/kg groups were significantly different from the obese control group. Relative to obese and lean placebo-treated control rats, these results correspond to dose-dependent reductions by 49%, 84%, and 100%.

Figure 2 - Effect of subcutaneous AVE0010 on blood glucose excursion in oral glucose tolerance test in ZDF rats.



Conclusions: A single subcutaneous injection of 5 or 10 µg/kg AVE0010 30 minutes before an oral glucose challenge significantly and dose-dependently improved oral glucose tolerance in obese, diabetic ZDF rats.

Effect of AVE0010 on Glucose-Stimulated Insulin Secretion in Isolated Pancreas of Chronically Treated Male Zucker Diabetic Fatty Rats ex vivo (Study MVT0013)

Study Design: (sponsor-generated summary)

Test: Glucose stimulated insulin secretion in chronically treated male Zucker Diabetic Fatty rats	**Test Article (batch):** AVE0010 (b) (4)	**Location in CTD:**
Species/Strain: Rats / obese (ZDF/Gmi-fa/fa) and lean (ZDF/Gmi-Fa/?) (b) (4)	**Vehicle:** phosphate-buffered saline, pH 4.5	**Study No.:** MVT0013
Gender/No. per Group: male / n = 6-7	**Treatment Schedule:** continuous perfusion	**Report No.:** MVT0013
Weight/Age: 360-390g (obese) and 240-260 g (lean) / 12 weeks	**Administration:** sc	**Study Period:** March – April 2005
	Doses: 50µg*kg^{-1}*day^{-1}	**GLP Compliance:** Not required

Results/Conclusions: Obese, male ZDF rats treated with AVE0010 for 6 weeks preserved GSIS compared to vehicle-treated obese rats. GSIS was not significantly altered in lean ZDF rats (AVE0010 treated group compared to vehicle, CTRL group).

Figure 1. Effect of AVE0010 on glucose-stimulated insulin secretion in isolated pancreas of chronically treated lean, male Zucker Diabetic Fatty rats ex vivo

Insulin secretion AUC(10-60min) (min*µg/L)			
	mean	SEM	n
lean, CTRL	896	294	6
lean, AVE	645	123	6
obese, CTRL	507	137	7
obese, AVE	2026	390	6

Effect of Chronic Treatment with Subcutaneous AVE0010 on Glycemic Control in Male Zucker Diabetic Fatty Rats (Study MVV0010)

Study Design: (sponsor-generated summary)

Test: Effect of sc AVE0010 in ZDF rats	**Test Article (batch):** AVE0010 (b) (4)	**Location in CTD:** in module 4
Species/Strain: Rats / obese (ZDF/Gmi-fa/fa) and lean (ZDF/Gmi-Fa/?) (b) (4)	**Vehicle:** water, 30 µL/day	**Study No.:** MVV0010
Gender/No. per Group: Male / n = 8	**Treatment Schedule:** continuous SC infusion	**Report No.:** (b) (4) MVV010
Weight/Age: 8 weeks at study start and 20 weeks at study end	**Administration:** continuous SC infusion via implanted osmotic minipumps	**Study Period:** 22-Jun-2004 – 15-Sep-2004
	Doses: 0.1, 1 and 10 nmol/kg*day AVE0010, reference: 1 nmol/kg*day exendin-4	**GLP Compliance:** Not required

Results: Introduction of a high-fat diet (HFD) to obese ZDF rats resulted in hyperglycemia, hyperinsulinemia, decreased oral glucose tolerance, and increased HbA1c as compared to lean ZDF rats. A dose of 10 nmol/kg/day AVE0010 administered to obese ZDF rats significantly decreased basal blood glucose during the diabetic phase and HbA1c at the end of the study (Sponsor-generated Figures 3 and 6). Oral glucose tolerance was improved at the time point of highest blood excursion (1 hour) after 5.5 weeks on HFD, when the obese ZDF cohort was overtly diabetic (Sponsor-generated Figure 5). This treatment-related improvement was not observed after 1 week of treatment, at which time obese ZDF rats were still normoglycemic. At study end, plasma insulin levels in the obese groups were significantly higher than in lean ZDF groups and untreated control groups (Sponsor-generated Figure 7). AVE0010 did not alter any of the metabolic parameters that were measured in lean rats. AVE0010 resulted in reduced food intake in both obese and lean ZDF rats that resulted in decreased body weight in the lean cohort only.

Figure 3 - Effect of AVE0010 on basal blood glucose in ZDF rat

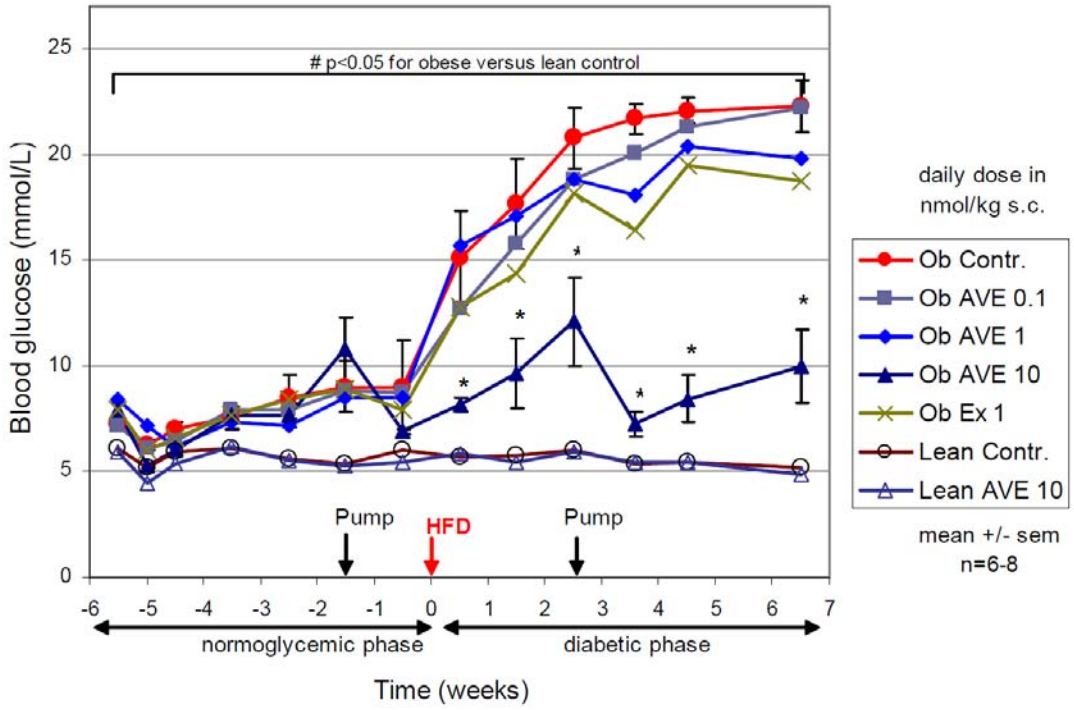


Figure 5 - Effect of 5.5 weeks AVE0010 on oral glucose tolerance in ZDF rats in diabetic phase (normalized by baseline values at t=0)

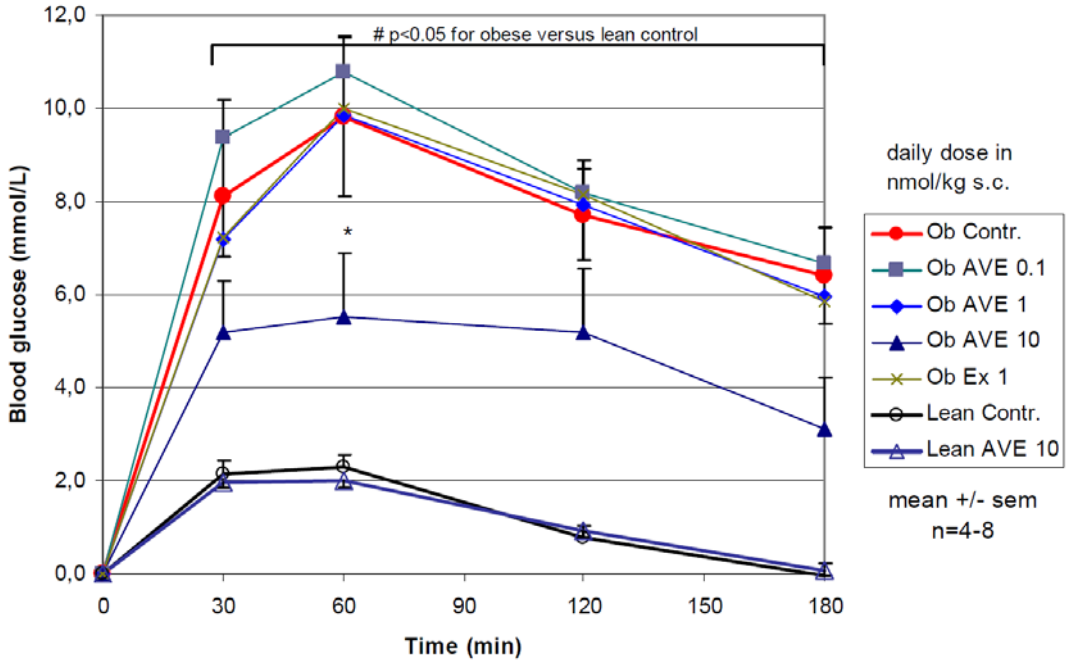


Figure 6 - Effect of AVE0010 on HbA1 in ZDF rat

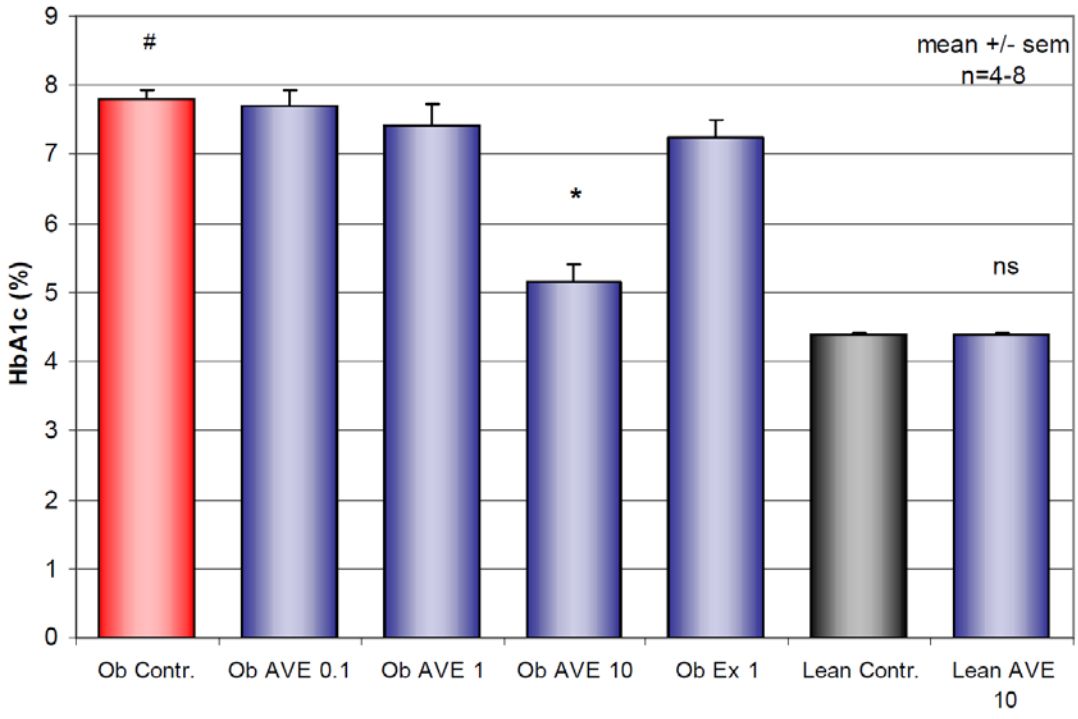
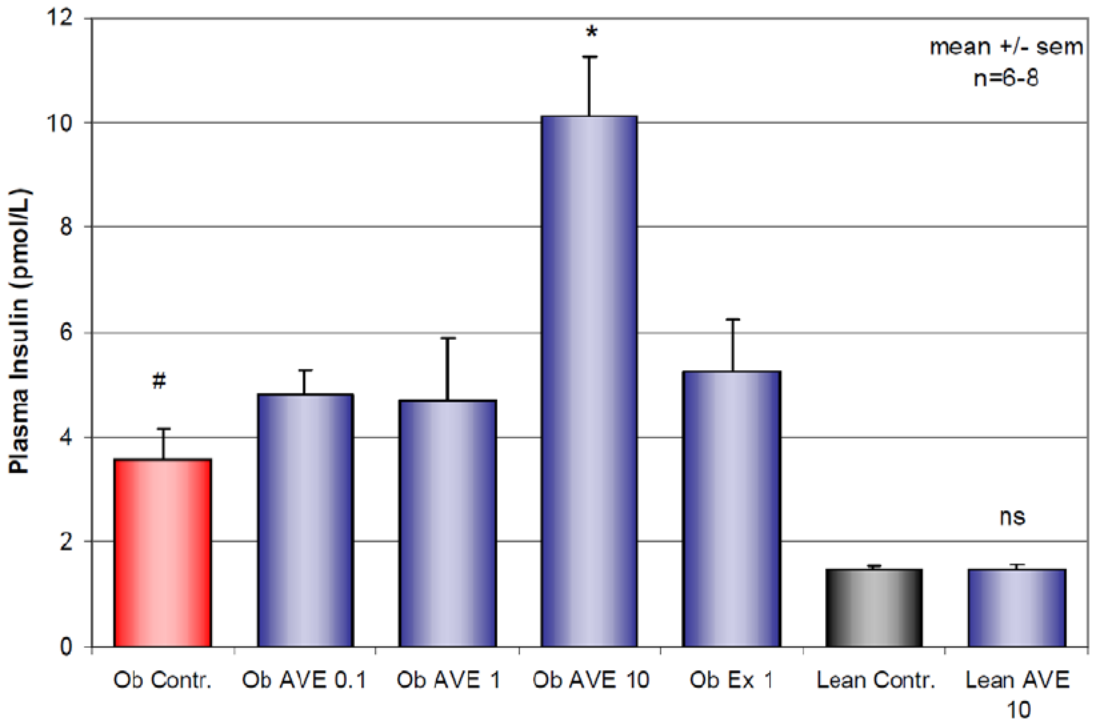


Figure 7 - Effect of AVE0010 on plasma insulin in ZDF rat



Conclusions: 10 nmol/kg/day AVE0010 significantly improved oral glucose tolerance, hyperglycemia, and HbA1c and preserved the pancreatic production of insulin and glucose responsiveness in obese ZDF rats, while the same dose did not induce hypoglycemia or insulin release in lean, normoglycemic ZDF rats.

AVE0010 Prevents Diabetic Progression in db/db Mice(Study MVV0003)

Study Design: (sponsor-generated summary)

Test: Chronic effects of ip AVE0010 on blood glucose levels, oral glucose tolerance, HbA_{1c} and pancreatic insulin mRNA in db/db mice	**Test Article (batch):** AVE0010 (b) (4)	**Location in CTD:** in module 4
Species/Strain: Mice / db/db BKS.Cg-m+/+ $Lepr^{db}$ (b) (4)	**Vehicle:** phosphate buffer containing 0.1% BSA, pH = 7.4, 5 ml/kg	**Study No.:** MVV0003 [# 01-010 (Zealand)]
Gender/No. per Group: Male / n = 9-11	**Treatment Schedule:** multiple dose, days 1-50 or 51-90 or 0-90	**Report No.:** (b) (4) MVV0003
Weight/Age: 37.3 ± 1.1 g / 6-10 weeks	**Administration:** i.p.	**Study Period:** Jan. – Jul. 2001
	Doses: 0 and 100 nmol/kg	**GLP Compliance:** Not required

Results: Mice received 100 nmol/kg AVE0010 from Day 1 to 50 (Group 2), from Day 51 to 90 (Group 3), or from Day 1 to 90 (Group 4). Body weights were similar between treated and control (Group 1) groups during Treatment Phase 1 (Day 1 to 50). During Treatment Phase 2 (Day 51 to 90), Group 4 animals gained more weight than controls. Fasting blood glucose (FBG) was significantly higher in controls compared with treated animals.

Group 2 mice, which were switched to vehicle on Day 51, had increased FBG compared with mice receiving AVE0010 throughout the entire study.

On Days 67 to 90, oral glucose tolerance of the three groups receiving AVE0010 was greater than the control group. Glucose tolerance was similar between the three treated groups during Phase 2. Group 4 animals had an increased expression of insulin mRNA after 90 days of treatment compared with Group 1. Group 2 and Group 4 animals had a similar degree of insulin mRNA expression, whereas Group 3 animals had expression levels more similar to untreated controls. A non-statistically significant decrease in HbA1c was observed for Group 4 (6.65%) compared with controls (7.99%).

Conclusions: Once daily treatment with 100 nmol/kg (i.p.) AVE0010 for 3 months prevented the progressive development of diabetes in db/db mice. AVE0010 significantly increased glucose tolerance, decreased FBG, and decreased water intake (Sponsor-generated Figures 3 and 4). Treatment also resulted in a non-statistically significant decrease in HbA1c and an increase in expression of insulin mRNA in pancreatic beta cells relative to control mice (Sponsor-generated Figures 5 and 6). Mice receiving AVE0010 only during Phase 1 had a sustained improvement in glucose tolerance, FBG, and water consumption.

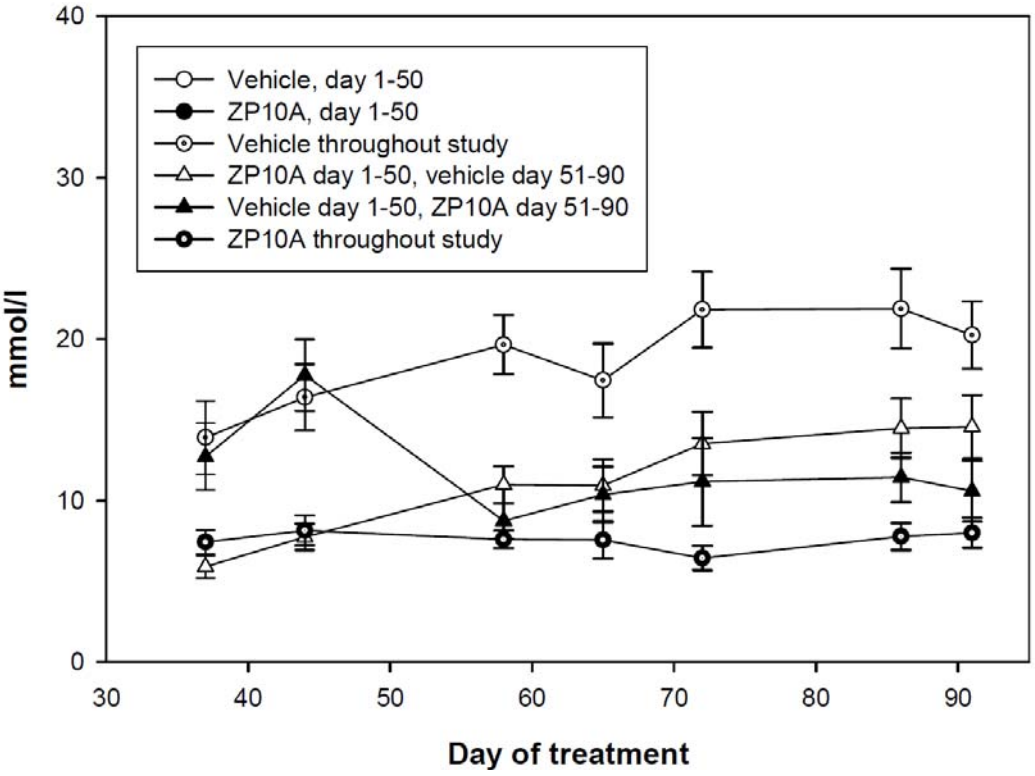


Figure 3. Effect of AVE0010 on Fasting Blood Glucose

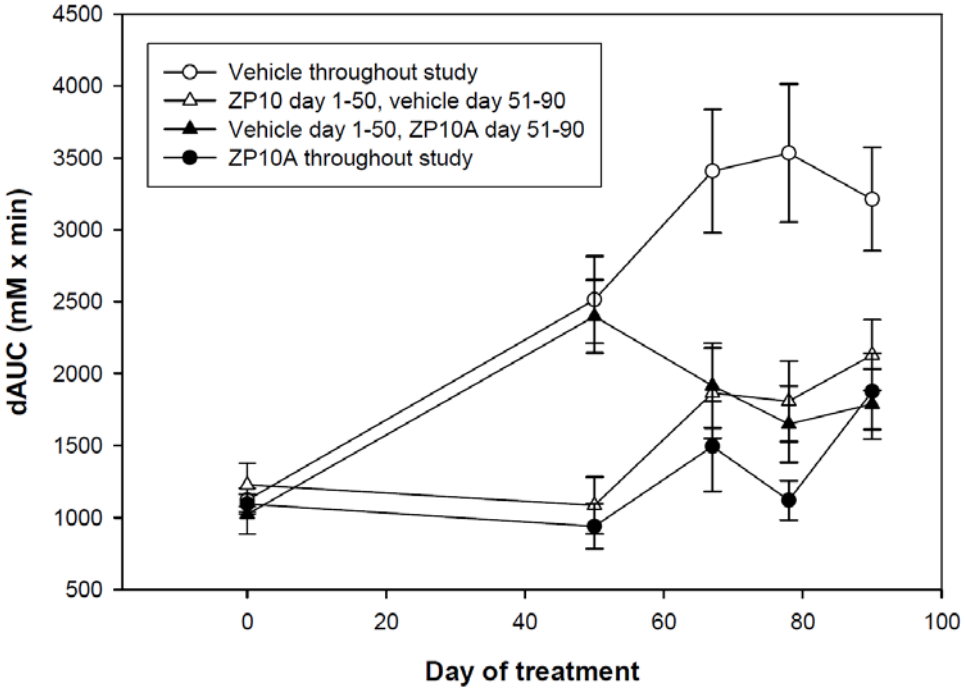


Figure 4. Effect of AVE0010 on Oral Glucose Tolerance

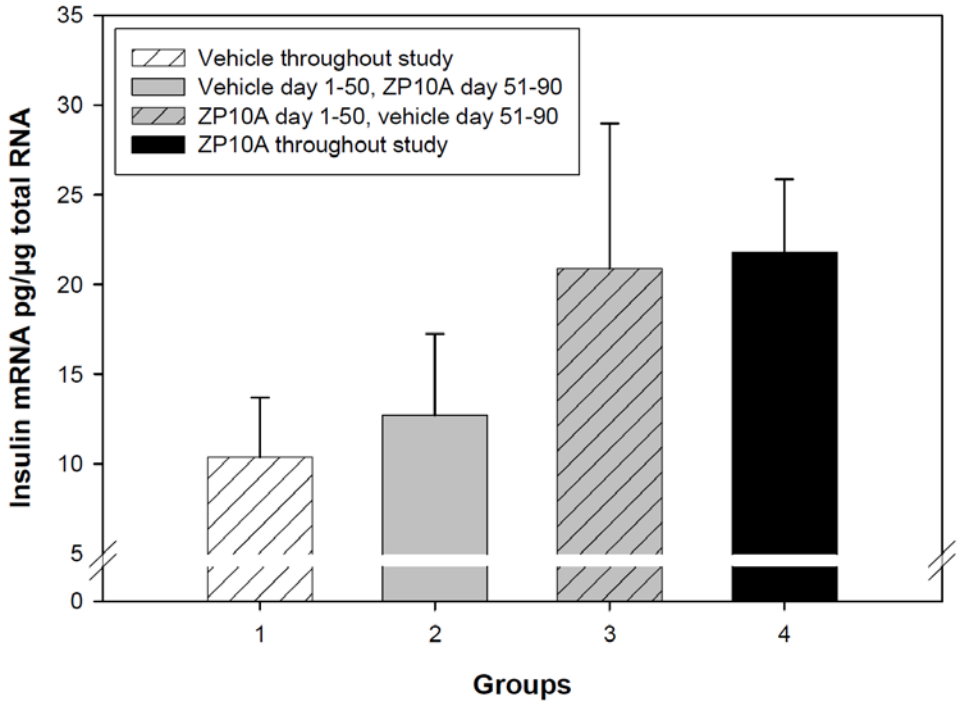


Figure 5. Effect of AVE0010 on Pancreatic Insulin mRNA

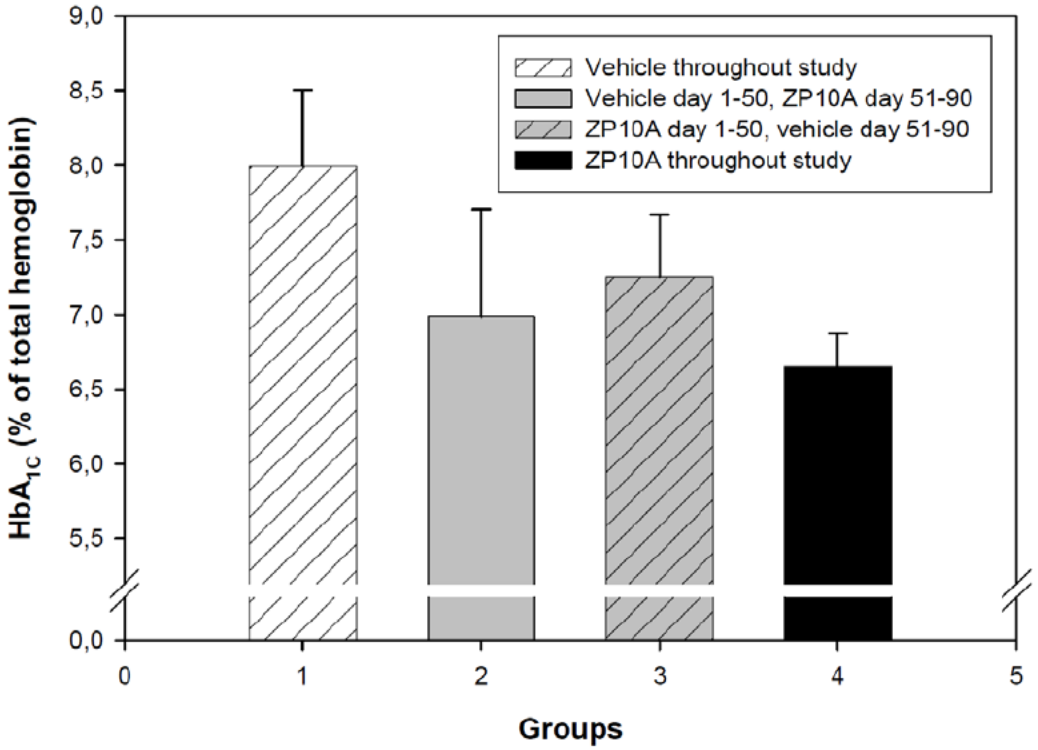


Figure 6. Effect of AVE0010 on HbA1c

Insulinotrophic Effect of AVE0010 after 42 Days of Twice Daily Dosing (Study MVV0006)

Study Design: (sponsor-generated summary)

Test: Chronic effects of twice daily ip AVE0010 on pancreatic β-cell volume in db/db mice	**Test Article (batch):** AVE0010 (b) (4)	**Location in CTD:** in module 4
Species/Strain: Mice / db/db BKS.Cg-m+/+ Lepr^{db} (b) (4)	**Vehicle:** phosphate buffer containing 0.1% BSA, pH = 7.4, 5 ml/kg	**Study No.:** MVV0006 (b) (4) (Zealand)]
Gender/No. per Group: Male / n = 5 out of a total of n = 15	**Treatment Schedule:** multiple dose, 42 days twice daily	**Report No.:** (b) (4) MVV0006
Weight/Age: 33.1 ± 0.9 g / 6-8 weeks	**Administration:** i.p.	**Study Period:** 19.05. – 03.08.2000
	Doses: 0 - 1 - 10 - and 100 nmol/kg BID	**GLP Compliance:** Not required

Results/Conclusions: Beta cells were intensively stained for insulin and exocrine cells were devoid of staining. Stereology analysis of beta cell volume showed a tendency towards a dose-dependent increase of the beta cell volume after administration of AVE0010 (Sponsor-generated Figure 2).

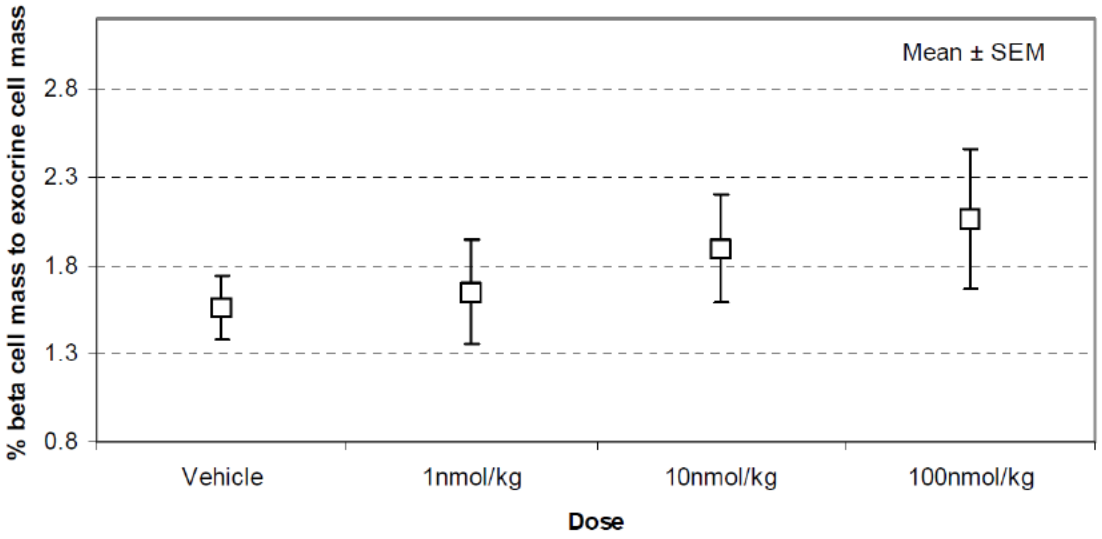


Figure 2: Effect of AVE0010 on beta cell mass after 6 weeks of treatment

Effect of 42 Days' i.p. Administration Twice Daily of Three Doses of AVE0010 on Blood Glucose Levels, Oral Glucose Tolerance, and HbA_{1c} in Diabetic db/db Mice (Study MVV0007)

Study Design: (sponsor-generated summary)

Test: Chronic effects of twice daily ip AVE0010 on blood glucose levels, oral glucose tolerance and HbA_{1c} in db/db mice	**Test Article (batch):** AVE0010 (b) (4)	**Location in CTD:** in module 4
Species/Strain: Mice / db/db BKS.Cg-m+/+ $Lepr^{db}$ (b) (4)	**Vehicle**: phosphate buffer containing 0.1% BSA, pH = 7.4, 5 ml/kg	**Study No.:** MVV0007 (b) (4) (Zealand)]
Gender/No. per Group: Male / n = 15	**Treatment Schedule:** multiple dose, 42 days twice daily	**Report No.:** SPRFU-MVV0007
Weight/Age: 33.1 ± 0.9 g / 6-8 weeks	**Administration:** i.p.	**Study Period:** 19.05. – 03.08.2000
	Doses: 0 - 1 - 10 - and 100 nmol/kg BID	**GLP Compliance:** Not required

Results: Water and food intake was reduced in treated groups, although body weight increases were similar across all groups. Administration of AVE0010 resulted in a dose-dependent decrease in HbA1c for all treated groups (Sponsor-generated Figure 4). Fasting blood glucose was also decreased for treated groups. In vehicle-treated mice, oral glucose tolerance was progressively impaired. By the end of the study, control mice given an oral glucose load had a glucose $AUC_{0\text{-}240min}$ level that was 7-fold higher than on the day of stratification. When given an oral glucose challenge at the end of the study, treated animals had similar or slightly greater (~2x) glucose AUC values than the pre-treatment values (Sponsor-generated Figure 6).

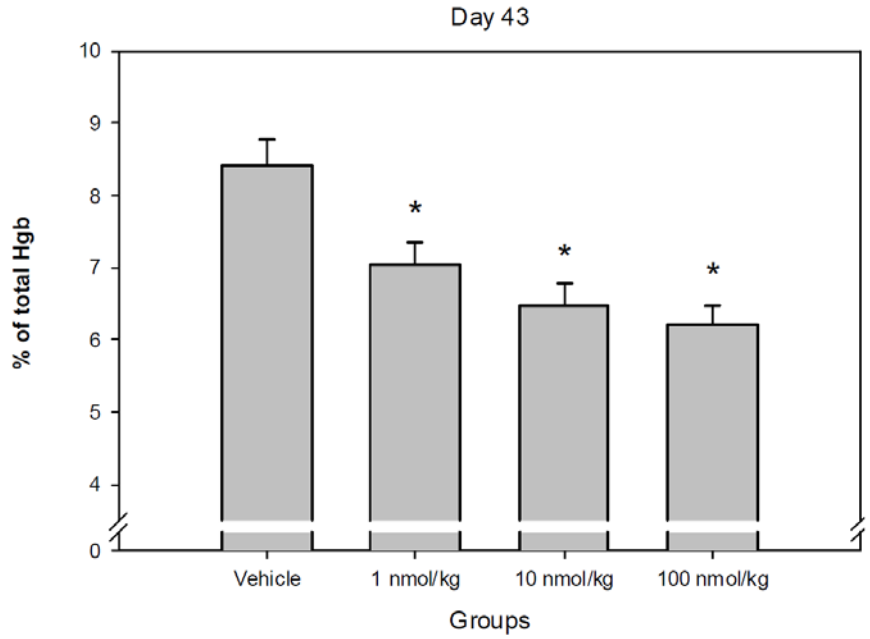


Figure 4. AVE0010 effects on HbA1c after 6 weeks of treatment

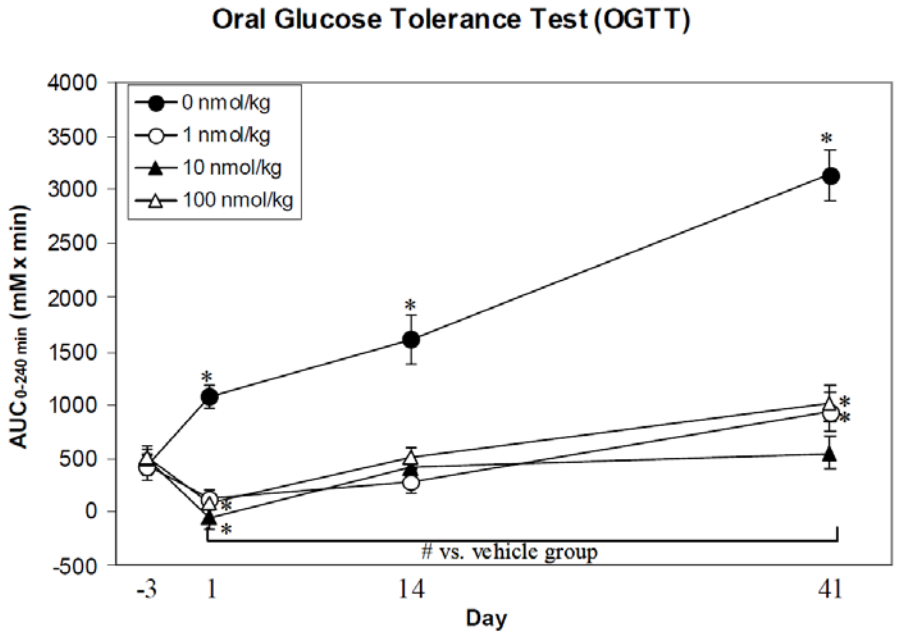


Figure 6. Effect of AVE0010 on the oral glucose tolerance test

Conclusions: AVE0010 prevented the progressive development of diabetes in db/db mice during 6 weeks of treatment. This was demonstrated by a highly significant improvement of oral glucose tolerance, decreased water intake, decreased fasting blood glucose, and decreased HbA1c values.

Acute Effect of Subcutaneous AVE0010 on Oral Glucose Tolerance in Male Dogs
(Study MVV0009)

Study Design: (sponsor-generated summary)

Test: Acute effect of sc AVE0010 on oral glucose tolerance (2g glucose/kg) in dogs	**Test Article (batch):** AVE0010 (b) (4) (b) (4)	**Location in CTD:**
Species/Strain: normoglycemic Beagle dogs (b) (4)	**Vehicle:** compound-free HOE901 placebo solution (b) (4)	**Study No.:** MVV009
Gender/No. per Group: Male / n = 6-9	**Treatment Schedule:** single dose	**Report No.:** (b) (4) MVV009
Weight/Age: ~10-17 kg	**Administration:** s.c.	**Study Period:** 30.03.-08.06.2004
	Doses: 0.03, 0.1, 0.3 and 1.0 nmol/kg	**GLP Compliance:** Not required

Results/Conclusions: After an oral glucose challenge, the blood glucose excursion and plasma insulin levels for dogs treated with AVE0010 were significantly different than controls (Sponsor-generated Figures 1 and 2). A similar observation was made with plasma c-peptide levels. The data indicate that single subcutaneous injections of 0.03 to 1.0 nmol/kg AVE0010 30 minutes prior to an oral glucose load significantly improved oral glucose tolerance in healthy, normoglycemic beagle dogs. The effect was similar to that of equivalent doses of exendin-4.

Figure 1 - Effect of subcutaneous AVE0010 on blood glucose in OGTT in Beagle dogs

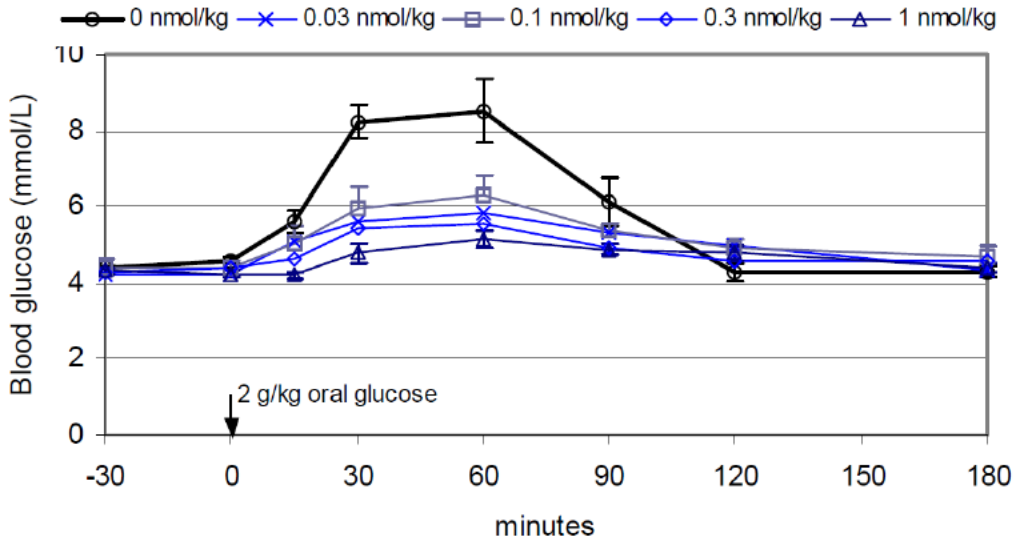
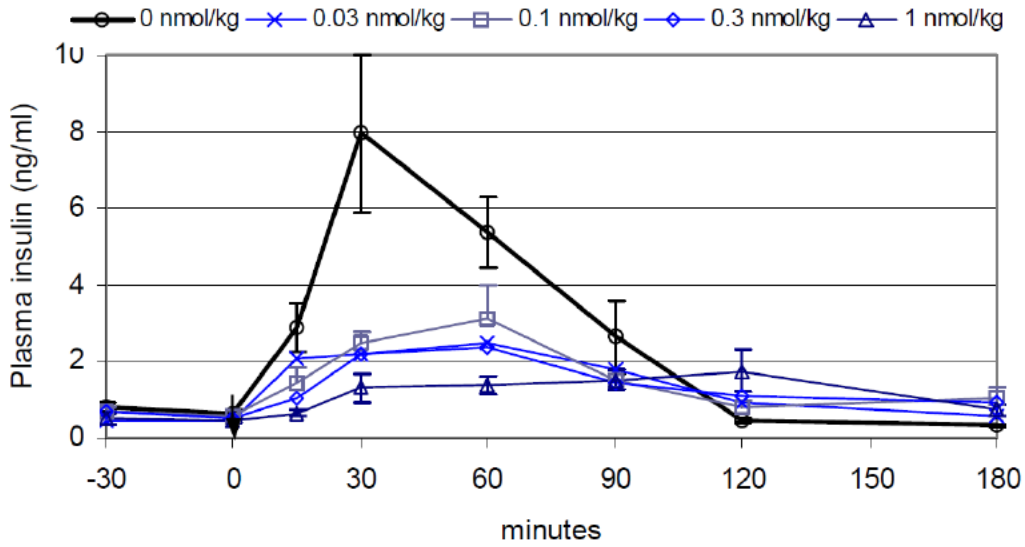


Figure 2 - Effect of subcutaneous AVE0010 on plasma insulin in OGTT in Beagle dogs



Effect of Single Subcutaneous Injection of Lixisenatide and Liraglutide on Oral Glucose Tolerance in Male Dogs (Study DIVV0006)

Study Design: (sponsor-generated summary)

Test: Oral glucose tolerance test (2g glucose/kg) in dogs	**Test Article (batch)**: Lixisenatide (b) (4) Liraglutide (AVE0010 (b) (4)	**Location in CTD**: module 4
Species/Strain: normoglycemic Beagle dogs (b) (4)	**Vehicle**: no vehicle, placebo: NaCl 0.9%	**Study No.**: D090311
Gender/No. per Group: Male / n = 6	**Treatment Schedule**: single dose	**Report No.**: DIVV0006
Weight/Age: ~12-18 kg	**Administration**: SC	**Study Period**: 11-Mar-2009 – 18-Mar-2009
	Doses: Lixisenatide 1 µg/kg, Liraglutide 50 and 100 µg/kg	**GLP Compliance**: Not required

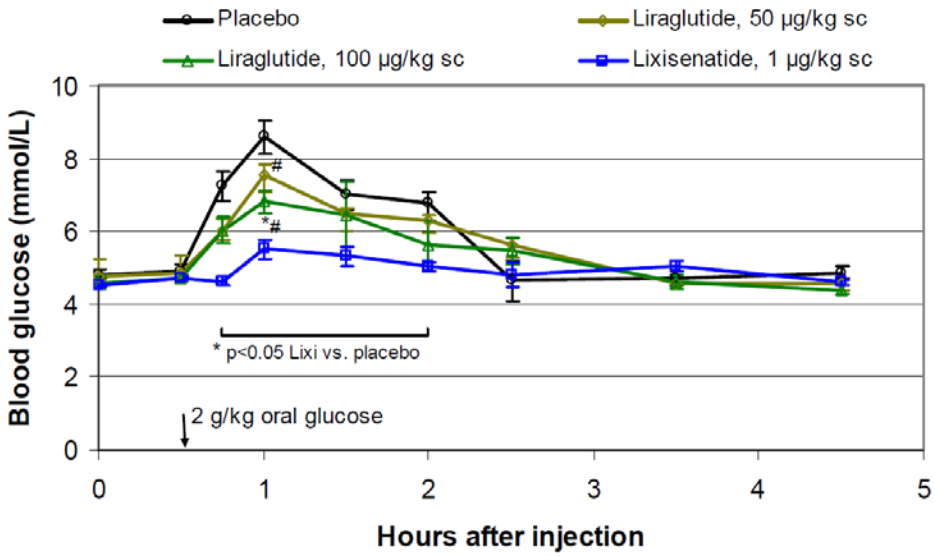
Results: Blood glucose concentrations after oral glucose challenge were significantly lower in the lixisenatide-treated group compared with controls at 0.75, 1.5, and 2 hours. After oral glucose challenge, both liraglutide-treated groups showed a blood glucose excursion that peaked at 1 hour and was still elevated at 2.5 hours compared with control. Blood glucose concentrations of both liraglutide groups were lower between 0.75 and 2 hours after treatment when compared with controls. Blood glucose concentrations for the lixisenatide-treated group were lower between 0.75 and 2.5 hours compared with both liraglutide groups (Sponsor-generated Figure 1).

Treatment with either 1 µg/kg lixisenatide or 50 or 100 µg/kg liraglutide resulted in a trend towards a decrease in glucagon in all three treatment groups before oral glucose challenge and glucagon levels remained lower than controls up to the last sampling point (4.5 hours) after glucose challenge (Sponsor-generated Figure 2).

In the placebo group, mean serum insulin and C-peptide increased rapidly after oral glucose challenge, reaching its maximum at 1 hour and then declining to baseline level by 2.5 hours. There was no significant difference for any treatment group compared with control for both serum insulin and C-peptide (Sponsor-generated Figures 3 and 4).

Conclusions: A single subcutaneous injection of 1 µg/kg lixisenatide 30 minutes before an oral dose load improved oral glucose tolerance in healthy, normoglycemic beagle dogs at 0.75 to 2 hours compared with control. The effect observed with lixisenatide was greater than for the 50 and 100 µg/kg liraglutide dose groups, which only showed statistical significance at 1 hour for the 100 µg/kg group. Both lixisenatide and liraglutide significantly prevented the oral glucose load-induced increase in serum glucagon levels with no major difference between the two drugs. Treatment did not result in significant effects on serum insulin or C-peptide levels during the oral glucose challenge by either treatment. Based on these results, the sponsor concluded that the improvement of oral glucose tolerance by lixisenatide was superior compared with liraglutide.

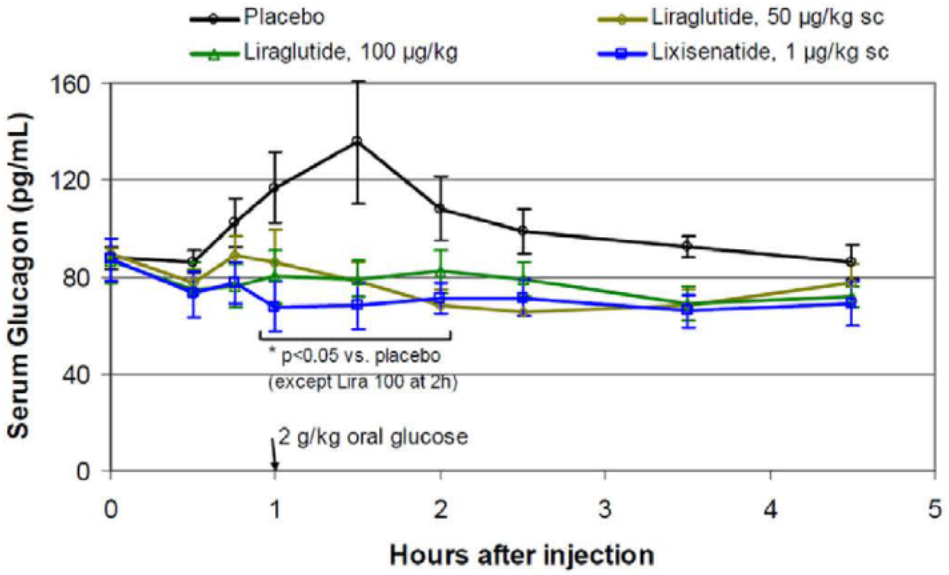
Figure 1 - Effect of 1 µg/kg SC lixisenatide compared to 50 or 100 µg/kg SC liraglutide on oral glucose tolerance in male Beagle dogs



Blood glucose concentration; mean +/- SEM; male Beagle dog, n = 6

* p<0.05 vs. placebo, # p<0.05 vs. lixisenatide 1 µg/kg SC, two-way ANOVA with Newman-Keuls posthoc analysis for time period 0.75h – 2.5h

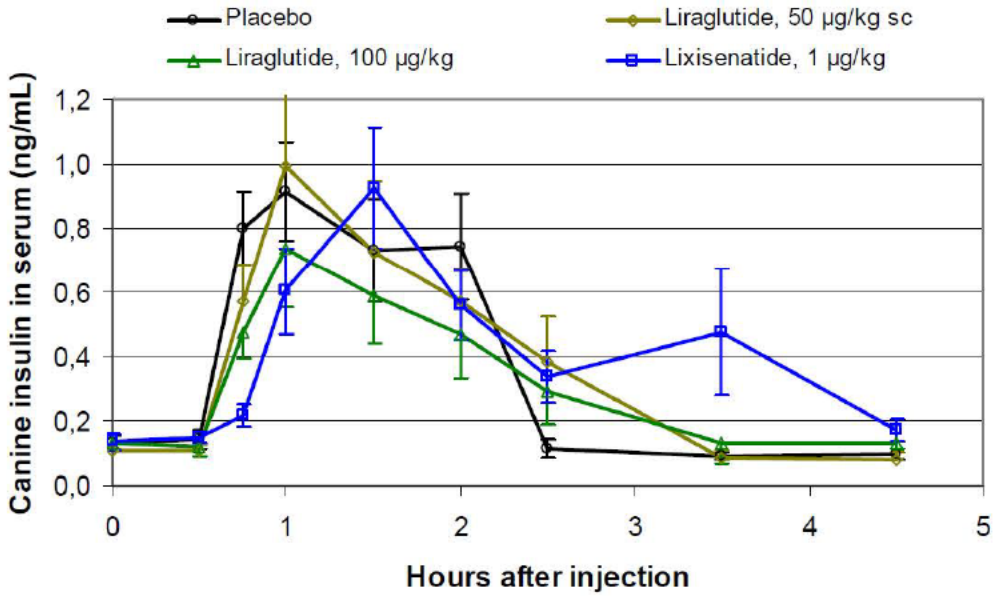
Figure 2 - Effect of 1 µg/kg SC lixisenatide and 50 or 100 µg/kg SC liraglutide on serum glucagon during OGTT in male Beagle dogs



Serum glucagon concentration; mean +/- SEM; male Beagle dog, n = 6

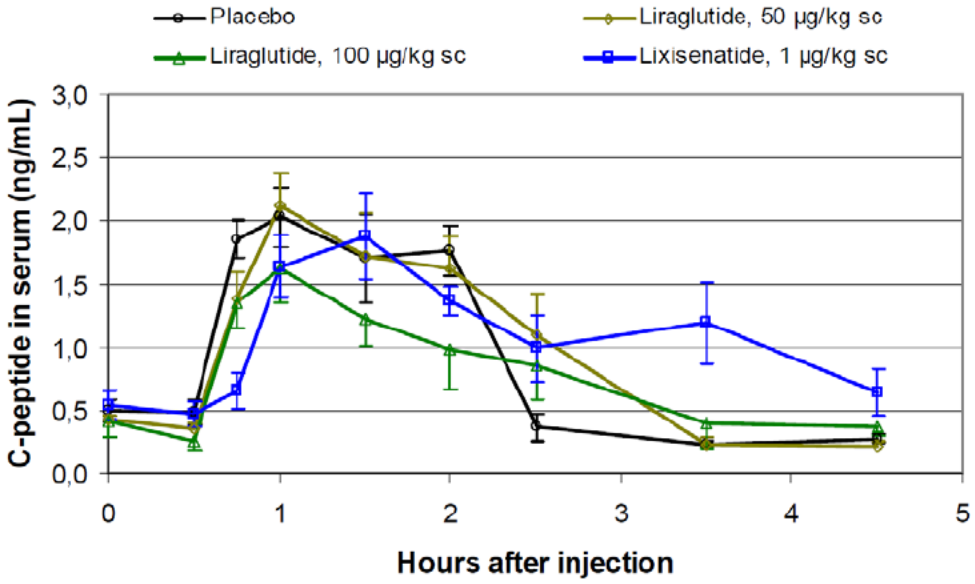
* p<0.05 vs. placebo, # p<0.05 vs. lixisenatide 1 µg/kg SC, two-way ANOVA with Newman-Keuls posthoc analysis for time period 0.5h – 4.5h

Figure 3 - Effect of 1 µg/kg SC lixisenatide and 50 or 100 µg/kg SC liraglutide on serum insulin during OGTT in male Beagle dogs



Serum insulin concentration; mean +/- SEM; male Beagle dog, n = 6

Figure 4 - Effect of 1 µg/kg SC lixisenatide and 50 or 100 µg/kg SC liraglutide on serum c-peptide during OGTT in male Beagle dogs



Serum c-peptide concentration; mean +/- SEM; male Beagle dog, n = 6

4.2 Secondary Pharmacology

Binding Affinity of AVE0010 to the GLP-1 Receptor (Study MVT 0011, MVT0011exta, MVT0011extb, and MVT0011extc)
(sponsor-generated summary)

Test: Selectivity of AVE0010 versus 91 different receptors

Test Article (batch): (b) (4) (b) (4)

Location in CTD: in module 4

(b) (4)

Vehicle: H_2O

Study No.: (b) (4)

No. of Preparations / Group: AVE0010 was tested in duplicate versus reference compounds for either specific receptor or function

Administration: in vitro in buffer media

Report No.: MVT0011

Study Period: 31-Jan – 02-Mar-2001, 16 – 26-Mar-2009 and 18-Feb – 23-Mar-2010

Concentration: 100 nmol/L

GLP Compliance: Not required

Other Protocol Information: ZS42-0010 = ZP10A = (b) (4); AVE0010 = Lixisenatide

Results: ZP10A did not inhibit (<10%) specific radioligand binding to the following receptors: A_1*(h)*, α_1(non-selective), α_2*(non-selective)*, β_1*(h)*, β_2*(h)*, NE transporter*(h)*, AT_2*(h)*, ANP, BZD*(central)*, BZD*(peripheral)*, bombesin*(non-selective)*, B_2*(h)*, CGRP*(h)*, Calcitonin*(h)*, CB_1*(h)*, CB_2*(h)*, CCK_A*(h)*, CCK_B*(h)*, D1*(h)*, D2*(h)*, D3*(h)*, D4.4*(h)*, D5*(h)*, DA transporter*(h)*, ET_B*(h)*, GABA*(non-selective)*, GAL1*(h)*, Glucagon*(h)*, PDGF, IL-8B*(h)*, TNF-α*(h)*, CCR_1*(h)*, H_1*(central)*, H_2, ML_1, M_2*(h)*, M_3*(h)*, M_4*(h)*, M_5*(h)*, NK_1*(h)*, NK_2*(h)*, Y_1*(h)*, Y_2*(h)*, NT_1*(h)*, δ*(h)*, κ*(h)*, μ*(h)*, ORL_1*(h)*, PAC_1*(h)*, PACAP, PCP, TXA_2/PGH_2*(h)*, PGI_2*(h)*, P2Y, 5-HT_{1A}*(h)*, 5-HT_{1B}, 5-HT_{2C}*(h)*, 5-HT_3*(h)*, 5-HT_{5A}*(h)*, 5-HT_6*(h)*, 5-HT_7*(h)*, σ*(non-selective)*, SST*(non-selective)*, VIP_1*(h)*, VIP_2*(h)*, Ca^{2+}-channel *(L, DHP site)*, Ca^{2+}-channel*(L, diltiazem site)*, Ca^{2+}-channel *(L, verapamil site)*, K^+_{ATP}-channel, K^+_V-channel, SK^+_{Ca}-channel, Na^+-channel *(site 1)*, Cl^--channel, nAChR subtype α7 *(N neuronal α-BGTX-sensitive)*, at 100 nmol/L, while AVE0010 at that concentration inhibited specific radioligand binding with 12 – 22% to the following receptors: A_{2A}*(h)*, A_3*(h)*, AT_1*(h)*, ET_A*(h)*, M_1*(h)*, NK_3*(h)*, P2X, Secretin*(h)*, 5-HT_{2A}*(h)*, 5-HT transporter*(h)*, V_{1A}*(h)*, Na^+-channel *(site 2)*, nAChR subtype α4β2 *(N neuronal α-BGTX-insensitive)*, nAChR N muscle-type*(h)*. AVE0010 inhibited specific radioligand binding to the Ca^{2+}-channel (N) with 71%. (b) (4)

Conclusion: The results of this study indicate that AVE0010 has low to very low affinity to a wide range of receptors. Of the 91 receptors tested at the high concentration of 100 nmol/L, only the Ca^{2+}-channel (N) showed inhibition of greater than 50%. Thus, AVE0010 appears to act as a selective agonist at the GLP-1 receptor.

Effect of AVE0010 at Native N-Type Calcium Channels Expressed in Rat Cultured Dorsal Root Ganglion Neurons: A Patch-Clamp Study (Study NVT0222)

(sponsor-generated summary)

[redacted] (b) (4)	Test Article (batch): AVE0010 [redacted] (b) (4)	Location in CTD:
Test system: cultured neurons of rat dorsal root ganglia (DRG)	Vehicle: water	Study No.: NVT0222
No. of Preparations / Group: 3 to 9	Administration: diluted in extracellular medium	Report No.: (b) (4) NVT0222
Study Period: June to July 2009	Concentration: 1 and 10µM	GLP Compliance: Not required

Results: AVE0010 exerted 10% and 52% of omega conotoxin GVIA effect at 1 and 10 µM, respectively, without affecting other calcium channel subtypes, as its effects were not additive to those of omega conotoxin GVIA. Therefore, it was concluded that AVE0010 is a weak and selective blocker of N-type calcium channels expressed by DRG neurons.

Receptor profiling of VIP, PACAP, and glucagon family receptors in rat, dog, and human epididymis and testis by quantitative real-time PCR (Study DIV1283)

Commercially available RNA samples of whole testis from rat, dog, and human (10 weeks, 4 years, and 59-93 years old, respectively) and whole epididymis from rat (pooled from adult tissue) and human (24-93 years old) were purchased to evaluate the expression levels of vasoactive intestinal polypeptide (VIP) receptors, pituitary adenylate cyclase-activating polypeptide (PACAP) receptors, and glucagon family receptors by real-time PCR. Whole tissue RNA from dog epididymis (14 months old) was isolated in-house.

Results:

GLP1R expression in human samples:

GLP1R expression in human testis samples obtained from a commercial supplier had a mean CT value of 32.9 ± 0.17 and samples prepared in-house had a mean CT value of 36.6 ± 2.60. CT values of greater than 35 are considered to exceed the limit of sensitivity. In the human epididymis, a mean CT value of 31.26 ± 0.06 was obtained. The CT mean values for the caput, corpus, and cauda segments of the epididymis were 33.9 ± 0.49, 34.2 ± 1.86, and 35.0 ± 1.18, respectively.

Species comparison of GLP1R expression:

Expression of GLP1R in the testis and epididymis from rat and human was generally low, often nearing or exceeding the limit of sensitivity of CT 35. The highest expression levels of GLP1R were observed in dog testis (CT 29.1) and the caput segment of the dog epididymis (CT 25.4). For dog testis, these values translated to a 100-fold higher expression (external sample) and a 138-fold higher expression (internal sample) than

observed in rat testis and 10-fold higher (external sample) and 3.3-fold higher (internal sample) than observed in human testis. GLP1R expression in human testis was approximately 10-fold higher (external sample) and 41-fold higher (internal sample) than in rat testis.

The expression levels in epididymis tissue of dog in comparison to the rat were 184-fold higher (external sample), 4147-fold higher (internal caput sample), 26-fold higher (internal corpus sample) and 21-fold higher (internal cauda sample). The expression levels in epididymis tissue from human in comparison to the rat were 18-fold higher (external sample), 1082-fold higher (internal caput sample), 291-fold higher (internal corpus sample) and 8-fold higher (internal cauda sample). The expression levels in epididymis tissue from human in comparison to the dog were 10-fold lower (external sample), 3.8-fold lower (internal caput sample), 11-fold higher (internal corpus sample) and 7.9-fold higher (internal cauda sample). Summaries of the expression data are presented in the sponsor-generated tables below.

Table 8 - TESTIS –RNA samples from external supplier

	Testis
Rat	SCTR > GIPR > VIPR2 > GLR = PACR > GLP2R > GHRHR > VIPR1 > **GLP1R**
Dog	PACR > VIPR1 > **GLP1R** > GIPR > SCTR > VIPR2 > GLR > GLP2R > GHRHR
Human	VIPR1 > PACR > GLP2R > VIPR2 > SCTR > **GLP1R** > GLR > GIPR > GHRHR
Target gene GLP1R is marked in bold	

Table 9 - EPIDIDYMIS –RNA samples from external supplier (beside dog)

	Epididymis
Rat	GHRHR > VIPR2 > VIPR1 > PACR > GLP2R > SCTR > GLR > **GLP1R** > GIPR
Dog	PACR > **GLP1R** > SCTR > GIPR > VIPR1 > VIPR2 > GHRHR > GLP2R > GLR
Human	VIPR1 > VIPR2 > PACR > **GLP1R** > GIPR > GLP2R > GLR > GHRHR > SCTR

Table 10 - TESTIS – whole organ, in-house preparation

	Testis
Rat	SCTR > VIPR2 > GIPR > GLR > PACR > GLP2R > GHRHR > VIPR1 > **GLP1R**
Dog	VIPR1 > **GLP1R** > SCTR > GIPR > PACR > VIPR2 > GLR >GHRHR > GLP2R
Human	VIPR2 > VIPR1 > GLR > PACR > SCTR > GLP2R > **GLP1R** > GHRHR > GIPR

Table 15 - Relative differences in receptor expression levels from human in comparison to dog

	GHRHR	GLP1R	GIPR	GLP2r	VIPR1	VIPR2	GLR	PACR	SCTR
Testis human	3.3*	-10	-33	20**	-2.4	1.1	2.5	-5.3	-3.6
Epididymis human	-1.3*	-10	-5.8	9.3**	15	22	13**	-11	-187*

* = CT mean values >35 in dog and human
** = CT mean value >35 in dog or human

Table 20 - Relative differences in receptor expression levels in human testis tissue in comparison to dog (in-house preparation)

Human versus dog	GHRHR**	GLP1R	GIPR	GLP2r**	VIPR1	VIPR2	GLR**	PACR	SCTR
Testis human	59*	-3.3*	-3.1*	265	-3.0	76	1372	5.1	2.0

* = CT mean values >35 in human
** = CT mean values >35 in dog

Table 11 - EPIDIDYMIS CAPUT REGION – in-house preparation

Epididymis caput	
Rat	VIPR2 > GLR > GLP2R > SCTR > PACR > GHRHR > VIPR1 >GIPR > **GLP1R**
Dog	**GLP1R** > PACR > SCTR > VIPR2 > VIPR1 > GIPR > GHRHR > GLR > GLP2R
Human	VIPR1 > VIPR2 > GIPR > GLR > PACR > **GLP1R** > GLP2R > GHRHR > SCTR

Table 21 - Relative differences in receptor expression levels in human epididymis tissue (caput region) in comparison to dog (in-house preparation)

Human versus dog	GHRHR	GLP1R	GIPR	GLP2r**	VIPR1	VIPR2	GLR**	PACR	SCTR
Epididymis caput human	-2*	-3.8	66	n.e.*	130	54	2432	1.3	-24*

n.e. = not expressed

* = CT mean values >35 in human
** = CT mean values >35 in dog

Table 12 - EPIDIDYMIS CORPUS REGION –in-house preparation

Epididymis corpus	
Rat	VIPR2 > VIPR1 > PACR > GLR > GLP2R > GHRHR > SCTR > GIPR > **GLP1R**
Dog	PACR > GIPR > SCTR > VIPR1 > VIPR2 > **GLP1R** > GHRHR > GLR > GLP2R
Human	VIPR1 > VIPR2 > GLR > **GLP1R** > GIPR > PACR > GLP2R > SCTR > GHRHR

Table 22 - Relative differences in receptor expression levels in human epididymis tissue (corpus region) in comparison to dog (in-house preparation)

Human versus dog	GHRHR	GLP1R	GIPR	GLP2r	VIPR1	VIPR2	GLR**	PACR	SCTR
Epididymis corpus human	5.4*	11	-1.8	n.e.*	104	119	1574	-8.6*	-13*

n.e. = not expressed

* = CT mean values >35 in human
** = CT mean values >35 in dog

Table 13 - EPIDIDYMIS CAUDA REGION –in-house preparation

	Epididymis cauda
Rat	GHRHR > VIPR2 > VIPR1 > PACR > GLP2R > GLR > SCTR > **GLP1R** > GIPR
Dog	PACR > VIPR2 > VIPR1 > SCTR >GIPR > **GLP1R** > GLP2R > GLR > GHRHR
Human	VIPR1 > VIPR2 > GLR > **GLP1R** > PACR > GIPR > GLP2R > SCTR > GHRHR

Table 23 - Relative differences in receptor expression levels in human epididymis tissue (cauda region) in comparison to dog (in-house preparation)

Human versus dog	GHRHR**	GLP1R	GIPR	GLP2r**	VIPR1	VIPR2	GLR**	PACR	SCTR
Epididymis cauda human	50*	7.9	1.3*	32*	65	25	1255*	-15*	-1.9*

* = CT mean values >35 in human
** = CT mean values >35 in dog

Conclusions:
The data of the present study demonstrate that the expression of VIP receptors, pituitary PACAP receptors, and glucagon family receptors in rat, dog and human epididymis and testis tissue is generally medium to low.

For GLP1R a clear and reproducible (internally and externally isolated RNA) ≥100-fold higher expression in dog testis could be observed in comparison to the rat. In the human testis a reproducible and at least 10-fold higher GLP1R expression was obtained in comparison to the rat. Besides GLP1R, only VIPR1 showed a clearly higher expression level in dog (≥464-fold) and human (≥189-fold) testis compared to rat testis.

In epididymis, clear expression differences between dogs/humans and rats were restricted to GLP1R expression, primarily in the epididymal caput: The expression levels in epididymis tissue of dog were 184-fold higher (external samples) to 4147-fold higher (internal samples) when compared with rat epididymal caput RNA. The expression levels in epididymal caput from human were 18-fold higher (external) and 1082-fold higher (internal) when compared with rat samples.

VIP1R showed a higher expression in human epididymis, in particular in the corpus, compared with rats, whereas dogs showed no major expression differences compared to rats. Other receptors showed only small or no consistent (e.g., PACR in dogs comparing segmental and whole tissue values) expression differences in dog/human epididymis compared to rats.

These results indicate that main expression differences in dogs versus rats are limited to the GLP1R in testes and epididymal caput and on VIPR1 in testes only. In comparison to the dog, the expression of GLP1R in human testis was 3.3- to 10-fold lower and the expression of VIPR1 was 2.4- to 3-fold lower in the testis or at least 15-fold higher in the epididymis. GLP1R expression levels in epididymis tissue from human in comparison to the dog was 10-fold lower in the whole tissue RNA sample, and 3.8-fold lower when specifically evaluating the caput segment.

Overall, GLP1R was among the receptors with the lowest expression in testis and all segments of the epididymis in rats. In dogs, GLP1R was among the receptors with the highest expression levels in testis and in the caput region of the epididymis. In human, GLP1R was among the receptors with a lower expression in testis and in epididymis.

Cardioprotective Effect of Lixisenatide on Ischemia/Reperfusion-Induced Injury in the Isolated Rat Heart after Chronic SC Treatment in Rats (Study divt0002)

Test: in vitro Heart Failure Model	**Test Article (batch):** - Lixisenatide, (b) (4) - - Liraglutide (b) (4)	**Location in CTD**
Test system: (b) (4) set up	**Vehicle:** 0.9% NaCl with 0.1 % BSA	**Study No.:** DIVT0002
No. of Preparations / Group: 4 groups, 10-11 preparations per group	**Administration:** perfusion	**Report No.:** (b) (4) DIVT0002
Study Period: 15-Jul-2010 – 01-Dec- 2010	**Concentration:** 0.3 nM	**GLP Compliance:** Not required

Results: The effect of AVE0010 on regional ischemia and reperfusion was evaluated in an isolated Langendorff perfused rat heart model. The left ventricular artery was occluded for 45 minutes. AVE0010 (0.3 nmol/L) was administered 10 minutes before and 120 minutes during reperfusion. The data indicated that AVE0010 administered prior to and during reperfusion significantly reduced the development of myocardial infarction induced by transient LAD occlusion and reperfusion. Similar effects were observed with the same concentrations of native GLP-$1_{(7\text{-}36)}$ amide and liraglutide.

Cardioprotective effects of chronic treatment with Lixisenatide in rats with heart failure induced by ischemia/reperfusion (Study divv0030)

Test: Heart Failure, induced by ischemia/reperfusion	**Test Article (batch):** Lixisenatide, (b) (4)	**Location in CTD:**
Species/Strain: Wistar Rats	**Vehicle:** 0.9% NaCl with 0.1 % BSA	**Study No.:** DIVV0030
Gender/No. per Group: male, n=10-20	**Treatment Schedule:** 10 weeks	**Report No.:** (b) (4) DIVV0030
Weight/Age: 250-300g	**Administration:** s.c. via osmotic minipumps	**Study Period:** 21.06. 2010 – 24.08. 2010
	Doses: 10 µg/kg/d	**GLP Compliance:** Not Required

Results: Rats with myocardial ischemia and long-term reperfusion (I/R) injury treated with 10 µg/kg/d lixisenatide for 10 weeks significantly attenuated I/R-induced changes in left ventricular end-diastolic pressure compared with I/R placebo group. Relative lung weight as a measure for congestion was normalized in lixisenatide-treated rats compared with I/R placebo rats. Serum levels of brain natriuretic peptide were normalized in lixisenatide-treated rats. The ACE inhibitor ramipril showed similar beneficial effects. Body weight was significantly reduced in I/R ramipril- and I/R lixisenatide-treated rats compared with placebo. The results of this study showed that lixisenatide treatment attenuated impairment in left ventricular heart function in rats with myocardial I/R injury, which was similar to the effect of the ACE inhibitor ramipril.

Effects of Chronic Subcutaneous Infusion of Lixisenatide on Atherosclerotic Plaque Formation in Male ApoE Knockout Mouse (Study divv0007)

Test: Anti-atherosclerotic effect of lixisenatide in male ApoE knockout mice	**Test Article (batch):** AVE0010_ (b) (4)	**Location in CTD**
Test system: In vivo study in mice	**Vehicle: placebo solution (batch):** AVE0010_10_1245	**Study No.:** DIVV0007
No. of Preparations / Group: 3	**Administration:** chronic SC. infusion with osmotic minipumps	**Report No.:** (b) (4) DIVV0007
Study Period: 03-Nov-2010 – 01-Mar-2011	**Concentration:** 1.5 g/l	**GLP Compliance:** Not required

Other Protocol Information: The study was conducted to investigate the anti-atherosclerotic effect after chronic SC infusion in male ApoE knockout mice.

Results: Lixisenatide treatment decreased serum cholesterol and reduced atherosclerotic plaque progression in the aorta of the ApoE knockout mouse.

4.3 Safety Pharmacology

Effects on Neurobehavior

Effects on general activity and behavior in the rat following intravenous administration (Study 20(b)(4)-1177)

Groups of male Wistar rats received a single, IV dose (bolus infusion of 1 mL/min) of vehicle or AVE0010 (ZS42-0010). Dose groups are shown in the sponsor-generated table below.

Group	Intravenous treatment	Dose level (µg/kg)	Formulation concentration (µg/mL)	Number of animals
1	Vehicle	-	-	6
2	ZS42-0010	50	50	6
3	ZS42-0010	150	150	6
4	ZS42-0010	500	500	6
5	Vehicle	-	-	6
6	ZS42-0010	0.1	0.1	6
7	ZS42-0010	1	1	6
8	ZS42-0010	10	10	6

Irwin observations were conducted at 5, 15, 30, 60, and 120 minutes after dosing and animals were observed for gross signs of toxicity and mortality for an additional 7 days.

There were no unscheduled deaths. There were no effects on behavior or physiological changes at 0.1 µg/kg. At 1 mg/kg, one animal displayed a slight decrease in body tone at 5 minutes post-dose, which was not observed at any other time point during the study. At 10 µg/kg, slight apathy and a slight decrease in locomotor activity and body tone were noted in a majority of animals. Abnormal dispersion within the home cage was also apparent for all animals. Slight to moderate impairment of the righting reflex was noted for half of the animals and was fully recovered by 60 minutes post dose. At 120 minutes, signs were limited to slight apathy and a slight decrease in body tone.

Doses of ≥50 µg/kg AVE0010 produced signs that were comparable in frequency and severity. Abnormal dispersion within the home cage was noted for the majority of animals and was fully recovered by 30 minutes or 60 minutes (500 µg/kg). A majority of animals displayed slight apathy and decreased body tone, which were still observed at 120 minutes. A slight impairment of righting reflex was noted for most animals, with full recovery by 60 minutes. Slightly decreased spatial locomotion and decreased grip strength were observed in approximately 50% of animals, but was not specifically dose related. A minority of animals showed a slight decrease in pain response, with full recovery by 60 minutes. Hypothermia was observed for one animal from each of the three highest dose groups at 60 minutes post dose. Slightly flattened posture and slightly decreased locomotor activity and transfer arousal were noted for animals treated at 50 µg/kg only.

A single observation was also made for each of the following: landing with splayed hind limbs (500 µg/kg, 5 minutes post dose), landing on side (150 µg/kg, 5 and 15 minutes post

dose), and clonic convulsion (50 µg/kg, 5 minutes post dose). A summary of neurobehavioral effects is presented in the sponsor-generated tables below.

Parameter	Number of animals receiving dose (µg/kg) of ZS420010, showing abnormalities at time post-dose:														
	0 - 5 minutes			15 minutes			30 minutes			60 minutes			120 minutes		
	0.1	1	10	0.1	1	10	0.1	1	10	0.1	1	10	0.1	1	10
Dispersion (slight)						2/6			4/6			2/6			
Dispersion (moderate)						2/6			2/6						
Dispersion (substantial)			6/6			2/6									
Decreased locomotor activity (slight)			3/6			5/6			1/6						
Apathy (slight)			4/6			5/6			5/6			4/6			2/6
Decreased transfer arousal (slight)															
Decreased spatial locomotion (slight)															
Decreased touch-escape (slight)															
Impaired righting reflex (slight)			1/6			2/6			1/6						
Impaired righting reflex (moderate)			1/6			1/6									
Decreased grip strength (slight															
Decreased grip strength (moderate)															
Decreased pain response (slight)															
Hypothermia (slight)															
Decreased body tone (slight)		1/6	5/6			6/6			6/6			6/6			6/6
Decreased body tone (moderate)															
Landed with splayed hind limbs															
Animal landed on its side															
Clonic convulsion															

Parameter	Number of animals receiving dose (µg/kg) of ZS420010, showing abnormalities at time post-dose:														
	0 - 5 minutes			15 minutes			30 minutes			60 minutes			120 minutes		
	50	150	500	50	150	500	50	150	500	50	150	500	50	150	500
Dispersion (slight)		2/6		2/6	2/6	2/6									
Dispersion (moderate)				2/6											
Dispersion (substantial)	2/6		2/6			2/6			6/6						
Decreased locomotor activity (slight)	6/6			2/6			2/6			2/6					
Apathy (slight)	5/6	5/6	4/6	6/6	6/6	6/6	6/6	4/6	6/6	5/6	4/6	5/6	3/6	5/6	3/6
Decreased transfer arousal (slight)	1/6			2/6											
Decreased spatial locomotion (slight)	2/6			2/6	3/6	3/6	2/6	2/6	1/6	3/6					
Decreased touch-escape (slight)	2/6			2/6	1/6		2/6			2/6					
Impaired righting reflex (slight)		5/6	2/6		3/6		2/6								
Impaired righting reflex (moderate)	1/6														
Decreased grip strength (slight)		1/6	2/6	2/6	3/6	2/6	1/6	2/6	2/6			1/6			
Decreased grip strength (moderate)	2/6														
Decreased pain response (slight)		1/6	3/6	2/6		1/6	3/6								
Hypothermia (slight)										1/6	1/6	1/6			
Decreased body tone (slight)	4/6	3/6	5/6	4/6	4/6	4/6	5/6	6/6	4/6	5/6	5/6	6/6	6/6	5/6	6/6
Decreased body tone (moderate)	2/6	3/6	1/6	2/6	2/6	2/6	1/6		2/6	1/6	1/6				
Flattened posture (slight)	2/6			2/6			2/6								
Landed with splayed hind limbs			1/6												
Animal landed on its side		1/6			1/6										
Clonic convulsion	1/6														

Subcutaneous general behavior study (Irwin profile) in male mice (Study 20 (b)(4)-1224)
Male CD-1 mice (8/group) received either vehicle (saline) or AVE0010 (0.02, 0.2, or 2 mg/kg) by SC injection. Clinical and behavioral observations were conducted for each mouse at 0.5, 1, 2, 5, and 24 hours after treatment. Assessments included endpoints for the autonomic system, alertness/reactivity, motor activity, tone/coordination, and mortality.

The results indicated that a single SC injection of dose up to 2 mg/kg had no effect on general behavior.

Effects on Cardiovascular Function:

Exploratory hERG channel affinity (IC50) assay (Study DSE 20(b)(4)-1521)

An exploratory in vitro study was conducted to evaluate the effects of AVE0010 on the cloned human cardiac K^+ channel hERG using the whole-cell patch-clamp technique in CHO cells stably expressing hERG. As summarized in the sponsor-generated table below, AVE0010 dose-dependently blocked hERG currents by 12.5% and 37.3% at 10 and 30 µg/mL, respectively.

Concentration (µg/ml)	HERG Current (%Control)					
	Cell #1	Cell #2	Cell #3	Cell #4	Average	SEM
0	100	100	100	100	100	0.0
10	91.8	88.0	97.0	73.5	87.5	5.0
30	55.6		66.4	66.1	62.7	3.5

Electrophysiology assay on isolated male rabbit purkinje fibers (Study 20(b)(4)-0098)

The potential effects of AVE0010 on resting membrane potential, amplitude, maximum rate of rise of action potential and action potential duration (APD50 and APD90) were evaluated in electrically-stimulated rabbit Purkinje fibers. AVE0010 was tested on 6 Purkinje fibers at the nominal concentrations of 0.01, 0.1, and 1 µg/mL sequentially applied every 30 minutes.

Analytical evaluation of the bath solution indicated that AVE0010 likely adsorbed on the silicone tubing or glass materials of the experimental set-up. Thus, only the highest concentration tested (nominal 1 µg/mL) was considered to be valid, giving an actual AVE0010 tested concentration of 0.57 µg/mL. At this highest concentration tested, AVE0010 did not induce any changes in resting membrane potential or action potential parameters of rabbit Purkinje fibers.

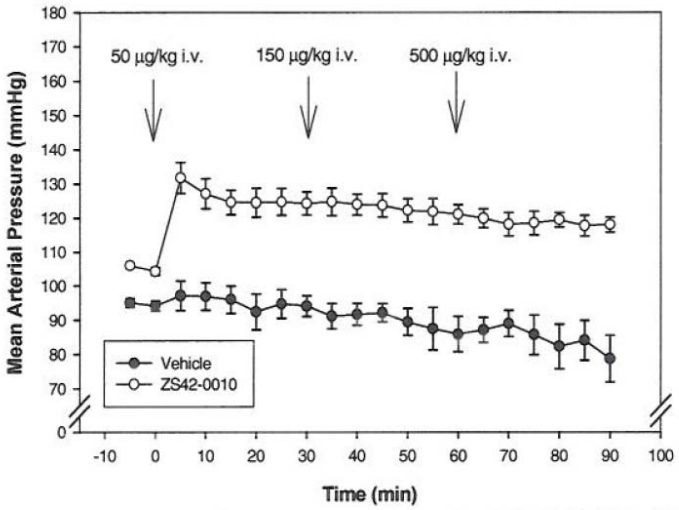
Cardiovascular effects of ZS42-0010 [AVE0010] in conscious rats (Study DIV1154)

In a first experiment, conscious Wistar rats (7/group) received a single IV dose of 50, 150, and 500 µg/kg AVE0010 sequentially at 30 minute intervals. Mean blood pressure (MAP) and blood glucose were measured in 5 minute increments.

In a second experiment, conscious Wistar (4/group) and Sprague-Dawley (4/group) rats were administered single IV dose either vehicle or 500 µg/kg AVE0010. Effects on blood pressure were evaluated for 1 hour post-dose.

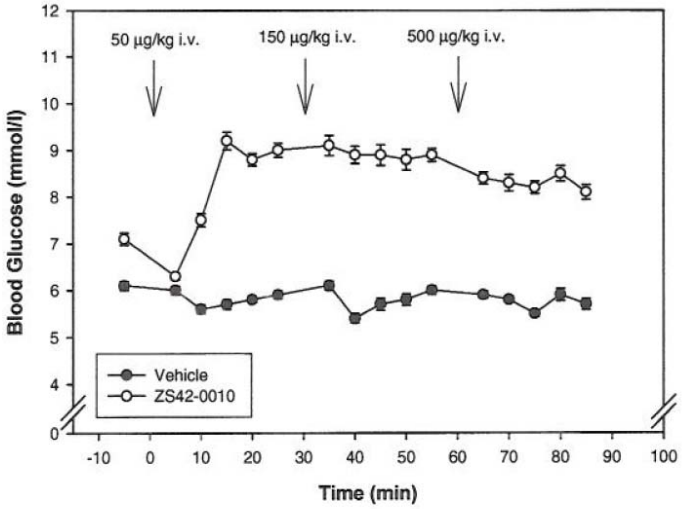
In experiment 1, administration of ≥50 µg/kg by IV injection resulted in a significant increase in MAP and blood glucose relative to vehicle-treated animals within 5 minutes. Higher cumulative doses did not produce any further increase in MAP or blood glucose (see sponsor-generated Figures 1 and 2 below).

Figure 1



MAP after i.v. administration of 50, 150 and 500 µg/kg ZS42-0010 in Wistar rats (n=7).

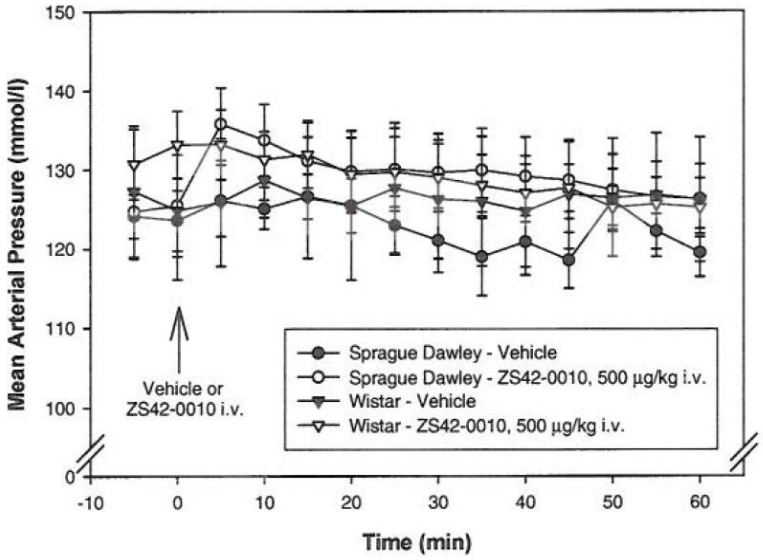
Figure 2



BG after i.v. administration of 50, 150 and 500 µg/kg ZS42-0010 in Wistar rats (n=7).

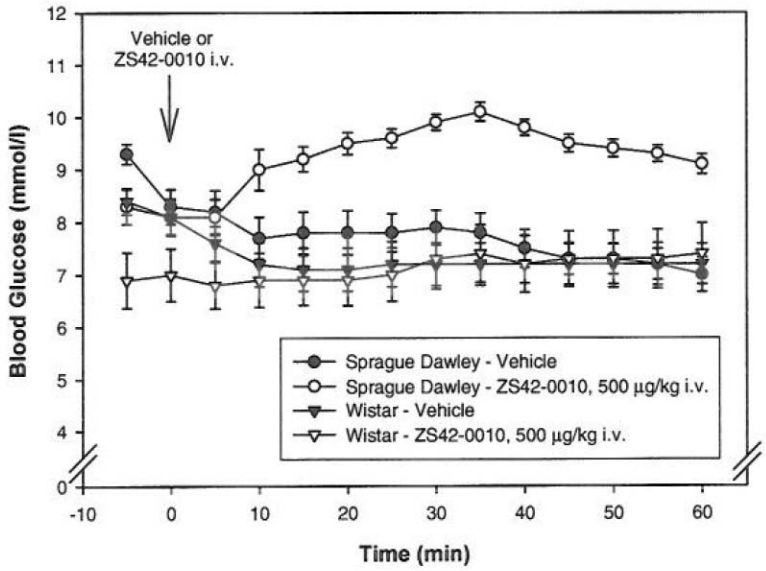
In experiment 2, effects on MAP or blood glucose were not observed for Wistar rats, but similar increases in MAP and blood glucose were observed in Sprague-Dawley rats (sponsor generated Figures 3 and 4).

Figure 3



MAP after i.v. administration of vehicle or 500 µg/kg ZS42-0010.

Figure 4



Blood glucose after i.v. administration of vehicle or 500 µg/kg ZS42-0010.

Conclusions: Based on the results of these experiments, the sponsor concluded that the previously observed behavioral effects in conjunction with effects on MAP and blood glucose suggest that high dose levels of AVE0010 enters the brain after IV administration, potentially resulting in sympathetic nerve activity and adrenal catecholamine release.

Cardiovascular and respiratory effects in the anesthetized dog following intravenous administration (Study 20(b)(4)-1182)

Four Beagle dogs/sex were anesthetized, instrumented, and dosed with ascending doses of test article or vehicle as shown in the sponsor generated table:

Group number	Group description	Dose number	Dose concentration (µg/mL)	Dose volume (mL/kg)	Dose level (µg/kg)
1	Vehicle treated group	1	0	2	0
		2	0	2	0
		3	0	2	0
2	Test article treated group	1	0.05	2	0.1
		2	0.5	2	1
		3	5	2	10

After the surgical procedures were completed, animals were allowed to stabilize for at least 30 minutes and then baseline values were collected. Vehicle or test article was then injected intravenously via a cannula placed in the jugular vein in ascending doses at intervals of at least 30 minutes. Cardiovascular parameters that were evaluated included: blood pressure (systolic, diastolic, and mean arterial), heart rate, left ventricular pressure and its derivative (dP/dt_{max}), femoral artery blood flow and resistance, and ECG intervals including QT, QT_{CB}, and QT_{CF}. Respiratory parameters included: peak inspiratory and expiratory flow, tidal volume, minute volume, rate of respiration, and blood oxygen.

The results indicated that AVE0010 administered to anesthetized dogs at single IV doses of up to 10 µg/kg did not significantly affect cardiovascular or respiratory function.

Insulin Glargine/AVE0010 Combination - Effect of a Single Intravenous Dose on Cardiovascular Function in Anesthetized Dogs (Study CVR0345)

This study was conducted in normoglycemic anesthetized Beagle dogs (9-12 months old) to assess the potential effect of a single administration of insulin glargine/AVE0010 combination, on ECG parameters (in particular QT interval duration) and on glycemia and kaliemia. A summary of the study design is shown in the sponsor-generated table 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

Dosing: Each dog received each of the 4 drug/vehicle combinations shown in the table above in a cross-over design, with at least 8 days of washout between each treatment. AVE0010 or its vehicle was administered via a 30-minute intravenous infusion followed by a bolus injection of insulin glargine or its vehicle using a catheter implanted in the saphenous vein. Dogs were anesthetized through an intravenous bolus injection of sodium thiopenthal, allowing endotracheal intubation. Anesthesia was then maintained by isoflurane inhalation.

Endpoints: ECG measurements were made before treatment, at the middle and end of the 30 minute infusion, then 15, 30, 45, 60, 90, 120, 150, 180, and 240 minutes after insulin glargine or vehicle administration. Blood samples were collected for the measurement of plasma glucose and potassium concentration before first treatment, between 5 to 1 minute before the end of infusion of the first treatment, and 15, 30, 45, 60, 90, 120, 150, 180, and 240 minutes after the IV bolus of the second treatment. Blood was also sampled for the measurement of serum drug concentrations before treatment, at the end of the 30 minute infusion, and 15 and 60 minutes after the second treatment administration.

Results: (sponsor-generated table)

Parameter	AVE0010	Insulin glargine	Combination: 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

TK analysis:
All mean vehicle and pre-treatment values for AVE0010 and insulin glargine were below the lower limit of quantitation (LLOQ; 12 pg/mL for AVE0010 and 8 µU for insulin glargine), as expected. AVE0010 concentrations ranged from 35,500 to 106,000 pg/mL at the end of infusion, 20,200 to 49,300 pg/mL 15 minutes after the second treatment, and 7,850 to 18,800 pg/mL 30 minutes after the second treatment (ranges combined for Groups 2 and 4). Insulin glargine levels ranged from 60.7 to 140 µU 15 minutes after insulin administration and <LLOQ to 22.1 µU/mL 30 minutes after insulin administration (ranges combined for Groups 3 and 4).

Conclusions:
The results show that a single intravenous dose of 10 µg/kg AVE0010 did not increase the mean QT, QTc, PQ, or QRS intervals. Mean heart rate was increased by 5 beats per minute midway through the infusion. Glucose was slightly reduced at the end of infusion whereas there was no effect on serum potassium at any time point. Insulin glargine alone resulted in increased mean heart rate by 15 beats per minute 45 minutes after bolus injection and increased QT (15 ms) and QTc (17-23 ms) intervals 15 minutes after bolus injection. Decreases in mean serum potassium and glucose were noted 15 minutes after bolus injection and 30 minutes after bolus injection, respectively. Some S-T segment morphology modifications, T wave morphology modifications, and T wave amplitude changes were observed for the insulin glargine alone groups, either when preceded by AVE0010 or vehicle. The addition of AVE0010 with insulin glargine did not enhance the cardiovascular or glucose effects observed for insulin glargine alone. Therefore, under the conditions of this study, the coadministration of AVE0010 and insulin glargine does not appear to result in a pharmacodynamic drug interaction. The sponsor states that the effects on ECG morphology were likely linked to potassium serum concentration modifications.

Effects on Gastric Emptying

Effect of AVE0010 and Exendin-4(1-39)-NH_2 on Rate of Gastric Emptying after i.p. Administration in NMRI Mice (Study MVV0005)

(sponsor-generated summary)

Test: Methyl red dilution method to test acute effect of ip AVE0010 on rate of gastric emptying	**Test Article (batch):** AVE0010: (ZS42-0010, (b) (4)) exendin-4: (b) (4)	**Location in CTD:** in module 4
Species/Strain: Mice / NMRI (b) (4)	**Vehicle:** PBS buffer (pH = 7.4)	**Study No.:** MVV0005 (b) (4) (Zealand)]
Gender/No. per Group: Male / n = 5-15	**Treatment Schedule:** single dose	**Report No.:** (b) (4) MVV0005
Weight/Age: 23g – 30 g	**Administration:** i.p.	**Study Period:** Aug. – Sep. 2000
	Doses: AVE0010: 0.0005 – 1000 nmol/kg (b) (4) 0.1 – 10000 nmol/kg	**GLP Compliance:** Not required

Results: The ED_{50} of AVE0010 on gastric emptying was estimated to be 6.39 nmol/kg (slope factor = 0.60). The ED_{50} of exendin-4(1-39)-NH_2 was estimated to be 12.9 nmol/kg (slope factor = 0.40).

Conclusions: Both AVE0010 and exendin-4 decreased the rate of gastric emptying in NMRI mice with similar ED_{50} values and widely overlapping confidence intervals when administered by IP injection. Therefore, it was concluded that there is no difference between these two compounds with respect to inhibition of gastric emptying in NMRI mice.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Methods of Analysis:
Analytical assays were developed and validated for the quantitation of lixisenatide in mouse, rat, rabbit, dog, and pig plasma. The lixisenatide LC/MS assay was used in initial nonclinical PK studies. A more sensitive ELISA method was used for TK and clinical studies. An ELISA method was also used for the measurement of anti-lixisenatide antibodies in animal plasma. A cell-based bioassay was developed to investigate the potential neutralizing effect of anti-lixisenatide antibodies in rat and human plasma. Information about the type of bioanalytical assays used for the nonclinical program is listed in the sponsor-generated tables below.

Table 1 – Analytical assay methods

Assay Type	Species (Strain)/Matrix	Limit of Quantitation	Assay Range	Study No. (see 2.6.5 [TS 2.6.5.1])
Sandwich Enzyme Immunoassay (lixisenatide)	Rat / plasma	50 pg/mL	50 - 1600 pg/mL	F2004KIN0107 F2004KIN0290 (b) (4) 332910)
Sandwich Enzyme Immunoassay (Total lixisenatide)	Rat / plasma	50 pg/mL	50 – 2000 pg/mL	F2005KIN0248 F2008KIN0010 (b) (4) 26642)
Sandwich Enzyme Immunoassay (lixisenatide)	Mouse / plasma	50 pg/mL	50 – 1600 pg/mL	F2004KIN0145 F2004KIN0289 (b) (4) 18568)
Sandwich Enzyme Immunoassay (Total lixisenatide)	Mouse / plasma	100 pg/mL	100 – 2000pg/mL	F2005KIN0284 F2007KIN0125 (b) (4) 28058)
Sandwich Enzyme Immunoassay (lixisenatide)	Dog / plasma	40 pg/mL	40 – 1280 pg/mL	F2004KIN0152 F2004KIN0306 F2005KIN0107 (b) (4) 19051)
Sandwich Enzyme Immunoassay (Total lixisenatide)	Dog / plasma	100 pg/mL	100 – 2000 pg/mL	F2005KIN0308 F2008KIN0011 (b) (4) 26643)
Sandwich Enzyme Immunoassay (lixisenatide)	Rabbit / plasma	40 pg/mL	40 – 1280 pg/mL	F2005KIN0019 (b) (4) 19052)
LC/MS	Dog / Plasma	3,4 nM	3,4 – 73,9 nM	Zealand: 00-107
LC/MS	Rat / Plasma	2,6 nM	2,6– 73,9 nM	Zealand:00-107
LC/MS	Pig / Plasma	4,1 nM	4,1 – 738,6 nM	Zealand:00-107
Direct ELISA (anti -drug antibody)	Rat / plasma	Cut-off approach	Not applicable	F2004KIN0265 (b) (4) 17838)
Sandwich Enzyme Immunoassay (influence of anti-drug antibodies on the drug assay)	Rat / plasma	50 pg/mL	50 - 1600 pg/mL	F2004KIN0246 (b) (4) 21011)
Direct ELISA (anti -drug antibody)	Mouse / plasma	Cut-off approach	Not applicable	F2006KIN0014 F2008KIN0012 (b) (4) 27229)
Direct ELISA (anti- drug antibody)	Dog / plasma	Cut-off approach	Not applicable	2003-1926 (b) (4) AAA19512)
ELISA (anti- drug antibody), bridge-assay	Dog / plasma	Cut-off approach	Not applicable	DOS1493
Cellular assay – biologically active concentration	Rat / plasma	40 pg/mL	40pg/mL – 800pg/mL	DOS1043; (b) (4) 071716.01

Table 2 - Stability of lixisenatide in plasma samples

Test species	Matrix	at 20 ± 5°C	At around –20°C	Following 3 freeze/thaw cycles
Mouse	Plasma	up to 3 h	up to 24 weeks	Stable
Mouse	Plasma-total	Bad*	up to 24 weeks	Stable
Rat	Plasma	up to 3 h	up to 24 weeks	Stable
Rat	Plasma-total	up to 3 h*	up to106 weeks	Stable
Dog	Plasma	up to 3 h	up to 106 weeks	Stable
Dog	Plasma-total	up to 3 h	up to 106 weeks	Stable
Rabbit	Plasma	up to 3 h	up to 24 weeks	Stable
Human	Plasma-total	Up to 2hours (at 20°C and 37°C)	Up to 104 weeks**	Stable

* benchtop storage at 4°C or on ice is recommended

**Long-term stability analysis in EDTA plasma demonstrated with the 1st generation assay validated at (b) (4) (VAA26641). The actual assay (DOH0498) in addition demonstrates 36 weeks stability at -75-80°C

Absorption:
Absorption of lixisenatide is rapid following a single SC or IP administration for all species tested, with mean T_{max} values between 0.25 and 3.75 hours after dosing. Absolute bioavailability for SC dosing was approximately 90% in dogs, 70% in pigs, 36% to 50% in db/db mice, ≥30% in rabbits, and only 3% in rats. A summary of mean PK parameter values obtained in the various tested species is presented in the sponsor-generated table below.

Repeat-dose absorption was measured during the toxicology studies and the data are presented with each toxicology study in Section 6 of this review. Additionally, three rabbits were implanted with SC osmotic minipumps to deliver a constant amount of lixisenatide (4 µg/h) for up to 76 hours (Study 03-013). A steady state plasma concentration of 485 ± 119 pmol/L (infusion rate normalized to 1 µg/kg/h) was reached. Plasma concentration curves were predicted by fitting observations to a one-compartment model with constant input and first order elimination. Estimates of the AUC were also in the range that had been expected on the basis of previous observations.

Table 3 - Summary of mean pharmacokinetic parameters of lixisenatide calculated from plasma concentrations following administration of lixisenatide to the mouse, rat, rabbit, dog and pig (analysed with the less sensitive LC/MS assay)

Species	Sex	n	Route	Dose [nmol/kg]	C_{max} [nM]	t_{max} [min]	$t_{1/2z}$ [min]	AUC [min x µM]	V_{ss} [mL/kg]	CL [mL/min /kg]	F [%]	Study Number (see 2.6.5 [TS 2.6.5.2])
Mouse (db/db)	M	16*	IV	100	1069	0	37	10.4	132	9.6		[00-077]
	M	9*	SC	100	50	28	ND	3.75			36	
	M	17*	SC	1000	332	49	69	52.7			51	
	M	9*	IP	100	94	14	40	6.9			66	
	M	18*	IP	1000	568	51	103	119			115	
Mouse (NMRI)	M	32*	IV	100	719	0	83	13.7	421	7.3		[00-085]
	M	18*	SC	100	39.5	14.4	24	2.08			15	
	M	17*	IP	100	2.9	48.6	ND	ND			ND****	
Rat (Sprague-Dawley)	M	1	IV	1231	21000	0	31	271	62.9	4.6		[00-062]
	M	3	SC	137	NA	19±7.4	37±3.1	0.3659-1.1845			1.2-3.9	
	M	3	SC	772	NA	15.5±0.8	29.1±4.9	3.4577-6.0815			2.0-3.6	
	M	2	SC	1231	NA	19.5±8.0	36.3 ±16.5	5.8785/ 11.8732			2.2/4.4	
	M	5	IV	100	1663±421	0	48.6 ±10.2	17.982 ±3.901	98±36	5.8±1.4		[00-074]
	M	6	SC	1000	113±41	18.1±4.1	30±10	8.335 ±4.355			4.3±1.9	
	M	6	IP	900	48±12	6.7±2.6	29±7.9	1.8±0.34			1.1±0.21	[01-012]
Rabbit (Ssc: CPH)	M	5	IV	200	4000±1700**	0	63±16	42±40**	270±260	5.3±2		[00-032]
	M	12	IV	137	2600±1370**	0	84±45	47.4±12.9	150±59	3.0±1.1		[00-044]
	M	4	IV	65	840±500	0	91±41	26±8.8	200±140	2.8±0.9		[00-139]
	M	2	SC	400	240±32	160±72	275±75	140±22			38±5/ 86***	
	F	3	SC	2	1.853±0.364	84±9	58±6	0.41±0.06#			ND	[02-139]
Dog (Beagle)	M / F	4 4	IV	21	216±44 226±50	30 (inf)	60±25 57±3	14.6±2.2 14.9±2.3	104±45 95±21	1.46 ±0.20 1.44 ±0.26		[00-141]
	M / F	4 4	SC	41	40±11 53±27	128±26 122±39	342±206 180±57	27±17 22.7±11			102±75 84±54	
Pig (cross-breed of Danish country)	F	3	IV	61-130	347-886	0	196-392	14.4-29.9	441-736	2.9-4.5		[00-45]
	F	3	SC	61-104	12-22	142-225	ND	4.22-6.73			>26	
	F	3	IV	19	85±6	30 (inf)	53±5	3.63-4.15	192±22	5.0±0.6		[00-083]
	F	3	SC	109	39±3	120±23	159±20	15.4±2.1			71±17	

F(%) = Bioavailability; ND = Not determined; IV = Intravenous; IP = Intraperitoneal; SC = Subcutaneous; t1/2 = Elimination half-life
NA = no specific information given in the report or protocol
The units in this table are given as in the original reports. One nmol/kg corresponds to 0.0049 mg/kg; 1 nM corresponds to 4900 pg/mL; 1 min x µM corresponds to 3374 pg*day/mL.
* non-serial sampling with 1 or 2 animals per time point ** data calculated from values given in the report
depending on calculation (using AUClast or AUCinf) * report estimates a value of 0.4 using Cmax

Distribution:

Protein Binding

Plasma protein binding was determined in vitro for human, rat, and dog (Study LPR1021) using an ultracentrifuge method and lixisenatide concentrations were determined using ELISA. Lixisenatide showed a mean binding of 55% to human plasma proteins in the concentrations between approximately 500 and 50,000 pg/mL, and a mean binding of 49% in dog and 62% in rat plasma between approximately 50 pg/mL and 10,000 ng/mL.

Tissue Distribution and Accumulation

Tissue distribution of radioactivity was studied following a single dose of 1 mg/kg ^{3}H-lixisenatide by SC or IV administration (Study DIS0474) or ^{14}C-lixisenatide (Study DIS0531) to Long Evans rats. Radioactivity (^{3}H) was widely distributed in the body within 5 minutes after IV administration, with highest amounts of radioactivity in the kidneys, thyroid, adrenals, salivary gland, lung, and liver. Distribution was not as extensive after SC administration, remaining mostly at the injection site at the earliest time points. At 0.25 hours postdose, the highest concentrations were found in the pancreas, renal cortex, lung, and glandular tissues. Blood, myocardium, and adrenals showed moderate levels of radioactivity. Radioactive levels in fat, skeletal muscle, testis, brain, and spinal cord were near background concentrations. The distribution pattern of lixisenatide at later time points could not be determined because of a weak labeling position leading to a significant fraction of $^{3}H_2O$ being formed.

Five minutes after IV administration of ^{14}C-lixisenatide, radioactivity was widely distributed within the body, with highest radioactivity concentrations in the kidneys. Radioactivity in the brain corresponded to the percentage of blood in the brain and therefore does not indicate a crossing of the blood-brain barrier at this early time point. Five minutes after SC administration, radioactivity in the brain was below the limit of quantitation. The highest radioactive levels were found at the site of administration, the kidneys, pancreas, and the adrenals. Noticeable distribution to cartilaginous areas (vertebral column, trachea, ribs) and to the peripheral region of the lens was found at later time points. As with the ^{3}H label, rapid release of volatile degradation products was noted (most likely $^{14}CO_2$; 43% of the radioactive dose within the first day), and therefore, the distribution data at later time points are likely not reliable.

An additional investigation of lixisenatide brain concentrations through the use of a more sensitive ELISA revealed that the concentration of lixisenatide in the brain is similar to the amount in plasma within the brain, again indicating that there does not appear to be a significant amount of lixisenatide that enters brain tissue.

Placental transfer

Placental transfer of radioactivity was studied following a single SC dose of ^{14}C-lixisenatide to female rats (Study PLT0236) and rabbits (Study PLT0237). Using a more sensitive detection assay due to observed radioactive label volatility, a fetus to dam plasma concentration ratio was found to be approximately 0.1% 15 minutes after administration on GD 17. No lixisenatide could be found in fetal plasma 24 hours after administration or in amniotic fluid after 0.25 or 24 hours. Twenty-four hours after SC administration to pregnant rabbits on GD 18, approximately 0.5% of the administered dose was found in total fetal tissues. The lixisenatide ratio in fetal plasma compared with doe plasma ranged from <0.01% to 0.3%. In conclusion, a very low amount of lixisenatide could be detected in the placenta of rats and rabbits. This suggests that the fetal findings in reproductive and developmental toxicity studies are likely secondary to maternal toxicity.

Metabolism

After incubation of lixisenatide in human S9 liver and kidney fractions, the percentage of parent lixisenatide remaining after 60 minutes was 8% and 11%, respectively (Study F2006KIN0001). Lixisenatide was less extensively metabolized in liver and kidney S9 from mouse and rat compared with rabbit, dog, and human. Overall, 28 metabolites of lixisenatide were detected in human liver and kidney fractions. All in vitro metabolites were degraded peptide products. These degraded peptide products are not expected to be pharmacologically active.

Lixisenatide did not significantly inhibit or induce the CYP isozymes tested.

The stability of lixisenatide was investigated in vitro in heparin-stabilized plasma from mouse, rat, rabbit, dog, pig, and human (Study 00-039). Fast degradation was observed in mice (db/db), rats (Sprague-Dawley), and dogs (Beagle) with estimated half-lives between 212 and 322 minutes. Lixisenatide was more stable in the plasma of Danish slaughter swine and mixed breed mice and rabbits (half-lives between 476 and 564 minutes. Slow metabolism was observed in human plasma (half-life of 2,094 minutes).

Elimination and Secretion

Terminal elimination half-lives of lixisenatide ranged between 0.5 and 6.5 hours after IV administration in mouse, rat, rabbit, dog, and pig, with no apparent relationship to body size. Terminal plasma half-lives after SC dosing tend to be shorter in the smaller animals (mouse, rat) and longer in the larger species (rabbit, dog, pig), suggestive of a prolonged absorption period.

Excretion of radioactivity in milk was studied following a single SC administration of 1 mg/kg of ^{14}C-lixisenatide to female lactating rats on postpartum day 11 (Study (MIL0066). It was estimated that approximately 9.4% of the administered radioactivity was excreted in milk within 24 hours. The radioactive distribution pattern in the pups showed only traces of radioactivity in the region of the stomach at up to 4 hours postdose. After 24 hours, radioactivity was widely distributed within the pup, with the highest level located in the stomach. The sponsor estimates that only 0.0004% of lixisenatide administered to the dam was found in the gastric content of the pups as unchanged lixisenatide. Of the radioactivity found in the stomach from pups, only 0.01% came from unchanged drug. Therefore, a very low amount of the radioactivity transferred to the pups via milk could be attributed to unchanged lixisenatide. In conjunction with minimal amounts of lixisenatide transferred across the placenta, these results also support that the effects seen in the peri-/postnatal developmental study were likely the indirect result of maternal toxicity.

5.2 Toxicokinetics

Effects of anti-drug antibodies on exposure and biological activity of AVE0010

Study Title: 13-week twice daily subcutaneous administration toxicokinetic study in the mouse (dpk0231)

CD1 mice (9 to 11 weeks) received 0 (0.9% NaCl) or 40 µg/kg BID twice daily by subcutaneous injection for 13 weeks. Samples were collected for TK and ADA analyses.

There was one unscheduled death (moribund sacrifice) on Day 19 at 40 µg/kg BID. No adverse clinical signs were noted. Animals receiving AVE0010 had greater mean body weight gain, particularly over the first 5 weeks.

Total AVE0010 was determined by using a double-antibody sandwich immunoassay technique. Monoclonal anti-WAVE002 antibody directed against the last 13 amino acids of lixisenatide C-terminus was pre-coated onto a microtiter plate.

Active AVE0010 was determined by using an in vitro cell-based assay for its function on the activation of GLP-1R by measuring cumulative cAMP production. The concentration of cAMP was measured by using the Cyclic AMP assay, Parameter ELISA Kit. The method was validated under (b) (4) study number (b) (4) 12411.F04. The cell line used in this assay was PSCGa16GLP-1R, a derivative of the HEK293 cell line with genetic modifications. The cell line contains the inserts of the human G-protein Gα16 gene and the human GLP-1R gene.

Summaries of the AVE0010 exposures and a comparison of active versus total AVE0010 are presented in the sponsor-generated tables below. Exposure values for Day 91 female samples for the active AVE0010 assay could not be calculated because of missing data points. Based on the results of this study, it was concluded that neutralizing antibodies were not present after dosing up to 13 weeks.

Analyte	Sex	Day	t_{max} [a] [h]	C_{max} [ng/mL]	AUC_{0-24} [ng•h/mL]
active AVE0010	Male	1	8.17	37.8	80.4
		91	8.33	35.6	70.7
	Female	1	8.33	28.1	67.7
total AVE0010	Male	1	8.33	24.6	48.6
		91	8.17	19.8	57.3
	female	1	0.330	26.6	47.3
		91	8.17	18.5	47.5

[a] relatively to the first daily dose
Values are rounded to 3 significant figures

Table 10 - AVE0010 active fraction (active AVE0010 / total AVE0010) for Cmax and AUC0-24

Sex	Day	Ratio active AVE0010 / total AVE0010	
		C_{max}	AUC_{0-24}
male	1	1.54	1.65
	91	1.80	1.23
female	1	1.06	1.43

Study Title: 13-week twice daily subcutaneous administration toxicokinetic study in the rat (dpk0230)

Sprague-Dawley rats (11 weeks) received 0 (0.9% NaCl) or 40 µg/kg BID twice daily by subcutaneous injection for 13 weeks. Samples were collected for TK analysis on Day 1 and Day 90. Samples were not collected for ADA analysis so the percentage of animals having ADAs and the ADA titer was not determined. Based on the results from other studies, 3 months is a sufficient amount of time for Sprague-Dawley rats to develop anti-AVE0010 antibodies that results in increased drug exposure.

There were no unscheduled deaths. Reduced activity, mouth rubbing, and salivation were observed post-dosing in treated animals. There was an initial decrease in body weight during the first week for treated males after which time body weight gain was similar to controls. There was no apparent treatment-related effect on females.

Total AVE0010 was determined by using a double-antibody sandwich immunoassay technique. Monoclonal anti-WAVE002 antibody directed against the last 13 amino acids of lixisenatide C-terminus was pre-coated onto a microtiter plate.

Active AVE0010 was determined by using an in vitro cell-based assay for its function on the activation of GLP-1R by measuring cumulative cAMP production. The concentration of cAMP was measured by using the Cyclic AMP assay, Parameter ELISA Kit. The method was validated under [(b) (4)] study number [(b) (4)] 12411.F04. The cell line used in this assay was PSCGa16GLP-1R, a derivative of the HEK293 cell line with genetic modifications. The cell line contains the inserts of the human G-protein Gα16 gene and the human GLP-1R gene.

Summaries of the AVE0010 exposures and a comparison of active versus total AVE0010 are presented in the sponsor-generated tables below.

Text Table 1: Summary of toxicokinetic parameters of AVE0010 in male and female rats

Analyte	Sex	Day	C_{max} [ng/mL]	AUC_{0-24} [ng•h/mL]
active AVE0010	Male	1	20.4	90.6
		90	39.4	305
	Female	1	23.9	93.5
		90	40.4	630
total AVE0010	Male	1	16.4	74.7
		90	39.2	423
	female	1	21.0	81.4
		90	48.6	636

Values are rounded to 3 significant figures

Sex	Day	Ratio active AVE0010 / total AVE0010	
		C_{max}	AUC_{0-24}
male	1	1.24	1.21
	90	1.00	0.722
female	1	1.14	1.15
	90	0.832	0.991

The active fraction (active AVE0010 / total AVE0010 for C_{max} and AUC_{0-24h}) ranged from 0.722 to 1.24 (mean: 1.04). No difference in exposure comparing active AVE0010 and total AVE0010 was observed, either on Day 1 or Day 90. The results of this study suggest that the majority of the AVE0010 in rat plasma is biologically active even in the presence of anti-AVE0010 antibodies.

Study Title: 13-week twice daily subcutaneous administration toxicokinetic study in the dog followed by a 4-week treatment-free period (dpk0232)

Two (control) or four Beagle dogs/sex/group (8 to 10 months) received 0 (0.9% NaCl), 5, or 100 µg/kg twice daily by subcutaneous injection for 13 weeks. There were no unscheduled deaths. Occasional post-dose salivation and vomiting were observed at ≥5 µg/kg BID. Initial body weight loss was observed at ≥5 µg/kg BID in a dose-related manner. After approximately the first month of dosing, body weight gain for treated groups appeared to be similar to controls. During the treatment-free period, treated groups gained slightly more weight than controls. The effects on body weight correlated with decreased food consumption during the first 1 to 2 weeks for males and up to 4 weeks for females.

Total AVE0010 was determined by using a double-antibody sandwich immunoassay technique. Monoclonal anti-WAVE002 antibody directed against the last 13 amino acids of lixisenatide C-terminus was pre-coated onto a microtiter plate.

Active AVE0010 was determined by using an in vitro cell-based assay for its function on the activation of GLP-1R by measuring cumulative cAMP production. The concentration of cAMP was measured by using the Cyclic AMP assay, Parameter ELISA Kit. The method was validated under (b) (4) study number (b) (4) 12411.F04. The cell line used in this assay was PSCGa16GLP-1R, a derivative of the HEK293 cell line with genetic modifications. The cell line contains the inserts of the human G-protein Gα16 gene and the human GLP-1R gene.

A summary of the AVE0010 exposures is presented in the sponsor-generated table below.

Text Table 1: Summary of toxicokinetic parameters of AVE0010 in male and female dogs

Analyte	Sex	Dose [µg/kg/day]	Day	C_{max} [ng/mL]	AUC_{0-24} [ng*h/mL]
active AVE0010	Male	10	1	5.63	44.7
			28	6.70	49.2
			60	6.34	50.3
			90	6.41	51.2
		200	1	95.6	917
			28	166	1500
			60	475	6670
			90	579	10600
	Female	10	1	6.48	46.2
			28	8.67	58.8
			60	11.0	123
			90	21.0	192
		200	1	92.2	914
			28	198	1980
			60	488	8680
			90	477	9300
total AVE0010	Male	10	1	4.58	41.8
			28	6.38	56.6
			60	5.88	58.2
			90	5.46	53.4
		200	1	76.4	769
			28	119	1250
			60	393	6640
			90	593	10300
	Female	10	1	5.26	43.6
			28	7.92	72.8
			60	13.3	155
			90	17.6	185
		200	1	68.3	731
			28	124	1570
			60	405	5350
			90	236	4430

From Day 27 onward, ADAs were detected in 25% of LD males and 50% of LD females, whereas 100% of HD animals were ADA positive. This resulted in a greater drug accumulation at the HD level compared with the LD level.

The active fraction (active AVE0010 / total AVE0010 for C_{max} and AUC_{0-24h}) ranged from 0.823 to 1.57 (mean: 1.17). No considerable difference in exposure comparing active AVE0010 and total AVE0010 was observed, irrespective of the observation day (1, 28, 60, or 90). The results of this study suggest that the majority of the AVE0010 in dog plasma is biologically active even in the presence of anti-AVE0010 antibodies.

6 General Toxicology

6.1 Single-Dose Toxicity

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (µg/kg) Route	Endpoints	Findings
Single dose with 14-day recovery 2004-1181 (Sanofi) (b) (4) GLP	Mouse/CD-1 Preliminary: 1/sex Main: 5/sex	500 Subcutaneous	-Mortality -Clinical signs -Body weights -Gross pathology	• No mortality or adverse clinical signs occurred. • There were no apparent adverse effects on body weight gain. • No macroscopic findings were noted on Day 15.
Single dose with 14-day recovery 2004-1180 (Sanofi) (b) (4) GLP	Mouse/CD-1 Preliminary: 1/sex Main: 5/sex	500 Intravenous	-Mortality -Clinical signs -Body weights -Gross pathology	• No mortalities occurred. • In the preliminary study, one male showed limited lethargy for approximately 2 hours after dosing. No adverse effects on behavior were noted in the main study. • There were no apparent adverse effects on body weight gain. • No macroscopic findings were noted on Day 15.
Single dose with 14-day recovery 2004-1179 (Sanofi) (b) (4) GLP	Rat/ Wistar Han Preliminary: 1/sex Main: 5/sex	5000 Subcutaneous	-Mortality -Clinical signs -Body weights -Gross pathology	• No mortalities occurred. • In the preliminary study, one male showed piloerection during the day after dosing. No adverse effects on behavior were noted in the main study. • Small losses in body weight or no gain was prevalent up to Day 6, which was slightly more evident in males. Body weight gains occurred during Week 2. • No macroscopic findings were noted on Day 15.

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (µg/kg) Route	Endpoints	Findings
Single dose with 14-day recovery 2004-1186 (Sanofi) (b) (4) GLP	Rat/Wistar Han Main: 5/sex Recovery: 5/sex TK: 10/sex	0, 50, 125, or 500 Subcutaneous	-Mortality -Clinical signs -Body weights -Food consumption -Hematology -Clinical chemistry -Gross pathology -Organ weights -Histopathology	• There were no mortalities, adverse clinical signs, or effects on body weight. • At the 24 hour necropsy, 5% decrease in packed cell volume in HD males; 90% (MD) and 180% (HD) increase in bilirubin (males only); 55% (MD males) and 36% (HD females) increase in glucose; and 32% decrease (HD males) in cholesterol; no changes noted after 14 day recovery. • Spleen weights were slightly decreased for HD females and LD and MD males. • No macroscopic or microscopic findings noted on Day 2 or Day 15.
Single dose with 14-day recovery 2004-1178 (Sanofi) (b) (4) GLP	Rat/ Wistar Han Preliminary: 1/sex Main: 5/sex	5000 Intravenous	-Mortality -Clinical signs -Body weights -Gross pathology	• No mortalities occurred. • Clinical signs included piloerection from 4 hours after dosing through Day 2, anogenital soiling up to Day 3, and staining of the snout on Day 2. External appearance returned to normal by Day 4. • All rats lost weight between Day 1 and 4, after which time weight gain occurred. • No macroscopic changes were noted on Day 15.
Single dose with 14-day recovery 2004-1184 (Sanofi) (b) (4) GLP	Rat/Wistar Han Main: 5/sex Recovery: 5/sex TK: 10/sex (no control group)	0, 1, 2.5, or 10 Intravenous	-Mortality -Clinical signs -Body weights -Food consumption -Hematology -Clinical chemistry -Gross pathology -Organ weights -Histopathology	• No mortality or clinical signs. • No body weight changes. • Two HD females had slightly elevated alkaline phosphatase on Day 2. There were no effects on clinical pathology parameters on Day 15. • No macroscopic or microscopic findings on Day 2 or Day 15.

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (µg/kg) Route	Endpoints	Findings
Single dose with 14-day recovery 2004-1187 (Sanofi) (b) (4) GLP	Dog/Beagle Main: 2/sex Recovery: 2/sex	0, 10, 40, or 200 Subcutaneous	-Mortality -Clinical signs -Body weights -Food consumption -Hematology -Clinical chemistry -Gross pathology -Organ weights -Histopathology	• There were no mortalities or adverse effects on clinical signs, body weight, or food consumption. • There were no apparent effects on clinical pathology, gross pathology, or organ weights on Day 2 or Day 15. • An increased incidence and severity of vacuolation of the pancreatic islet cells was noted in HD females on Day 15. There were no other noteworthy microscopic findings on Day 2 or Day 15.
Single dose with 14-day recovery 2004-1185 (Sanofi) (b) (4) GLP	Dog/Beagle Main: 4/sex	0, 5, 20, or 100 Intravenous	-Mortality -Clinical signs -Body weights -Food consumption -Hematology -Clinical chemistry -Gross pathology -Organ weights -Histopathology	• There were no mortalities or adverse effects on clinical signs, body weight. • A slight decrease in food intake was noted for HD animals for the first 2 days after treatment. • There were no apparent effects on clinical pathology, gross pathology, organ weights, or histopathology on Day 2 or Day 15.

6.2 Repeat-Dose Toxicity

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (µg/kg) Route	Endpoints	Findings
2-week toxicity study* DSE 2003-1952 Non-GLP	Mouse /CD-1 Main: 10/sex	0, 20, 200, 1000, or 2000 BID Subcutaneous	-Mortality -Clinical signs -Body weight -Food consumption -Water consumption -Clinical pathology -Gross pathology -Organ weights -Histopathology (limited) -TK	• There were no unscheduled deaths or adverse clinical signs. • Body weight gains were lower for the two highest dose groups between Days 1 and 5, after which time BWs rebounded. Effects on BW correlated with decreased food and water consumption. • Decreased RBC parameters were observed in HD males. • There were no remarkable treatment-related effects on histopathology, gross pathology, or organ weights. • Mean TK (females had lower exposures than males) Day 1: C_{max} / AUC_{0-24}; Day 14: C_{max} / AUC_{0-24} 40: 11 ng/mL / 14 ng·h/mL / 19 / 24 400: 120 / 124 / 136 / 211 2000: 468 / 787 / 525 / 950 4000: 842 / 1380 / 1082 / 2056 • The NOAEL was 2000 µg/kg BID, the highest dose tested.
13-week toxicity* with 4-week recovery DSE 2004-0062 (Sanofi); (b) (4) GLP	Mouse /CD-1 Main: 10/sex TK: 65/sex	0, 20, 200, 1000, or 2000 BID (8-hr interval) Subcutaneous	-Mortality -Clinical signs -Body weight -Food consumption -Water consumption -Clinical pathology -Gross pathology -Organ weights -Histopathology -TK	• No drug-related mortality or clinical signs occurred. • Transient decreases in BW gain were observed at all dose levels and were associated with decreased FC. This was followed by greater BW gain and FC for males compared with controls. • No adverse drug-related effects were seen in clinical pathology or histopathology. • Increased vacuolation, suggestive of glycogen accumulation, was noted in liver for HD females. Liver weights were slightly increased, but a clear dose response was not seen. • A slight increase in the extent of subdermal fibrosis/ inflammation was seen at injection sites for all treated females.

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/strain
Number/group</th><th>Dose Levels
(µg/kg)
Route</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td>DSE 2004-0062, continued</td><td></td><td></td><td></td><td>• At Week 13, drug exposures were high and variable and were not felt to be valid due to ADA interference.
• The NOAEL was 2000 µg/kg BID, resulting in mean AUC_{0-24} values of 2848 and 2387 ng·h/mL in males and females, respectively, on Day 1.</td></tr>
<tr><td>13-week TK and antibody assessment study*

DSE 2005-0443 (Sanofi); (b) (4)

GLP</td><td>Mouse /CD-1

TK: 66/sex
Ab: 20/sex

Study intended to complement the data from Study DSE 2004-0062</td><td>0, 200, 1000, or 2000

BID

Subcutaneous</td><td>-Mortality
-Clinical signs
-Body weight
-Food consumption
-TK
-Anti-drug Ab</td><td>• Unscheduled deaths occurred in the control group (1 male), LD (1 male), MD (3 males), and HD (6 males and 2 females). The cause was undetermined for most deaths.
• Mean BWs were higher for treated animals compared with controls by the end of the study.
• FC was dose-dependently decreased for the first 2 to 3 weeks for all treated groups.
• On Day 92, positive antibody rates (% animals) in each dose group were:
Control: 0% (males and females)
LD: 40% (males and females)
MD: 60% (males), 90% (females)
HD: 100% (males), 60% (females)
• For MD and HD, mean exposures increased by 9X to 13X from Day 1 to Day 92 due to anti-drug Abs.</td></tr>
</table>

Dose levels [µg/kg BID]	Study Days	Sex	C_{max} [ng/mL]	t_{max} [hr]	C_{24hr} [ng/mL]	$AUC_{(0-24hr)}$ [ng*hr/mL]
200	1	M	136	0.33	< 0.10	192
		F	121	0.17	< 0.10	145
	92	M	229	0.33	0.16	351
		F	162	0.33	0.11	337
1000	1	M	602	0.33	0.35	927
		F	597	0.33	0.38	787
	92	M	972	16.00	971.59	9521
		F	3017	1.00	45.45	6980
2000	1	M	1023	0.33	0.82	1590
		F	1018	0.17	0.57	1490
	92	M	4216	1.00	35.11	20351
		F	2597	1.00	0.79	13039

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (µg/kg) Route	Endpoints	Findings
5-day toxicity study DSE 2003-1276 Non-GLP (no QA audit)	Rat / Sprague-Dawley (b) (4) Main: 5/sex TK: 12/sex	0, 70, 1000, or 2000 BID Subcutaneous	-Mortality -Clinical signs -Body weight -Food consumption -Clinical pathology -Gross pathology -Organ weights -Histopathology (limited, no thyroid or pancreas) -TK	• No mortalities or adverse clinical signs occurred. • Dose-related, decrease in BW or BW gain observed at all dose levels, which correlated with decreased FC. • Decreased reticulocyte counts were observed for all male treatment groups and HD females. HD males had slightly reduced neutrophils. • There were no apparent effects on organ weights or macroscopic findings. • Microscopically, treatment-related, minimal to moderate inflammation of mainly medium sized portal triad was observed in the liver of all treatment groups. Inflammation was characterized by infiltration of mixed inflammatory cells around the arteries and bile ducts with beginning fibrosis mainly around arteries. The sponsor states that similar findings were not observed in other rat strains or the same strain purchased from other breeders (see Study 2003-1276). • The NOAEL was 2000 µg/kg BID. • Mean TK values on Day 14 were: C_{max} (ng/mL) / AUC_{0-24} (ng·h/mL) Male / Female / Male / Female LD 31.2 / 38.7 / 50 / 45 MD 143 / 130 / 357 / 393 HD 127 / 185 / 483 / 532
5-day toxicity study (follow-up to #2003-1276) DSE 2003-1986 Non-GLP (no QA audit)	Rat / (b) (4) Sprague-Dawley [Hsd:SD] or (b) (4) Sprague-Dawley Crl:SD) Main: 5/sex/group/ animal source	0 or 1000 BID Subcutaneous	-Mortality -Clinical signs -Body weight -Food consumption -Clinical pathology -Gross pathology -Organ weights -Histopathology (limited, no thyroid or pancreas)	• No unscheduled deaths occurred. • Treated animals showed hypoactivity during the first 3 days. • Dose-related, decrease in BW or BW gain observed at all dose levels, which correlated with decreased FC. • Statistically significantly reduced neutrophils were observed in treated Hsd:SD rats, but not Crl:SD rats. • Decreased thymus weights were observed in treated animals of both strains. Treated Crl:SD rats showed increased absolute adrenal weights. • There were no apparent treatment-related macroscopic findings.

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (µg/kg) Route	Endpoints	Findings
DSE 2003-1986, continued				• Microscopically, 2/5 treated rats showed mild inflammation in liver, mainly at medium sized portal triads, which exhibited features of chronic inflammation. • On the basis of the totality of rat data, the sponsor believes that the hepatic findings are the result of stress-related, breeder-specific toxicity.
5-day toxicity study (follow-up to #2003-1276) DSE 2003-1987 Non-GLP (no QA audit)	Rat / Wistar (b) (4) Main: 5/sex	0 or 1000 BID Subcutaneous	-Mortality -Clinical signs -Body weight -Food consumption -Clinical pathology -Gross pathology -Organ weights -Histopathology (limited, no thyroid or pancreas)	• No unscheduled deaths occurred. • Treated animals were hypoactive for the first 3 days. • A dose-related decrease in BW or BW gain was observed at all dose levels, which correlated with decreased FC. • Statistically significantly reduced neutrophils were noted. • Treated rats had reduced spleen weights, which is consistent with the observation of decreased extramedullary hematopoiesis in the spleen. • No microscopic alterations were observed in the liver.
2-week toxicity study* with 2-week recovery DSE 2003-1925 and Amendment 1 GLP	Rat / Sprague-Dawley Main: 10/sex Recovery: 5/sex (control and HD) TK: 12/sex (3 per time point)	0, 2, 20, and 200 BID Subcutaneous	-Mortality -Clinical signs -Body weight -Food consumption -Water consumption -Hematology -Clinical chemistry -Urinalysis -Gross pathology -Organ weights -Histopathology -TK -Antibody analysis	• No unscheduled deaths occurred. Hypoactivity was observed at the beginning of the study in some females and MD and HD males. • Dose-related, transient decreases in BW gain and FC with reversibility were observed at all dose levels. • Water consumption was transiently reduced during the first week of treatment. • There were no remarkable changes in clinical pathology parameters except higher eosinophil counts in MD and HD treated males, which may have been related to local irritation at the injection sites. • MD and HD males showed decreased mean thymus weights and increased adrenal weights. • Microscopically, injection site reaction was the major finding, mainly seen in HD treated animals and was partially reversed after a 2-week recovery period. • Anti-AVE0010 antibodies were detected in treated animals in the following percentage of animals per group following 14 days of dosing:

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/strain
Number/group</th><th>Dose Levels
(µg/kg)
Route</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td>DSE 2003-1925,
continued</td><td></td><td></td><td></td><td>Control: 0%, M and F
LD: 5% and 0%, M and F;
MD: 0% and 6%, M and F;
HD: 68% and 25%, M and F.
• The NOAEL was 400 µg/kg/day.
• Mean TK values on Day 14
<table><tr><th></th><th colspan="2">C_{max} (ng/mL)</th><th colspan="2">AUC_{0-24} (ng·h/mL)</th></tr><tr><th></th><th>Male</th><th>Female</th><th>Male</th><th>Female</th></tr><tr><td>LD</td><td>0.45</td><td>0.34</td><td>1.22</td><td>0.76</td></tr><tr><td>MD</td><td>8.46</td><td>6.27</td><td>24.09</td><td>11.52</td></tr><tr><td>HD</td><td>66.9</td><td>98.3</td><td>154.5</td><td>326.6</td></tr></table></td></tr>
<tr><td>4-week toxicity study*

2003-2059
(Sanofi)

DIV1156
(Ab report)

GLP</td><td>Rat / Sprague-Dawley

Main: 10/sex
TK: 10/sex</td><td>0, 3, 10, or 30

BID
(8 hours between 1st and 2nd doses)

Intravenous (bolus)</td><td>-Mortality
-Clinical signs
-Body weight
-Food consumption
-Water consumption
-Ophthalmic exam
-Clinical pathology
-Gross pathology
-Organ weights
-Histopathology
-TK
-Antibody analysis</td><td>• There were no unscheduled deaths.
• Treatment-related clinical signs included abnormal gait, piloerection, hypoactivity, irregular/fast respiration and hunched body posture. Incidence and severity tended to decrease over the course of the study.
• Treated animals showed increased water consumption
• There were no drug-related effects on body weight, FC, ophthalmology, clinical hematology, serum chemistry and organ weight.
• Higher incidences of foreign body granuloma in the lungs in treated animals, which were considered to be related to the mode and frequency of administration rather than a direct test-article effect.
• No anti-drug antibodies were detected.
<table><tr><th>Sex</th><th>Dose (µg/kg/bid)</th><th>C_{5min} (ng/mL) [a] Day 1</th><th>C_{5min} (ng/mL) [a] Day 28</th><th>AUC_{0-8h} (ng.h/mL) [a] Day 1</th><th>AUC_{0-8h} (ng.h/mL) [a] Day 28</th></tr><tr><td>male</td><td>3</td><td>3.95</td><td>4.34</td><td>1.64</td><td>2.05</td></tr><tr><td></td><td>10</td><td>13.3</td><td>16.0</td><td>6.36</td><td>8.07</td></tr><tr><td></td><td>30</td><td>66.1</td><td>74.1</td><td>24.9</td><td>29.3</td></tr><tr><td>female</td><td>3</td><td>3.35</td><td>4.27</td><td>1.37</td><td>1.65</td></tr><tr><td></td><td>10</td><td>16.4</td><td>16.0</td><td>6.74</td><td>7.01</td></tr><tr><td></td><td>30</td><td>54.9</td><td>68.7</td><td>20.2</td><td>26.3</td></tr></table></td></tr>
</table>

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (µg/kg) Route	Endpoints	Findings
13-week toxicity* with 4-week recovery DSE 2004-0063, Amendment 1 GLP	Rat/ Sprague Dawley Main: 10/sex Recovery: 5/sex (C, MD, HD only) TK: 18/sex (3/time point) Ab: all main study animals	0 (0.9% NaCl), 5, 20, 200, 1000, or 2000 BID Subcutaneous injection	-Mortality -Clinical signs -Body weight -Food consumption -Ophthalmology -Hematology -Clinical chemistry -Urinalysis -Gross pathology -Organ weights -Histopathology (complete list of tissues) -TK -Antibody analysis	• There were no deaths; mild lethargy occurred at ≥5 µg/kg BID immediately to 30 min after the first daily dose from Day 2 to around 2 weeks. Paddling, salivation, and mouth rubbing occurred transiently after dosing from Day 8 through the end of the study and occurred predominantly at higher doses. • Decreased mean BW gain occurred for males at all dose levels during the dosing period, primarily between Weeks 1 to 4 (↓24%, 22%, 22%, 20%, and 18% at LD1, LD2, MD1, MD2 and HD, respectively). BWs rebounded during the 4-week recovery period. Effects on BW in males correlated with reduced FC. • Decreased mean WBC and lymphocytes occurred at all doses at Week 4 and at all male doses at Week 13. These effects were fully reversible. No remarkable changes in serum chemistry parameters were noted. • Decreased mean urine volume and total urine sodium and potassium occurred in all treated male groups at Week 4 and 13; compensatory changes in these parameters were noted during the recovery period. • Mean absolute and relative kidney weights were increased in females at ≥ 5 µg/kg BID. This effect was still noticeable after the 4-week recovery period. • Potentially treatment-related microscopic findings included slightly increased incidence of papillary mineralization, inflammatory cell foci and corticomedullary mineralization in the female kidneys at 2000 µg/kg BID (4/10 vs 1/10 C). These changes were not observed in the recovery group animals. There was a greater number of control females in proestrus (6) compared with the HD group (1) and there were more HD females in diestrus (5) compared with controls (2). • Anti-AVE0010 antibodies were detected at all dose levels in Week 14: 80% animals at 5 µg/kg/BID; 95% animals at 20, 200 and 2000 µg/kg BID; 80% animals at 1000 µg/kg BID.

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/strain
Number/group</th><th>Dose Levels
(µg/kg)
Route</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td>DSE 2004-0063,
Amendment 1, continued</td><td></td><td></td><td></td><td>

No overt signs of toxicity were observed; therefore, the NOAEL was considered to be 2000 µg/kg BID.
A summary of TK data is shown in the sponsor-generated table below. Exposure increased by up to 180-fold between Day 1 and Week 13, due to the presence of ADAs. By Week 13, mean exposures were similar between the LD and HD groups. Exposures were greatest in the MD group. Because of Ab interference, a second bioanalytical method was validated to detect both unbound and Ab-bound test article, the data from which were presented in an amended study report.

Values following the two doses in Week 13
<table>
<tr><th rowspan="2">Dose nominal mg/kg/day</th><th rowspan="2">Dose adjusted mg/kg/day</th><th rowspan="2">Gender</th><th colspan="4">AVE0010</th></tr>
<tr><th>C_{max} [ng/mL]</th><th>t_{max} [hr]</th><th>C_{24hr} [ng/mL]</th><th>$AUC_{(0-24hr)}$ [ng*hr/mL]</th></tr>
<tr><td>0.4</td><td>0.33</td><td>M</td><td>1837</td><td>0.33</td><td>739.11</td><td>17642</td></tr>
<tr><td></td><td></td><td>F</td><td>1149</td><td>3.00</td><td>769.08</td><td>20637</td></tr>
<tr><td>2</td><td>1.66</td><td>M</td><td>5374</td><td>1.00</td><td>135.94</td><td>39235</td></tr>
<tr><td></td><td></td><td>F</td><td>2461</td><td>1.00</td><td>1364.20</td><td>28916</td></tr>
<tr><td>4</td><td>3.31</td><td>M</td><td>1215</td><td>1.00</td><td>87.32</td><td>14348</td></tr>
<tr><td></td><td></td><td>F</td><td>2643</td><td>0.33</td><td>109.40</td><td>15144</td></tr>
</table>
</td></tr>
<tr><td>57-day dose escalation, dose range finding toxicity study

DSE 2005-0233

Non-GLP</td><td>Dog/Beagle

Main: 2/sex</td><td>0 (0.9% NaCl), or rising dose
2.5 µg/kg start
500 µg/kg end
Dose escalated every 3 days

BID

Subcutaneous injection</td><td>-Mortality
-Clinical signs
-Body weight
-Food consumption</td><td>

There were no deaths.
Liquid feces and vomiting were observed occasionally for the dosed group.
Decreased BW gain was noted for treated males and BW loss for treated females.
Decreased FC was noted for one treated female.
The high dose of 500 µg/kg BID was well tolerated and was recommended for longer duration studies.

</td></tr>
</table>

Study Type Study No. GLP Status	Species/strain Number/group	Dose Levels (µg/kg) Route	Endpoints	Findings
4-week toxicity study DSE 2003-2060 DIV1157 (Ab report) GLP	Dog/Beagle Main: 3/sex	0 (0.9% NaCl), 10, 40, or 200 BID Subcutaneous injection	-Mortality -Clinical signs -Body weight -Food consumption -Ophthalmology -Electrocardiograph -Clinical pathology -Hematology -Clinical chemistry -Urinalysis -Gross pathology -Organ weights -Histopathology (complete list of tissues) -TK -Antibody analysis	• There were no mortalities or adverse clinical signs. • Slightly decreased BW or BW gain at all female doses during the first week and decreased BW gain at HD for both genders during the second week of dosing. Effects on BW correlated with decreased FC. • A slight decrease in mean epididymis and testis weight occurred at HD. Slightly decreased mean liver weight was noted at all female doses and slightly decreased heart weight occurred at all male doses. • Increased incidence of macroscopic hemorrhagic areas in MD females and HD males and females. • No definitive signs of systemic toxicity were observed. Dilated, congested vascular space of aortic valve was seen at the HD. A slight increase in subcutaneous fibroblast proliferation was seen at the injection sites. • No anti-drug antibodies were detected. • The NOAEL was determined to be 200 µg/kg BID.
13-week toxicity* with 4-week recovery DSE 2003-1926 Amendment 1 (Sanofi): (b) (4) GLP	Dog/Beagle Main: 3/sex Recovery: 2/sex	0 (0.9% NaCl), 20, 300/100, or 1000/400/250 (dose levels reduced after 1 month due to body weight loss) BID Subcutaneous injection	-Mortality -Clinical signs -Body weight -Food consumption -Ophthalmology -Electrocardiograph -Hematology -Clinical chemistry -Urinalysis -Gross pathology -Organ weights -Histopathology (complete list of tissues) -TK -Antibody analysis	• There were no mortalities; dose-related emesis, diarrhea, and thin appearance occurred at all doses. • Sharply decreased BW at MD and HD occurred during early dosing resulting in a dose reduction at the end of the first month (MD: 300 → 100 µg/kg BID; HD: 1000 → 400 → 250 µg/kg BID). Over the dosing period, a remarkable decrease in BW gain was seen in MD and HD males and HD females; BWs in HD groups did not fully recover. • Decreased FC was seen during the first 4 weeks in all treated animals and then improved by using moist food and additional food supplement (HD). • Lower heart rates were noted in all treated groups after dosing in Weeks 4 and 13 with large variability, and the reversibility was unknown. • No significant findings were noted for clinical pathology, gross pathology, or organ weights. • Treatment-related microscopic findings were observed in the liver (decreased hepatocellular glycogen vacuoles, MD and HD), testes (segmental sperm

<table>
<tr><th>Study Type
Study No.
GLP Status</th><th>Species/strain
Number/group</th><th>Dose Levels
(µg/kg)
Route</th><th>Endpoints</th><th>Findings</th></tr>
<tr><td rowspan="2">DSE 2003-1926
Amendment 1, continued</td><td></td><td></td><td></td><td>stasis, MD and HD), and at injection sites (slightly increased subdermal inflammation/fibrosis in all treated groups). After recovery, liver and testes findings were fully recovered, but the grade of injection site subdermal inflammation/fibrosis was still higher at the HD, suggesting partial reversal.
• Anti-AVE0010 antibodies were detected in all treated groups at Weeks 4 and 13 resulting in increased drug exposure; the percent animals with positive antibodies in C, LD, MD and HD detected are:
o Week 4: 0, 83, 100 and 90%;
o Week 13: 10, 100, 100 and 70%;
o Week 17 (R): 0 (C), 50% (HD).
• NOAEL was <20 µg/kg BID, based on BW decrement, ↓ HR, and injection site lesions at ≥ 20 µg/kg BID.
• TK results are below (sponsor-generated tables).</td></tr>
<tr><td colspan="3"></td><td>
Median values following the two doses on Day 1
<table>
<tr><th rowspan="2">Dose nominal µg/kg BID</th><th rowspan="2">Gender</th><th colspan="4">Total AVE0010</th></tr>
<tr><th>C_{max} [ng/mL]</th><th>t_{max} [hr]</th><th>C_{24} [ng/mL]</th><th>$AUC_{(0-24hr)}$ [ng*hr/mL]</th></tr>
<tr><td>20</td><td>M</td><td>22</td><td>2.00</td><td>0.17</td><td>215</td></tr>
<tr><td></td><td>F</td><td>24</td><td>2.00</td><td>0.12</td><td>197</td></tr>
<tr><td>300</td><td>M</td><td>122</td><td>2.00</td><td>3.12</td><td>1019</td></tr>
<tr><td></td><td>F</td><td>193</td><td>3.00</td><td>0.58</td><td>1395</td></tr>
<tr><td>1000</td><td>M</td><td>300</td><td>1.00</td><td>41.36</td><td>3578</td></tr>
<tr><td></td><td>F</td><td>341</td><td>1.00</td><td>4.68</td><td>3225</td></tr>
</table>
Median values following the two doses on Day 91
<table>
<tr><th rowspan="2">Dose nominal µg/kg BID</th><th rowspan="2">Gender</th><th colspan="4">Total AVE0010</th></tr>
<tr><th>C_{max} [ng/mL]</th><th>t_{max} [hr]</th><th>C_{24} [ng/mL]</th><th>$AUC_{(0-24hr)}$ [ng*hr/mL]</th></tr>
<tr><td>20</td><td>M</td><td>160</td><td>3.00</td><td>120.43</td><td>2700</td></tr>
<tr><td></td><td>F</td><td>77</td><td>3.00</td><td>18.34</td><td>957</td></tr>
<tr><td>100</td><td>M</td><td>1098</td><td>2.00</td><td>863.62</td><td>21607</td></tr>
<tr><td></td><td>F</td><td>239</td><td>0.50</td><td>66.47</td><td>3252</td></tr>
<tr><td>250</td><td>M</td><td>470</td><td>3.00</td><td>156.60</td><td>8289</td></tr>
<tr><td></td><td>F</td><td>618</td><td>2.00</td><td>344.76</td><td>11072</td></tr>
</table>
</td></tr>
</table>

*Initially reviewed by Dalin Yao. BID = twice daily dosing; BW = body weight; C = control; FC = food consumption; GLP = good laboratory practice; HD = high dose; LD = low dose; MD = mid dose; NOAEL = no observed adverse effect level; TK = toxicokinetics; WBC = white blood cells.

Repeat-Dose Toxicity - Pivotal Studies

Study title: AVE0010: 6-month subcutaneous toxicity study in rats with a 4-week recovery period

Study number:	DSE 2005-0085 (Sanofi)
Study report location:	Module 4.2.3.2
Conducting laboratory:	(b) (4)
Date of study initiation:	17 January 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, Batch #PPL-A VE100404B, and purity 98.4%

Key Study Findings

- No unscheduled mortalities occurred. There was a dose-related increase in squeaky noises during dosing for males and females, indicating signs of discomfort.
- Lower mean body weights associated with decreased FC were observed at all dose levels with reversibility after a 4-week recovery period.
- There were no remarkable findings in clinical pathology parameters.
- There were no test-article related microscopic findings in females. In males, an 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 2000 µg/kg BID. These effects were shown to be mostly 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.
- All animals from the MD and HD groups developed anti-AVE0010 antibodies by Day 28, whereas LD group rats did not have meaningful titers until the next time point (Day 154), at which point approximately 50% of LD males and 20% of LD females tested positive. At the end of recovery, 100% of HD animals still had positive titers.
- Exposure ($AUC_{0\text{-}24h}$) increased by approximately 20 times between Day 7 and Day 183 for the MD and HD groups, likely due to anti-AVE0010 antibody production.
- The NOAEL was determined to be 2000 µg/kg BID for females and 100 µg/kd BID for males, based on the testicular findings.

Methods

Species/Strain:	Rat / Sprague-Dawley (b) (4)
Frequency of dosing:	Twice daily (approximately 8 hours after the first dose)
Route of administration:	Subcutaneous
Formulation/Vehicle:	Sterile isotonic 0.9% sodium chloride
Age:	6-7 weeks at arrival
Weight:	151-192 g (males) and 139-169 g (females)
Doses and Groups:	see sponsor-generated table below

Group	Number of main group animals		Number of recovery animals		Number of toxicokinetic animals		Dose level	Dose conc.	Dose vol.
	Male	Female	Male	Female	Male	Female	(µg/kg BID)	(µg/mL)	(mL/kg BID)
1	15	15	5	5	3	3	0	0	0.5
2	15	15	-	-	18	18	5	10	0.5
3	15	15	-	-	18	18	100	200	0.5
4	15	15	5	5	18	18	2000	4000	0.5
Total	60	60	10	10	30	30			

Protocol deviations: There were no deviations from the protocol that might have affected the quality or integrity of the study.

Observations and Results

Mortality: Evaluated twice daily during treatment and once daily during recovery.

Unscheduled deaths occurred for 1/23, 2/33, 1/33, and 1/38 males and 0/23, 0/33, 1/33, and 3/38 females in the control, LD, MD, and HD groups, respectively. All of these deaths occurred in the toxicokinetic groups except for one main group HD female that was found dead on Day 33. The cause of death for this animal was not determined. Overall, there did not appear to be a treatment-related increased incidence in early mortality.

Clinical Signs: Evaluated twice daily during treatment and once daily during recovery.

Summary of Treatment-Related Clinical Signs

Dose (µg/kg BID)	0		5		100		2000	
Observation Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Squeaky noises during administration								
-Number of observations:	0	0	2	1	78	100	179	227
-Number of animals:	0	0	2	1	14	12	20	18
-Days from - to:	NA	NA	117 153	102 102	51 180	50 178	50 180	48 178

F = female; M = male; NA = not applicable.

Body Weights: Measured once weekly

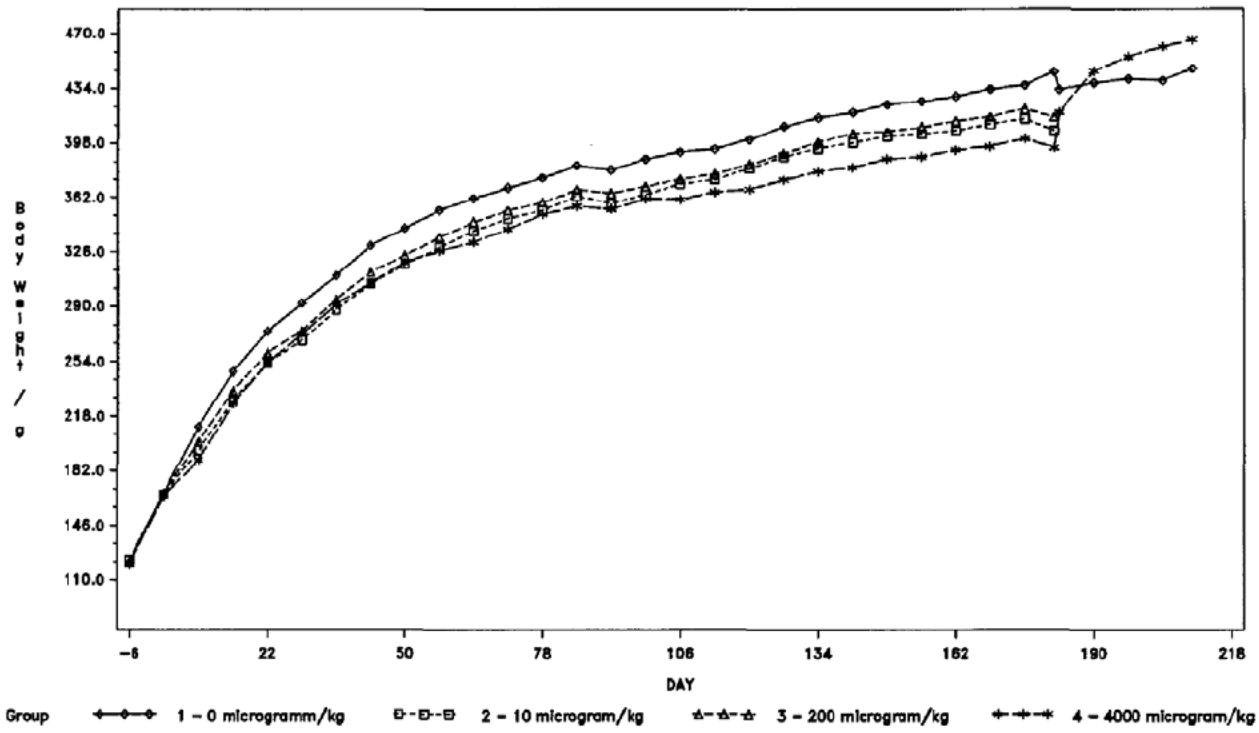
Summary of Body Weights

Dose (µg/kg BID)	0		5		100		2000	
Sex	M	F	M	F	M	F	M	F
Body Weight Gain: Days 1-8								
Weight gain (g)	44.2	20.0	29.4*	21.1	34.0*	20.2	24.2*	17.2
Diff from control (g)			-14.8	1.1	-10.2	0.2	-20.0	-2.8
% diff from control			↓33%	↑6%	↓23%	-	↓45%	↓14%
Final Body Weight: Day 176								
Body weight (g)	436.1	284.2	414.1	276.2	420.6	265.1*	400.9*	253.3*
Diff from control (g)			-22.0	-8.0	-15.5	-19.1	-35.2	-30.9
% diff from control			↓5%	↓3%	↓4%	↓7%	↓8%	↓11%

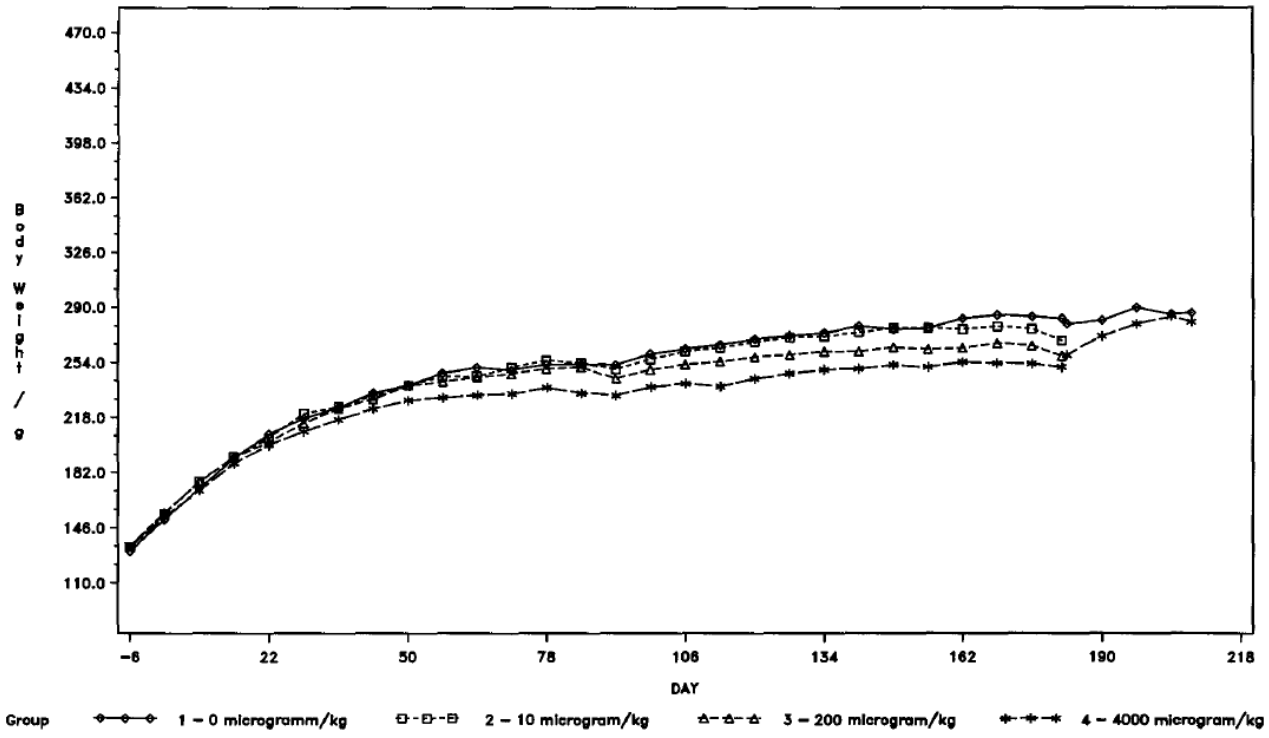
*p<0.05; F = female; M = male.

A dose-related decrease in body weight gain was noted for both males and females. The greatest effect for males occurred during the first week of treatment, after which body weight deficits remained mostly stable. For females, the effect was similar across all treatment weeks and gradually became more noteworthy by the end of the study. As shown in the sponsor-generated figures below, body weights rebounded during the treatment-free recovery period, with male weights exceeding control weights by the end of the study. Decreased body weight gain correlated with decreased food consumption and the increase in body weight gain during recovery correlated with increased food consumption.

Males

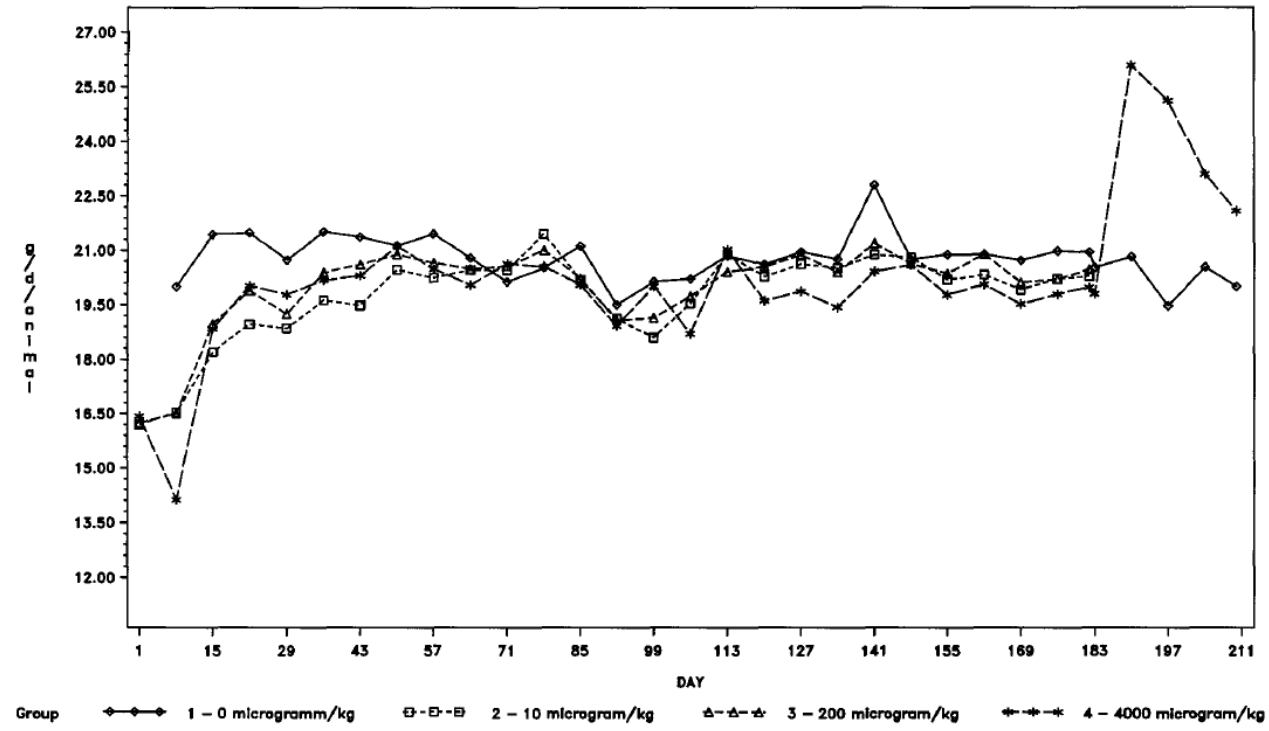


Females

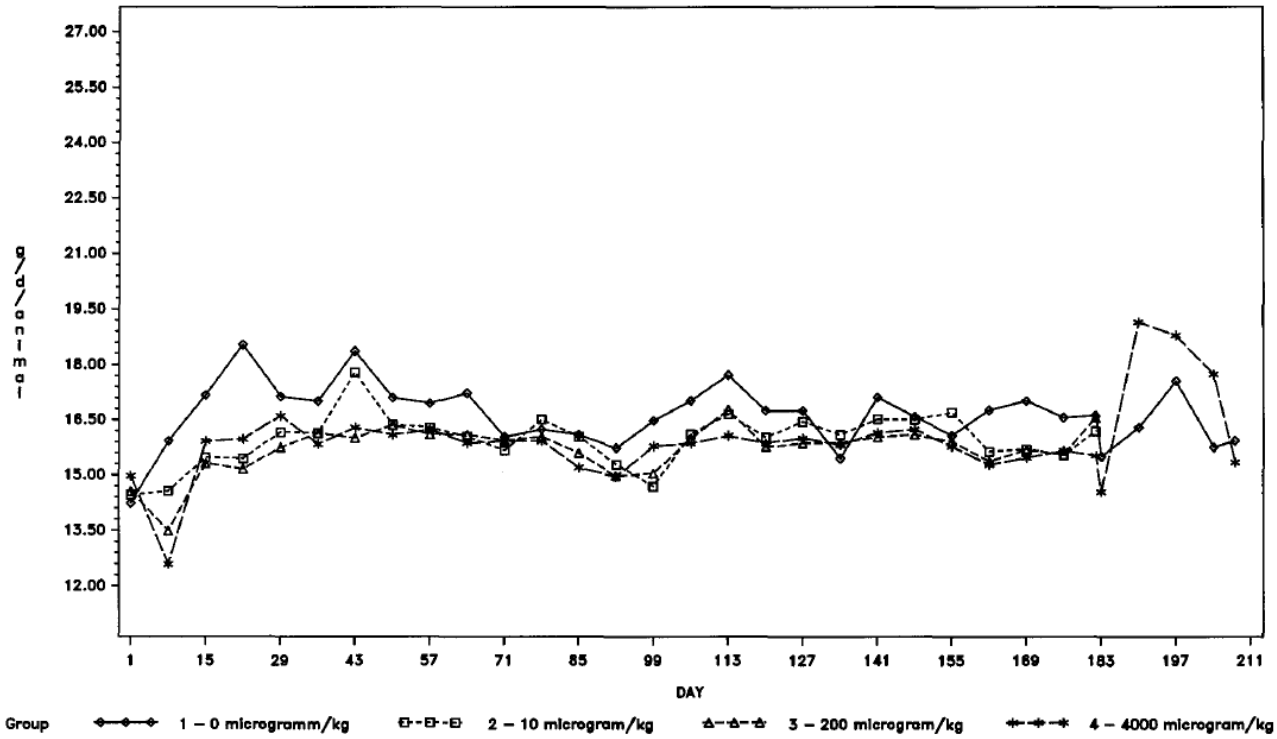


Feed Consumption: Measured once weekly

Males



Females



Ophthalmoscopy: Conducted before first treatment and during Week 12/13 (F/M).

There were no treatment-related findings observed during ophthalmic exams.

Hematology: Evaluated during Weeks 17 (non-fasted), 27 (non-fasted), and 30/31 (F/M; fasted).

Small, but statistically significant changes in some white blood cell parameters were observed either at the interim time point, at the end of the dosing period, or both. At the end of dosing, mean lymphocytes (absolute and percentage) in the HD male group were increased by 15%. These values were still higher than controls after the recovery period. In HD females, lymphocytes were increased by 12% (absolute) and 5% (percentage) at the end of the dosing period; values were similar to controls at the end of recovery. The observed changes in hematology parameters were not considered to be toxicologically meaningful.

Clinical Chemistry: Evaluated during Weeks 14 (non-fasted), 27 (non-fasted), and 30/31 (F/M; fasted).

Dose (µg/kg BID)	0		5		100		2000	
Sex	M	F	M	F	M	F	M	F
Total bilirubin (µmol/L)	10.43	-	10.57	-	9.29*	-	8.97* ↓14%	-
Triglycerides (mmol/L)	0.565	-	0.575	-	0.499	-	0.330* ↓42%	-
Glucose (mmol/L)	-	5.667	-	5.467	-	5.113*	-	4.860* ↓14%

F = female; M = male; "-" = no meaningful difference from control.

Urinalysis: Conducted in overnight metabolism cages during Weeks 14 (non-fasted), 27 (non-fasted), and 30/31 (F/M; fasted).

No toxicologically meaningful, treatment-related changes to urinalysis parameters were observed.

Gross Pathology: All main and recovery animals

The testes and/or epididymides of 4 HD males were considered to be small. The testicular and epididymal weights for these animals were lower than controls and other animals in this group. The seminal vesicles of one of these animals were also small.

Organ Weights: All main and recovery animals

In the HD group, 3 males had testicular weights less than 2.0 grams whereas the other animals in that group and in all other groups had testicular weights greater than 3.0 grams, with the exception of a single male from the control and LD groups (2.774 and 2.658 g, respectively). A similar trend was observed for these 3 animals with epididymal weights. These animals had the second, third, and seventh lowest body weights at the end of the treatment period for this treatment group. One of five recovery animals in the HD group had testicular weights of less than 2.0 grams (1.021 g), whereas all other animals from the HD and control recovery groups had testicular weights of greater than 3.0 grams. Because decreased nutritional status can delay sexual maturation, it is not clear whether the apparent treatment-related increased incidence in small testes was directly related to the test article or secondary to the effects on body weight and food consumption.

Histopathology: All main and recovery animals

Adequate Battery: Yes

Peer Review: Yes, all tissues from 2 control and HD animals/sex and testes and injection sites from all rats

Histological Findings (sponsor-generated table)

Sex		Males			
Dose Group		CO	D1	D2	D3
No. Animals per Dose Group		15	15	15	15
EPIDIDYMIS	No.Examined	15	15	15	15
– Infiltrate: lymphocyte	GRADE 1	–	–	–	2
	TOTAL AFFECTED	–	–	–	2
	MEAN SEVERITY	–	–	–	1.0
– Oligospermia	GRADE 2	–	–	–	1
	GRADE 3	–	1	–	1
	GRADE 4	–	–	–	1
	TOTAL AFFECTED	–	1	–	3
	MEAN SEVERITY	–	3.0	–	3.0
– Aspermia	GRADE 5	–	–	–	3
	TOTAL AFFECTED	–	–	–	3
	MEAN SEVERITY	–	–	–	5.0
– Granuloma: spermatic	GRADE 3	–	1	–	–
	GRADE 4	–	1	–	–
	TOTAL AFFECTED	–	2	–	–
	MEAN SEVERITY	–	3.5	–	–
SEMINAL VESICLE	No.Examined	15	–	1	15
– Atrophy	GRADE 3	–	–	–	1
	TOTAL AFFECTED	–	–	–	1
	MEAN SEVERITY	–	–	–	3.0
TESTIS	No.Examined	15	15	15	15
– Atrophy: seminiferous tubule	GRADE 1	1	1	–	–
	GRADE 2	1	–	–	1
	GRADE 3	–	–	1	–
	GRADE 4	–	1	–	2
	GRADE 5	–	–	–	2
	TOTAL AFFECTED	2	2	1	5
	MEAN SEVERITY	1.5	2.5	3.0	4.0
– Stasis: spermatid	GRADE 1	1	1	1	1
	GRADE 3	–	–	–	2
	TOTAL AFFECTED	1	1	1	3
	MEAN SEVERITY	1.0	1.0	1.0	2.3

Sex		Males			
Dose Group No. Animals per Dose Group		CO 15	D1 15	D2 15	D3 15
TESTIS	cont.d	15	15	15	15
– Mineralization	GRADE 1	–	–	1	1
	GRADE 3	–	–	–	2
	GRADE 4	–	–	–	1
	TOTAL AFFECTED	–	–	1	4
	MEAN SEVERITY	–	–	1.0	2.8
– Necrosis: seminiferous tubule	GRADE 5	–	–	–	1
	TOTAL AFFECTED	–	–	–	1
	MEAN SEVERITY	–	–	–	5.0
– Inflammation: chronic	GRADE 4	–	–	–	1
	TOTAL AFFECTED	–	–	–	1
	MEAN SEVERITY	–	–	–	4.0
INJECTION SITE	No.Examined	15	15	15	15
– Hemorrhage	GRADE 1	–	–	2	1
	GRADE 2	–	1	–	2
	TOTAL AFFECTED	–	1	2	3
	MEAN SEVERITY	–	2.0	1.0	1.7
– Reaction: fibroblastic	GRADE 2	1	1	6	6
	GRADE 3	–	1	2	2
	TOTAL AFFECTED	1	2	8	8
	MEAN SEVERITY	2.0	2.5	2.3	2.3
– Necrosis: panniculus muscle	GRADE 1	–	1	2	–
	TOTAL AFFECTED	–	1	2	–
	MEAN SEVERITY	–	1.0	1.0	–
– Abscess	GRADE 5	–	–	–	1
	TOTAL AFFECTED	–	–	–	1
	MEAN SEVERITY	–	–	–	5.0

Recovery – Males (sponsor-generated table)

SUMMARY INCIDENCE OF GRADINGS BY ORGAN/GROUP/SEX
Necropsy Status: RECOVERY PHASE EUTHANASIA (R1), Incl. Deaths

Sex		Males			
Dose Group		CO	D1	D2	D3
No. Animals per Dose Group		5	–	–	5
EPIDIDYMIS	No.Examined	5	–	–	5
– Infiltrate: lymphocyte	GRADE 1	1	–	–	–
	GRADE 2	–	–	–	1
	TOTAL AFFECTED	1	–	–	1
	MEAN SEVERITY	1.0	–	–	2.0
– Aspermia	GRADE 5	–	–	–	1
	TOTAL AFFECTED	–	–	–	1
	MEAN SEVERITY	–	–	–	5.0
INJECTION SITE	No.Examined	5	–	–	5
– Infiltrate: mononuclear cell	GRADE 1	–	–	–	1
	TOTAL AFFECTED	–	–	–	1
	MEAN SEVERITY	–	–	–	1.0
– Granuloma: foreign body	GRADE 1	3	–	–	1
	GRADE 2	–	–	–	1
	TOTAL AFFECTED	3	–	–	2
	MEAN SEVERITY	1.0	–	–	1.5

Toxicokinetics:

Samples for TK analysis were collected from the retrobulbar venous plexus on Days 1, 7, and 183 at 0.17, 0.33, 1, 3, 8, and 24 hours after the first daily dose (3 animals/sex/time point).

Summaries of TK results from Days 1, 7, and 183 are shown in the sponsor-generated tables below. Exposures increased with increasing duration of dosing, especially at the high dose. Between Day 1 and Day 183, mean $AUC_{0\text{-}24hr}$ values increased by approximately 2-fold, 30-fold, and 63-fold for the LD, MD, and HD animals. Across the different days and dose levels, females tended to have slightly lower exposures than males, although there was not a definitive gender difference.

I: Values following the two doses on Day 1					
Dose µg /kg BID	Gender	AVE0010			
		C_{max} [ng/mL]	t_{max} [hr]	C_{24hr} [ng/mL]	$AUC_{(0-24hr)}$ [ng*hr/mL]
5	M	4.70	0.17	< 0.050	8.77
	F	4.39	0.33	< 0.050	7.74
100	M	57.1	0.17	< 0.050	143
	F	57.2	0.33	< 0.050	110
2000	M	250	0.33	0.068	711
	F	386	0.33	0.089	697

II: Values following the two doses on Day 7					
Dose µg /kg BID	Gender	AVE0010			
		C_{max} [ng/mL]	t_{max} [hr]	C_{24hr} [ng/mL]	$AUC_{(0-24hr)}$ [ng*hr/mL]
5	M	5.22	0.17	< 0.050	10.5
	F	6.08	0.33	< 0.050	11.7
100	M	78.7	0.33	< 0.050	146
	F	68.1	0.33	< 0.050	132
2000	M	537	1.00	0.164	2815
	F	622	0.33	0.209	2298

III: Values following the two doses on Day 183					
Dose µg /kg BID	Gender	AVE0010			
		C_{max} [ng/mL]	t_{max} [hr]	C_{24hr} [ng/mL]	$AUC_{(0-24hr)}$ [ng*hr/mL]
5	M	6.42	0.17	0.11	15.7
	F	6.81	0.17	< 0.050	12.8
100	M	334	1.00	99.2	2735
	F	427	0.33	69.2	3914
2000	M	3303	0.17	1662	52597
	F	3301	0.17	2037	35563

Antibody analysis:

Samples were taken from all main and recovery animals for the measurement of anti-drug antibodies before treatment, on Days 7, 28, and 154 of treatment and at the end of the recovery period.

Pre-treatment

	male			female		
	negative	positive	total	negative	positive	total
Control	19	1	20	19	1	20
low dose	14	1	15	15	0	15
mid dose	15	0	15	15	0	15
top dose	14	1	15	15	0	15

Post-treatment (Day 7)

	male			female		
	negative	positive	total	negative	positive	total
Control	19	1	20	19	1	20
low dose	14	1	15	15	0	15
mid dose	15	0	15	15	0	15
top dose	13	2	15	15	0	15

Post-treatment (Day 28)

	male			female		
	negative	positive	total	negative	positive	total
Control	19	1	20	19	1	20
low dose	14	1	15	15	0	15
mid dose	0	15	15	0	15	15
top dose	0	20	20	0	20	20

Post-treatment (Day 154)

	male			female		
	negative	positive	total	negative	positive	total
Control	20	0	20	19	1	20
low dose	8	7	15	12	3	15
mid dose	0	15	15	0	15	15
top dose	0	20	20	2	17	19

Post-treatment (Day 210/208)

	male			female		
	negative	positive	total	negative	positive	total
Control	5	0	5	5	0	5
low dose						
mid dose						
top dose	0	5	5	0	4	4

Dosing Solution Analysis: Formulation samples were taken on Days 1, 7, 57/59 (F/M), 118/120 (F/M), 177/179 (F/M), 178/180 (F/M), and 183

Most samples were within the acceptable range of 90% to 110% of nominal concentration. Some samples from Day 1 and Day 7 were 117% to 127% of nominal concentration. These deviations did not affect the validity or integrity of the study.

Study title: 12-month twice a day (BID) subcutaneous toxicity study in dogs

Study number:	DSE 2004-0064, Amendment 1
Study report location:	Module 4.2.3.2
Conducting laboratory:	Sanofi-Aventis Deutschland GmbH, Drug Safety Evaluation (Frankfurt), Mainzer Landstrasse 500 Kastengrund, 65795 Hattersheim, Germany
Date of study initiation:	14 June 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, Lot # PPL-AVE100404A, and 87.0% pure

Key Study Findings

- No compound-related deaths occurred throughout the study.
- Mean BWs were lower in all treated groups. In males, BWs were dose-dependently reduced compared with control (significant changes at ≥200 µg/kg BID). In female dogs, mean BW was lower compared with control (significant changes at 1000 µg/kg BID), but was not dose dependent. Lower weights in treated animals correlated with lower FC.
- No compound-related changes were seen in clinical pathology, ECG parameters, or ophthalmoscopy.
- 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.
- Macroscopically, an increased incidence of a red focus/area at injection sites was noted.
- Microscopic findings were observed at injection sites (dose-related increase in inflammation and fibrosis at ≥200 µg/kg BID) and epididymis/testis (moderate to severe hypospermatogenesis in seminiferous tubules; epididymal dilation, degeneration, oligospermia or aspermia in testis at ≥200 µg/kg BID).
- 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.
- TK data showed significantly increased AUC values from Day 1 to 6 months (up to 12X), which was due to the anti-AVE0010 antibody formation.
- Based on the treatment-related effects on testes at ≥200 µg/kg BID, the NOAEL for males was considered to be 2 µg/kg BID. The NOAEL for systemic toxicity in females was 1000 µg/kg BID.

Methods

Species/Strain:	Dog/Beagle
Number/Sex/Group:	4
Satellite groups:	None
Dose Levels:	0, 2, 200, and 1000 µg/kg/dose
Dosing Volume:	0.5 mL/kg
Frequency of dosing:	Twice daily (2nd dose given 8 hours after the 1st dose)

Route of administration:	Subcutaneous injection
Formulation/Vehicle:	0.9% (w/v) sodium chloride aqueous solution
Age:	7 to 8 months
Weight:	8.3 to 9.1 kg (males) and 7.4 to 8.5 kg (females)
Unique study design:	To achieve the mid- and high-dose levels without inducing a severe degree of body weight loss, a dose escalation approach was taken in which doses were increased in small increments every 3 days until the desired dose level was reached. Mid-dose level escalation: 2, 5, 10, 20, 40, 70, 100, 125, 150, 175, and 200 µg/kg/dose BID; the definitive dose level was reached on Day 21. High-dose level escalation: 2, 5, 10, 20, 40, 70, 100, 125, 150, 175, 200, 225, 250, 275, 300, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, and 1000 µg/kg/dose BID; the definitive dose level was reached on Day 85.
Protocol deviations:	No unforeseen circumstances occurred that may have affected the quality or integrity of the study.
Reason for amendment:	A more in depth evaluation of testis and epididymis was conducted and the conclusions regarding a potential malnutrition effect on testis and epididymis were modified based on the 8-month juvenile toxicity study that implemented a pair-fed control group.

Observations and Results

Mortality: (evaluated daily)

One LD female was euthanized prematurely after 6 months of dosing due to an eating disorder. This death was not felt to be related to the test article.

Clinical Signs: (twice daily; sponsor-generated tables)

Males

Days 2-380

Group	Group 1	Group 2	Group 3	Group 4
Dose (BID)	0ug/kg	2ug/kg	200ug/k	1000ug/
Number of animals at the start of study	4	4	4	4
Number of animals at the end of observation period	4	4	4	4
Vomiting; Brown				
Number of animals with sign	2	4	4	4
Mean number of days with sign	7	4	3	12
Range of days seen	77-369	3-337	3-343	2-361
Vomiting; Green				
Number of animals with sign	0	0	1	1
Mean number of days with sign	-	-	1	1
Range of days seen	-	-	334-334	271-271
Vomiting; White				
Number of animals with sign	1	0	1	2
Mean number of days with sign	1	-	2	2
Range of days seen	271-271	-	31-54	20-355
Vomiting; Yellow				
Number of animals with sign	0	1	2	2
Mean number of days with sign	-	1	1	2
Range of days seen	-	333-333	319-320	23-307

Females

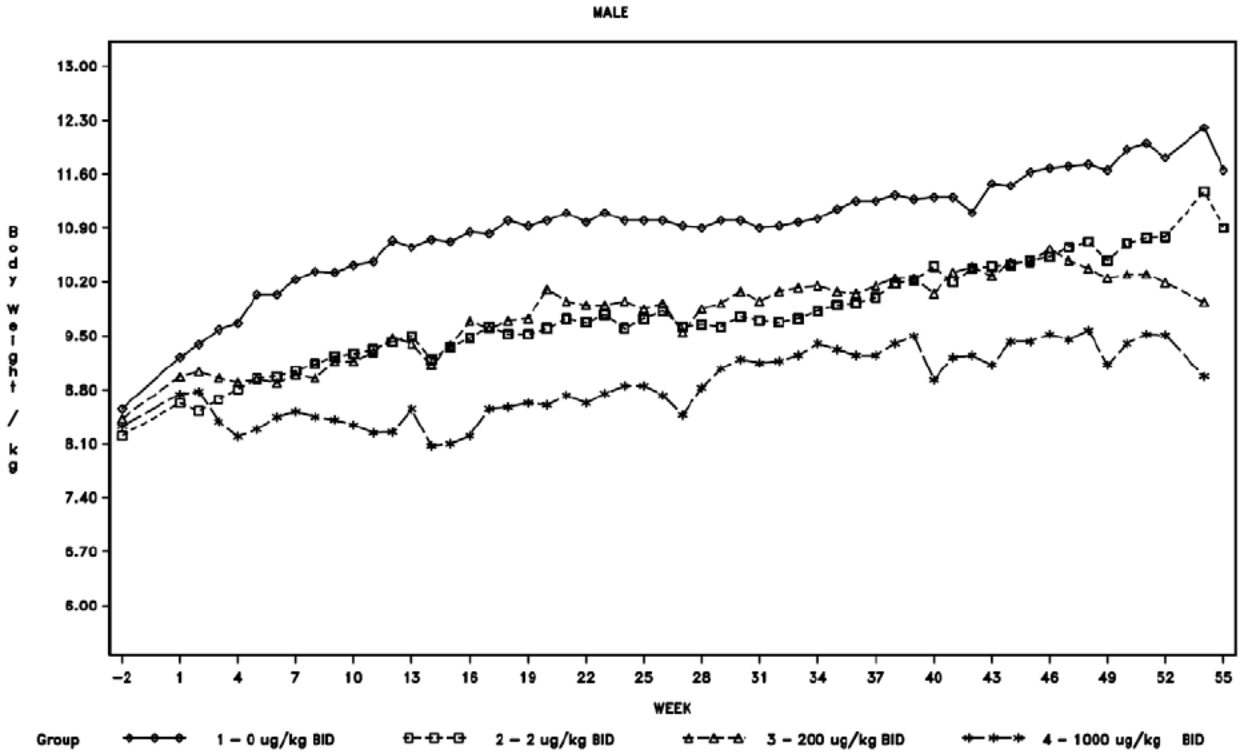
Days 2-380

Group	Group 1	Group 2	Group 3	Group 4
Dose (BID)	0ug/kg	2ug/kg	200ug/k	1000ug/
Number of animals at the start of study	4	5	4	4
Number of animals at the end of observation period	4	5	4	4
Vomiting; Brown				
Number of animals with sign	1	3	4	4
Mean number of days with sign	1	4	3	16
Range of days seen	14-14	14-342	19-343	11-371
Vomiting; Green				
Number of animals with sign	0	1	0	0
Mean number of days with sign	-	1	-	-
Range of days seen	-	313-313	-	-
Vomiting; White				
Number of animals with sign	0	1	1	3
Mean number of days with sign	-	1	1	1
Range of days seen	-	314-314	225-225	99-102

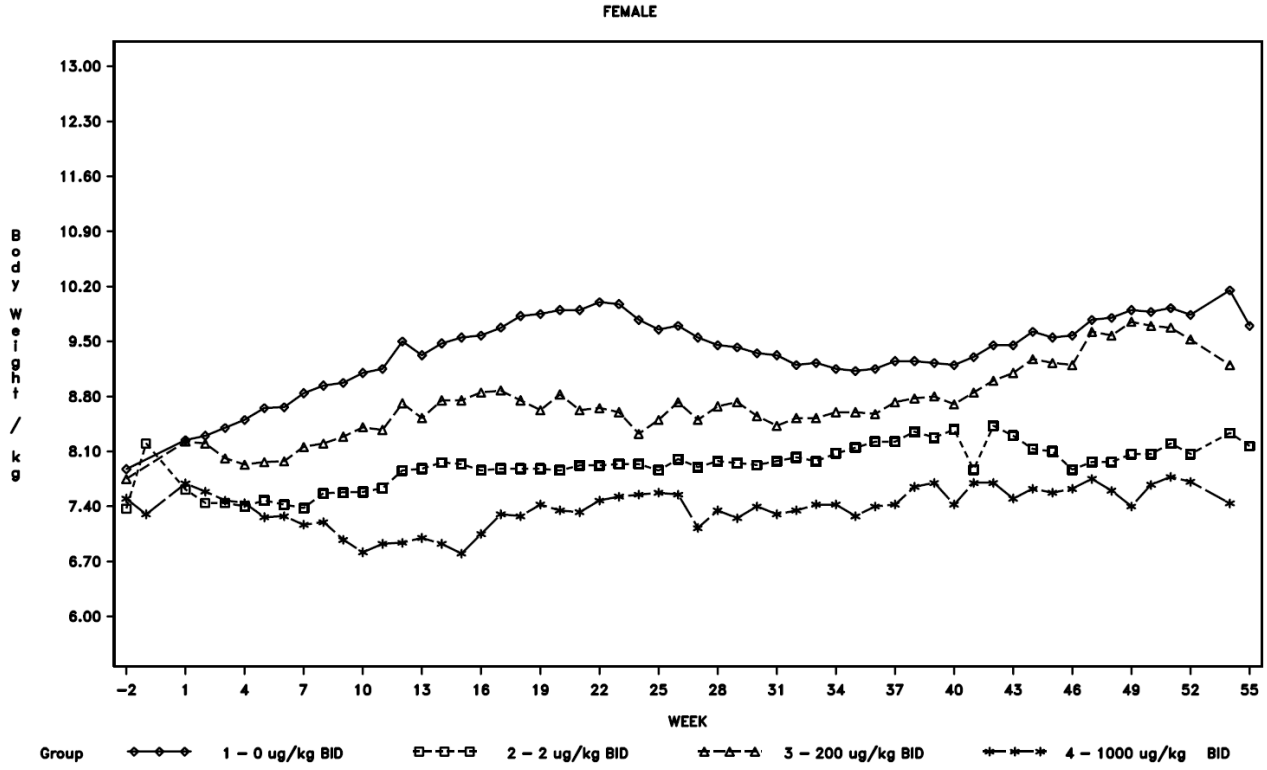
Body Weights: (every 3 days during dose escalation and once weekly thereafter)

Dose (µg/kg BID)	0		2		200		1000	
Sex	M	F	M	F	M	F	M	F
Weight (g) - Day 372	12.20	10.15	11.38	8.33	10.08*	9.25	9.10*	7.43*
Diff from control (g)			-0.82	-1.82	-2.12	-0.90	-3.1	-2.72
% diff from control			↓7%	↓18%	↓17%	↓9%	↓25%	↓27%

*p<0.05; F = female; M = male.



(sponsor-generated figure)



(sponsor-generated figure)

Feed Consumption: (daily)

(sponsor-generated table)

Table – Group Mean Food Consumption (FC)				
Group	**Dose level µg/kg BID**	**Gender**	**Absolute Food Consumption Day 1 to Day 377 [gram]**	**Relative FC* [%]**
1	0	Male	126,000	100
		Female	102,351	100
2	2	Male	122,156	97
		Female	100,836**	92**
3	200	Male	116,785	93
		Female	101,770	99
4	1000	Male	104,483	83
		Female	89,458	87

* Food consumption relative to concurrent control group
** n = 3 (females No 5265, No 5266, No 5267)

Ophthalmoscopy: (prior to start of dosing and Weeks 12, 26, 40, and 52)

No test article-related effects were noted.

ECG: (prior to start of dosing, and pre-dose and 2 hours after first daily dose (t_{max}) during Weeks 12, 26, 39, and 52)

No test article-related effects on ECG parameters were observed.

Hematology: (prior to start of dosing and Weeks 12, 26, 39, and 53)

There were no treatment-related effects on hematology or coagulation parameters.

Clinical Chemistry: (prior to start of dosing and Weeks 12, 26, 39, and 53)

There were no treatment-related effects on clinical chemistry parameters.

Urinalysis: (overnight collection prior to start of dosing and Weeks 12, 26, 39, and 53)

There were no treatment-related effects on urinalysis parameters.

Gross Pathology: (sponsor-generated table)

NUMBER OF ANIMALS WITH NECROPSY FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS

Sex	Males				Females			
Dose Group	C0	D1	D2	D3	C0	D1	D2	D3
Animals Examined	4	4	4	4	4	5	4	4
INJECTION SITE								
– focus/area, light/pale, red, focal.	–	–	1	–	–	–	–	–
– focus/area, light/pale, red.	–	–	1	–	–	–	–	–
– focus/area, red.	–	2	1	4	2	1	2	3

Group C0, control, males: AVE 0010 (0 µg/kg); females: AVE 0010 (0 µg/kg)
Group D1, 2 µg/kg BID, males: AVE 0010 (4 µg/kg); females: AVE 0010 (4 µg/kg)
Group D2, 200 µg/kg BID, males: AVE 0010 (400 µg/kg); females: AVE 0010 (400 µg/kg)
Group D3, 1000µg/kg BID, males: AVE 0010 (2000 µg/kg); females: AVE 0010 (2000 µg/kg)

Organ Weights

Dose (µg/kg BID)	0		2		200		1000	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Uterus (g)	NA	8.79	NA	6.69	NA	22.21 ↑153%	NA	18.53 ↑111%
(% body weight)	NA	0.089	NA	0.799	NA	0.235 ↑164%	NA	0.244 ↑175%
Ovary (g)	NA	1.02	NA	0.855	NA	1.29 ↑26%	NA	1.21 ↑19%
(% body weight)	NA	0.011	NA	0.010	NA	0.014 ↑30%	NA	0.016 ↑50%
Thymus (g)	-	4.40	-	4.54	-	5.23	-	2.03* ↓54%
(% body weight)	-	0.0455	-	0.0529	-	0.0567	-	0.0276 ↓39%

*p<0.05; "-" = no noteworthy difference from control; F = female; M = male; NA = not applicable.

Histopathology

Adequate Battery: Yes

Peer Review: Yes, conducted by board-certified pathologist from Sanofi-Aventis

Histological Findings: (sponsor-generated tables)

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL PHASE EUTHANASIA (K0), Incl. Deaths

Sex	Males				Females			
Dose Group	C0	D1	D2	D3	C0	D1	D2	D3
No. Animals per Dose Group	4	4	4	4	4	5	4	4
INJECTION SITE,LEFT No.Examined	4	4	4	4	4	5	4	4
– Infiltrate: mononuclear cell	3	3	4	3	1	2	3	3
Grade 1	1	1	–	–	–	1	1	–
Grade 2	2	2	1	–	–	–	–	1
Grade 3	–	–	1	1	–	1	1	–
Grade 4	–	–	2	2	1	–	1	2
– Infiltrate: mixed inflammatory cell	2	1	2	3	3	1	2	2
Grade 1	2	1	–	–	–	–	–	–
Grade 2	–	–	1	–	–	–	–	–
Grade 3	–	–	–	–	–	1	–	–
Grade 4	–	–	1	3	2	–	1	1
Grade 5	–	–	–	–	1	–	1	1
– Infiltrate: mononuclear	–	–	2	3	–	1	–	2
Grade 1	–	–	–	1	–	1	–	1
Grade 2	–	–	1	1	–	–	–	–
Grade 3	–	–	–	1	–	–	–	–
Grade 4	–	–	1	–	–	–	–	1
– Infiltrate: mixed inflammatory cell	–	–	2	2	–	1	1	2
Grade 1	–	–	–	–	–	1	–	–
Grade 2	–	–	2	1	–	–	–	1
Grade 3	–	–	–	1	–	–	1	1
– Fibrosis: panniculus muscle	–	–	–	1	–	–	–	–
Grade 3	–	–	–	1	–	–	–	–
– Regeneration: panniculus muscle	–	–	–	1	–	–	–	–
Grade 2	–	–	–	1	–	–	–	–
– Fibrosis: subcutaneous connective tissue	–	–	4	4	3	1	3	3
Grade 1	–	–	1	–	–	–	–	–
Grade 2	–	–	1	–	1	–	–	–
Grade 3	–	–	1	–	1	1	1	1
Grade 4	–	–	–	2	1	–	1	2
Grade 5	–	–	1	2	–	–	1	–
INJECTION SITE,LEFT cont.d	4	4	4	4	4	5	4	4
– Granuloma: foreign body giant cell	1	1	1	1	–	1	2	3
Grade 1	1	1	1	–	–	–	1	2
Grade 2	–	–	–	1	–	1	1	1
– Hemorrhage	–	–	2	1	1	–	1	–
Grade 1	–	–	1	–	–	–	–	–
Grade 3	–	–	1	1	–	–	–	–
Grade 4	–	–	–	–	1	–	1	–

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL PHASE EUTHANASIA (K0), Incl. Deaths

Sex	Males				Females			
Dose Group	C0	D1	D2	D3	C0	D1	D2	D3
No. Animals per Dose Group	4	4	4	4	4	5	4	4
INJECTION SITE,RIGHT No.Examined	4	4	4	4	4	5	4	4
– Infiltrate: mononuclear cell	4	4	4	3	2	4	4	4
Grade 1	1	2	1	–	1	4	–	–
Grade 2	2	2	1	–	–	–	1	–
Grade 3	1	–	2	1	–	–	–	1
Grade 4	–	–	–	2	1	–	3	3
– Infiltrate: mixed inflammatory cell	3	–	1	3	3	–	2	2
Grade 1	1	–	–	–	1	–	–	–
Grade 2	2	–	–	1	–	–	–	1
Grade 3	–	–	1	1	–	–	2	1
Grade 4	–	–	–	1	2	–	–	–
– Infiltrate: mononuclear cell	–	2	3	3	–	–	1	3
Grade 1	–	1	2	2	–	–	–	–
Grade 2	–	1	1	1	–	–	1	1
Grade 3	–	–	–	–	–	–	–	1
Grade 4	–	–	–	–	–	–	–	1
– Infiltrate: mixed inflammatory cell	–	–	–	1	–	–	–	1
Grade 1	–	–	–	–	–	–	–	1
Grade 2	–	–	–	1	–	–	–	–
– Regeneration: panniculus muscle	–	1	–	1	–	–	1	–
Grade 1	–	1	–	1	–	–	–	–
Grade 2	–	–	–	–	–	–	1	–
– Fibrosis: subcutaneous connective tissue	1	1	3	4	3	–	4	4
Grade 2	1	1	1	–	–	–	–	–
Grade 3	–	–	1	1	3	–	1	1
Grade 4	–	–	1	–	–	–	2	2
Grade 5	–	–	–	3	–	–	1	1
– Granuloma: foreign body giant cell	1	1	2	–	–	1	2	1
Grade 1	1	1	–	–	–	1	1	1
Grade 2	–	–	2	–	–	–	1	–
– Hemorrhage	–	–	1	4	1	–	2	2
Grade 1	–	–	–	–	–	–	1	1
Grade 2	–	–	–	1	–	–	–	1
Grade 3	–	–	1	2	1	–	1	–
Grade 4	–	–	–	1	–	–	–	–
SKIN/SUBCUTIS No.Examined	4	4	4	4	4	5	4	4
– Granuloma	–	–	–	–	–	–	–	1
Grade 2	–	–	–	–	–	–	–	1
PANCREAS No.Examined	4	4	4	4	4	5	4	4
– Infiltrate: mononuclear cell	–	–	–	1	–	–	–	–
Grade 1	–	–	–	1	–	–	–	–
THYROID GLAND No.Examined	4	4	4	4	4	5	4	4
– Hyperplasia: C-cell-focal	–	–	–	–	–	1	–	–
Grade 2	–	–	–	–	–	1	–	–

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL PHASE EUTHANASIA (K0), Incl. Deaths

Sex		Males				Females			
Dose Group		C0	D1	D2	D3	C0	D1	D2	D3
No. Animals per Dose Group		4	4	4	4	4	5	4	4
VAGINA	No.Examined	–	–	–	–	4	5	4	4
– Estrus cycle: proestrus		–	–	–	–	–	–	1	1
	Grade 3	–	–	–	–	–	–	1	1
– Estrus cycle: estrus		–	–	–	–	–	–	–	1
	Grade 3	–	–	–	–	–	–	–	1
– Estrus cycle: metestrus		–	–	–	–	2	1	3	1
	Grade 3	–	–	–	–	2	1	3	1
– Estrus cycle: diestrus		–	–	–	–	2	4	–	1
	Grade 3	–	–	–	–	2	4	–	1
– Cyst		–	–	–	–	1	–	–	–
	Grade 4	–	–	–	–	1	–	–	–
EPIDIDYMIS	No.Examined	4	4	4	4	–	–	–	–
– Oligospermia		–	–	4	4	–	–	–	–
	Grade 3	–	–	2	–	–	–	–	–
	Grade 4	–	–	1	3	–	–	–	–
	Grade 5	–	–	1	1	–	–	–	–
– Aspermia		–	–	1	1	–	–	–	–
	Grade 5	–	–	1	1	–	–	–	–
TESTIS	No.Examined	4	4	4	4	–	–	–	–
– Atrophy: seminiferous tubule		2	1	3	3	–	–	–	–
	Grade 1	2	1	1	–	–	–	–	–
	Grade 3	–	–	–	3	–	–	–	–
	Grade 4	–	–	2	–	–	–	–	–
– Vacuolation: tubule		3	–	2	3	–	–	–	–
	Grade 1	2	–	–	–	–	–	–	–
	Grade 2	1	–	1	–	–	–	–	–
	Grade 3	–	–	–	3	–	–	–	–
	Grade 4	–	–	1	–	–	–	–	–
– Stasis: spermatid		2	–	2	4	–	–	–	–
	Grade 1	2	–	2	–	–	–	–	–
	Grade 2	–	–	–	1	–	–	–	–
	Grade 3	–	–	–	3	–	–	–	–
– Hypospermatogenesis		3	4	4	4	–	–	–	–
	Grade 2	1	2	–	–	–	–	–	–
	Grade 3	2	2	2	1	–	–	–	–
	Grade 4	–	–	2	3	–	–	–	–
– Fibrosis: tubule		–	–	–	1	–	–	–	–
	Grade 2	–	–	–	1	–	–	–	–

Group C0, control, males: AVE 0010 (0 µg/kg); females: AVE 0010 (0 µg/kg)
Group D1, 2 µg/kg BID, males: AVE 0010 (4 µg/kg); females: AVE 0010 (4 µg/kg)
Group D2, 200 µg/kg BID, males: AVE 0010 (400 µg/kg); females: AVE 0010 (400 µg/kg)
Group D3, 1000µg/kg BID, males: AVE 0010 (2000 µg/kg); females: AVE 0010 (2000 µg/kg)

Special Evaluation: (Re-evaluation of testis and epididymis, reported in amended report)

NUMBER OF MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL PHASE EUTHANASIA (K0), Incl. Deaths

Sex		Males			
Dose Group		C0	D1	D2	D3
No. Animals per Dose Group		4	4	4	4
EPIDIDYMIS	No.Organs	8	8	8	8
– Oligospermia		–	–	5	6
	Grade 3	–	–	3	–
	Grade 4	–	–	1	5
	Grade 5	–	–	1	1
– Aspermia		–	–	1	1
	Grade 5	–	–	1	1
– Dilation: initial segment and efferent ducts		–	–	6	6
	Grade 2	–	–	2	1
	Grade 3	–	–	4	5
– Degeneration: epithelium of initial segment		–	–	4	7
	Grade 2	–	–	–	2
	Grade 3	–	–	4	5

Group C0, control, males: AVE 0010 (0 µg/kg)
Group D1, 2 µg/kg BID, males: AVE 0010 (4 µg/kg)
Group D2, 200 µg/kg BID, males: AVE 0010 (400 µg/kg)
Group D3, 1000µg/kg BID, males: AVE 0010 (2000 µg/kg)

Toxicokinetics: 0 (only Day 1), 0.5, 1, 2, 3, and 8 hours after the first daily dose and 0.5, 1, 2, 3, 8, and 16 hours after the second daily dose on Day 1 (Groups 1 and 2), Day 31 (Group 3), Day (Group 4), after 26 weeks of dosing at the definitive dose, and at the end of the dosing period (sponsor-generated table)

Sex	Dose (µg/kg/day)	Dose (µg/kg BID)	C_{max} (ng/mL)[a]			AUC_{0-24} (ng.h/mL)[a]		
			Day 1,31,85	Day 184,216, 268	Day 371,370, 370	Day 1,31,85	Day 184,216, 268	Day 371,370, 370
male	4	2	2.05	12.0	7.16	19.3	150	126
	400	200	217	2060	1300	2650	33300	25600
	2000	1000	17400	13500	7270	120000	247000	161000
female	4	2	1.95	3.01	3.25	18.6	24.7	21.7
	400	200	263	2060	1630	3230	31000	33800
	2000	1000	42300	3050	1950	339000	43700	33900

a Values are rounded to 3 significant figures.

Anti-Drug Antibody Analysis: Before the start of dosing, after 26 weeks of dosing at the definitive dose, and at the end of the dosing period.

Pre-treatment

	male			female		
	negative	positive	total	negative	positive	total
Control	4	0	4	4	0	4
low dose	3	1	4	4	0	4
mid dose	4	0	4	4	0	4
top dose	3	1	4	4	0	4

Mid term treatment (Day 184, 216, 268)

	male			female		
	negative	positive	total	negative	positive	total
Control	3	1	4	2	2	4
low dose	1	3	4	2	3	5
mid dose	0	4	4	0	4	4
top dose	0	4	4	0	4	4

End of treatment (Day 370, 371)

	male			female		
	negative	positive	total	negative	positive	total
Control	0	4	4	3	1	4
low dose	1	3	4	1	3	4
mid dose	0	4	4	0	4	4
top dose	0	4	4	0	4	4

Dosing Solution Analysis

Most dosing formulations prepared over the 12-month dosing period were found to be within the nominal concentration acceptance range of 90% to 110%. Therefore, results of the formulation analyses were found to be acceptable.

Histopathology Inventory for NDA #204961

Study / Species	6 Month Rat	1 Year Dog
Study Number	DSE 2005-0085	DSE 2004-0064
Adrenals	X*	X*
Aorta	X	X
Bone Marrow (in bone section)	X	X
Bone (femur with joint)	X	X
Brain	X*	X*
Cecum	X	X
Colon	X	X
Duodenum	X	X
Epididymides	X*	X*
Esophagus	X	X
Eyes	X	X
Gall bladder		X
Gross lesions	X	X
Harderian gland	X	
Heart	X*	X*
Ileum	X	X
Injection sites	X	X
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	X	X
Larynx	†	†
Liver	X*	X*
Lungs	X	X*
Lymph nodes, retropharyngeal		X
Lymph nodes, mand bular	X	
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity	†	†
Optic nerves	X	X
Ovaries	X*	X*
Oviducts	X	X
Pancreas	X	X
Peyer's patches		
Pharynx		
Pituitary	X*	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland – (sub)mandibular	X	X
Salivary gland - parotid	X	X
Salivary gland - sublingual	X	X
Sciatic nerve	X	X
Seminal vesicles (weighed w/ prostate)	X*	
Skeletal muscle (femoris - rat) (quadriceps and diaphragm - dog)	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X*	X
Sternum with bone marrow	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X	X*
Thyroid + parathyroid	X*	X*
Tongue	X	X
Trachea	X	X
Ureters	X	X
Urinary bladder	X	X
Uterus + cervix	X	X*
Vagina	X	X
Zymbal gland		

X, histopathology performed; *, organ weight obtained;
†tissues collected but not evaluated microscopically

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Reverse mutation in four histidine-requiring strains of *salmonella typhimurium* and one tryptophan-requiring strain of *escherichia coli*

Key findings:

- ZS42-0010 (AVE0010) did not induce mutations in the absence or presence of metabolic activation in any of the *S. typhimurium* or *E. coli* strains.

Study number: 2004-1183 (Sanofi); (b) (4)
Study type: *In vitro* bacterial mutagenesis (Ames test)
Location: Module 4.2.3.3.1
Conducting laboratory: (b) (4)
Date of study initiation: 01 September 2000
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: ZS42-0010 (AVE0010), batch # 1022/3330, 96.63% pure
Formulation/vehicle: white powder in solution for injection/vehicle 100 mM SPB pH 7.4

Methods:
Strains: *S. typhimurium*: TA98, TA100, TA1535, TA1537; *E. coli*: WP2 uvrA
Dose selection criteria:
Basis of dose selection: used limit dose of 5000 µg/mL for the definitive studies.
Range finding studies: not performed.
Test agent stability: no data.
Metabolic activation system: SD rat liver S9 fraction induced with Aroclor 1254
Controls: Vehicle: 100 mM sodium phosphate buffer (SPB)
Negative controls: SPB solution
Positive controls: as shown in the sponsor-generated table below:

Chemical S	ource	Stock * concentration (mg/mL)	Final concentration (µg/mL)	Use Strain(s) S-9	
2-nitrofluorene (2NF)	(b) (4)	0.5	25	TA98	!
4-nitroquinoline 1-oxide (NQO)		0.02	1	TA100	!
N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)		0.05 0.15	2.5 7.5	TA1535 WP2 uvrA	! !
ICR-191		0.02	1	TA1537	!
2-aminoanthracene (AAN)		0.8 0.2 0.14	20 5 3.5	WP2 uvrA TA1535 and TA98 TA100 and TA1537	+ + +

Study design:

Because AVE0010 is a peptide that contains the amino acids histidine and tryptophan, a "treat and plate" method was used instead of the standard plate incorporation or preincubation test. The "treat and plate" method is similar to the preincubation method except the bacterial cells are centrifuged after the incubation period so that the test article can be removed before the bacterial are added to the molten soft agar. Two independent experiments were conducted.

Exposure conditions:

Incubation and sampling times: incubation was performed with 0.1 ml of a dilution of test article or control treatment and followed by the addition of 0.5 ml of 10% S9 mix or buffer solution for 60 min at 37±1°C. Cells were centrifuged to remove the treatment mixture, rinsed in 100 mM phosphate buffer, and then resuspended in 100 mM phosphate buffer. The cells were added to 2 mL molten agar and then poured onto the surface of a Vogel-Bonner E agar plate. After agar was added, the plates were incubated at 37°C for 2 to 3 days.

Doses used in definitive study:

Experiment 1: 0, 1.6, 8, 40, 200, 1000 and 5000 µg/mL
Experiment 2: 156.25, 312.5, 625, 1250, 2500 and 5000 µg/mL

Analysis:

Number of replicates: triplicate plates/dose
Counting method: an automatic colony counter (Seescan plc) or manual count
Criteria for a valid assay:

The mean negative control counts were comparable with the normal range.
The positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains, and an active S-9 preparation.
No more than 5% of the plates were lost through contamination or some other unforeseen event.

Criteria for positive results:

The assay meets the acceptance criteria
The data show a significant dose correlation in induced mutant frequency
The positive response is reproducible

Summary of individual study findings:

Study validity: A limit dose of 5000 µg/plate was used as the maximum dose; the test compound was completely soluble. The solvent and positive controls gave results within the historical control data ranges. The study was considered to be valid.

Study outcome: Slight toxicity (slight thinning of the background lawn) was only observed at the highest concentration in Experiment 2. AVE0010 was judged to be negative in the Ames test as it did not induce mutation in the absence or the presence of metabolic activation in any of the *S. typhimurium* or *E. coli* strains.

Study title: AVE0010: Bacterial reverse mutation test

Key findings:

- This study was conducted to qualify a new clinical batch of test material and was conducted by the "treat and plate" method as described in the study above. Therefore, this study will only be briefly described.
- AVE0010 did not result in relevant or dose-dependent increases in the number of revertants in any of the bacterial strains either in the presence or absence of metabolic activation.

Study no: 2004-1342 (Sanofi); PT04-0239 (test lab number)
Study type: *In vitro* bacterial mutagenesis (Ames test)
Location: Module 4.2.3.3.1
Conducting laboratory: Aventis Pharma Deutschland, GmbH, Mainzer Landstrasse 500, D-65795 Hattersheim, Germany
Date of study initiation: 01 September 2004
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: AVE0010, batch # PPL-AVE100402A, and purity 85.8% (as is, no correction)

Study title: AVE0010: Bacterial reverse mutation test

Key findings:

- This study was conducted to qualify a new clinical batch of test material and was conducted by the "treat and plate" method as described in the study above. Therefore, this study will only be briefly described.
- AVE0010 did not result in relevant or dose-dependent increases in the number of revertants in any of the bacterial strains either in the presence or absence of metabolic activation.

Study no: 2005-0234 (Sanofi)
Study type: *In vitro* bacterial mutagenesis (Ames test)
Location: Module 4.2.3.3.1
Conducting laboratory: Aventis Pharma Deutschland, GmbH, Mainzer Landstrasse 500, D-65795 Hattersheim, Germany
Date of study initiation: March 18, 2005
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: AVE0010, batch # A003363205A, and purity 88.3% as is, 98.4% with correction factor

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In vitro Mammalian Chromosome Aberration Test in Human Lymphocytes

Key Study Findings

- Under the conditions of this study, AVE0010 showed no evidence of clastogenicity.

Study number:	maf0100
Study report location:	Module 4.2.3.3.1
Conducting laboratory:	(b) (4)
Date of study initiation:	06 August 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ZP10A (AVE0010), Batch (b) (4), 94.04% pure

Methods

Cell line:	Human peripheral lymphocytes
Concentrations in definitive study:	Test 1 -S9: 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50 µM +S9: 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50 µM Test 2 -S9: 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, and 50 µM +S9: 3.13, 6.25, 12.5, 25, and 50 µM
Basis of concentration selection:	Debris on slides at higher doses
Negative control:	Sterile water
Positive control:	Mitomycin C (-S9), Cyclophosphamide (+S9)
Formulation/Vehicle:	Sterile water
Metabolic activation:	Aroclor 1254-treated male SD rats
Incubation & sampling time:	Test 1 -S9: 3 hour treatment plus 17 hour incubation +S9: 3 hour treatment plus 17 hour incubation Test 2 -S9: 20 hour treatment and incubation +S9: 3 hour treatment plus 17 hour incubation

Study Validity

The study was considered to be valid if the negative and positive control values were within the current historical control range.

The test substance was considered to cause a positive response if the following conditions were met:

- Statistically significant increase in the frequency of metaphase spreads with aberrant chromosomes (excluding gaps) at one or more test concentration.

- The increases exceed the negative control range of the laboratory, taken at the 99% confidence limit.
- The increases were reproducible between replicate cultures.
- The increases were not associated with large changes in osmolality of the treatment medium or extreme toxicity.
- Evidence of a dose-relationship was considered to support the conclusion.

Results

Test 1

In the absence of S9, AVE0010 caused a reduction in the mitotic indices by 62% and 41% of the control values at 25 and 50 µM, respectively. However, these levels could not be selected for the metaphase analysis due to AVE0010 causing clotting, with resulting debris on the slides. Therefore, the next three lowest concentrations were selected for scoring. In the presence of S9, the mitotic index was 76% of the control value at 50 µM.

No increase in polyploidy was observed. Treatment with ZP10A did not cause a statistically significant increase in the proportion of cells with chromosomal aberrations at any dose, either with or without metabolic activation. Both positive control compounds caused large, statistically significant increases in the proportion of aberrant cells.

Test 2

In the absence of S9, treatment with AVE0010 did not result in a reduction in the mitotic index at any dose level. However, due to debris on the slides, the three highest concentrations could not be scored. In the presence of S9, treatment with AVE0010 did not result in a reduction in the mitotic index at any dose level.

No increase in polyploidy was observed. Treatment with AVE0010 did not cause a statistically significant increase in the proportion of cells with chromosomal aberrations at any dose, either with or without metabolic activation. Both positive control compounds caused large, statistically significant increases in the proportion of aberrant cells.

Study title: Chromosome Aberration Test in Human Lymphocytes in vitro

Key Study Findings

- This study was conducted to qualify a new clinical batch of test material; therefore, this study will only be briefly described.
- Under the conditions of this study, AVE0010 showed no evidence of clastogenicity.

Study number:	2004-1343; (b) (4) #S 4859 11
Study report location:	Module 4.2.3.3.1
Conducting laboratory:	(b) (4)
Date of study initiation:	10 August 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, Batch PPL-AVE100402A, 98.3% pure ((b) (4))

Study title: Chromosome Aberration Test in Human Lymphocytes in vitro with AVE0010

Key Study Findings

- This study was conducted to qualify a new clinical batch of test material; therefore, this study will only be briefly described.
- Under the conditions of this study, AVE0010 showed no evidence of clastogenicity.

Study number:	2005-0386; (b) (4) #881900
Study report location:	Module 4.2.3.3.1
Conducting laboratory:	(b) (4)
Date of study initiation:	07 April 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, Batch PPL-AVE100404B, 98.4% pure ((b) (4))

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mouse Micronucleus Test

Study no:	Mut0212
Study report location:	Module 4.2.3.3.2
Conducting laboratory:	(b) (4)
Date of study initiation:	15 August 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ZP10A (AVE0010), Batch (b) (4), 94% pure

Key Study Findings

- Under the conditions of this study, AVE0010 did not show any evidence of causing chromosome damage or bone marrow toxicity after a single intravenous injection.

Methods

Doses in definitive study:	0, 1.25, 2.5. and 5 mg/kg
Frequency of dosing:	Once
Route of administration:	Intravenous
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.9% NaCl, sodium citrate pH 5.3
Species/Strain:	Mouse / CD-1 (males only)
Number/Sex/Group:	7 males/group – 24 hour time point 7 males/group – 48 hour time point (vehicle control and HD only) 5 males/group (positive con) – 24 hour time point
Satellite groups:	None
Basis of dose selection:	Preliminary micronucleus test
Negative control:	0.9% NaCl, sodium citrate pH 5.3
Positive control:	12 mg/kg Mitomycin C, orally

Study Validity

The criteria for the study to be considered valid were not discussed in the report.

A response was considered to be positive for genotoxicity if a statistically significant, dose-related increase in the incidence of micronucleated immature erythrocytes was observed compared with the concurrent control group; individual and/or group means should exceed the laboratory historical control group. The assay was considered to be negative for genotoxicity when individual and group mean incidences were not statistically significantly greater than the concurrent control group.

Results

2000 immature erythrocytes from each animal were evaluated for the presence of micronuclei. The proportion of immature erythrocytes was determined to assess for bone marrow toxicity by examining at least 1000 erythrocytes from each animal.

There were no mortalities or clinical reactions to treatment. Incidences of weight loss were noted for all AVE0010-treated animals at 24 hours. No weight loss was observed for the 5 mg/kg AVE0010 group at the 48 hour sampling time.

AVE0010 did not induce an increase in the number of micronucleated immature erythrocytes 24 or 48 hours after treatment, whereas the positive control induced a robust increase in micronucleated immature erythrocytes. AVE0010 also did not cause an increase in the number of micronucleated mature erythrocytes. AVE0010 or mitomycin C did not induce a significant decrease in the proportion of immature erythrocytes.

Therefore, under the conditions of this study, AVE0010 did not show any evidence of causing chromosome damage or bone marrow toxicity after a single intravenous injection.

8 Carcinogenicity

Study title: Subcutaneous carcinogenicity study in mice, Amendment 1

Study number:	CAR0085
Study report location:	Module 4.2.3.4.1
Conducting laboratory:	Sanofi-Aventis Deutschland GmbH, Mainzer Landstrasse 500, Hattersheim, Germany
Date of study initiation:	Not stated in study report
First day of dosing:	19 Sep 2006 (males), 20 Sep 2006 (females)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010 #PPL-AVE100501A (until 31 Oct 2007) - 98.5% to 100.5% (b) (4) pure #B002 (from 01 Nov 2007 to 19 Aug 2008) - 100.8% #B004 (from 20 Aug 2008 to study end) - 98.6%
CAC concurrence:	The Committee conditionally concurred with the sponsor's proposed doses, which were based on the AUC exposure ratio in mice relative to that in humans. Concurrence was contingent upon demonstration that the anti-AVE0010 antibodies are not neutralizing in rodents and that the clinical dose does not change such that the ratio is no longer greater than 25-fold. Otherwise the 2-year studies may be considered to be inadequate. Development of a bioassay in a rodent species is recommended to address the neutralization as well as determination of cross reactivity of anti-AVE0010 antibodies to endogenous GLP-1 in human and rodent.

Key Study Findings

- There were no treatment-related effects on mortality. There were no noteworthy differences in deaths due to tumors or non-tumor causes between control and treated animals.
- Treatment-related clinical signs included an increased incidence in distended abdomens for all treated groups and a slight increase in corneal opacity for high-dose males and females.
- A dose-related increase in body weight gain from Week 1 to Week 100 was noted for treated groups compared with control groups.
- Macroscopically, an increased number of gall bladders from treated animals were noted as large, especially females. Microscopically, this observation was noted as dilation with no other treatment-related microscopic change. The sponsor noted that the increased gall bladder size was not considered compound related because gall bladder size was very variable in all groups, including controls.

- **Thyroid c-cell adenomas** were noted in 1, 1, and 4 male animals from the 40, 200, and 1000 µg/kg BID groups compared with 0 and 0 from 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.
- A slight increase in **Harderian gland adenomas** was noted for high-dose males (6 vs. 2 each for the two control groups), but the increase was not statistically significant for trend (pair-wise analytical data were not presented in the sponsor's statistical report). 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. Exposures after 6 months in this study were 3-4 times higher than those observed after 3 months in the 3-month range-finding study. This difference was likely due to anti-drug antibody (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 NOEL for thyroid c-cell adenomas was determined to be 200 µg/kg BID for males and 1,000 µg/kg BID for females. Exposures at the male and female NOELs are approximately 272X and 5,000X higher than the anticipated clinical exposure of 20 µg/day. The uterine adenocarcinomas appear incidental because statistical significance was only found for the mid-dose group when compared to the combined control group data.

Adequacy of Carcinogenicity Study

The design of the study was conditionally approved by the ECAC prior to study initiation. The ECAC noted that it should be determined whether ADAs had neutralizing activity; however the sponsor stated that a receptor binding test was not performed to investigate neutralization. The test article did appear to be pharmacologically active throughout the study because a statistically significant increase in thyroid c-cell adenomas was noted for high-dose males and the development of GLP-1 receptor agonist-induced c-cell tumors is believed to occur through a pharmacodynamic mechanism. Because the high-dose was based on a 25-fold clinical exposure margin rather than a maximum tolerated dose or maximum feasible dose, the ECAC also noted

that the study may not be considered adequate if the exposure margin fell below 25 times. Based on available clinical PK data, the mean exposure at the high dose in this study is approximately 500-fold higher than the exposure at the highest anticipated clinical dose, based on BID clinical dosing. Therefore, the carcinogenicity study was considered to be adequate.

Appropriateness of Test Model

The test model was found to be appropriate.

Evaluation of Tumor Findings

A summary of tumors that showed a numerical trend for increased incidence in treated animals compared with controls is shown in the sponsor-generated tables, which include statistical results. **A statistically significant increase in thyroid c-cell adenomas was observed for high-dose males**. Statistical analysis for Harderian gland adenomas in males did not indicate that these tumors were treatment related. The increase in uterine adenocarcinomas was only statistically significant at the mid-dose when using combined control group data (statistical analyses were not verified by our stats group; ECAC members did not feel that an internal statistical analysis was needed).

Table 3 - CAR0085 - Individual tumor incidence analysis - M Significant findings

Tumor by tissue/organ	Group Dose(µg/kg/BID)	1 0	2 0	3 40	4 200	5 1000
THYROID GLAND	Examined tissues	58	59	59	59	60
Adenoma: C-cell	Non lethal tumors	0	0	1	1	4
	Treated vs. Dual	**0.0042**		0.1524	0.1524	**0.0039**
	Treated vs. Ctrl1	**0.0152**		0.3406	0.3406	**0.0143**
	Treated vs. Ctrl2		**0.0177**	0.3720	0.3720	**0.0166**

The p-values under the controls are from upper-tailed Peto trend tests.
The p-values under each treated group are from upper-tailed Peto pairwise comparisons to the control.
Significant p-values are presented in bold at 2.5% level for trend tests and 5% level for pairwise comparisons.
: difference between the two controls is statistically significant at 5% level.
23 Nov 2009, 8:37

Table 4 - CAR0085 - Individual tumor incidence analysis - F Significant findings

Tumor by tissue/organ	Group Dose(µg/kg/BID)	1 0	2 0	3 40	4 200	5 1000
UTERUS	Examined tissues	59	60	60	60	60
Adenocarcinoma: endometrium	Non lethal tumors	0	0	0	3	1
	Lethal tumors	0	0	0	0	1
	Treated vs. Dual	**0.0250**		0.3951	**0.0224**	0.0857
	Treated vs. Ctrl1	0.0686		0.6478	0.0615	0.2035
	Treated vs. Ctrl2		0.0642	0.6300	0.0577	0.1919

The p-values under the controls are from upper-tailed Peto trend tests.
The p-values under each treated group are from upper-tailed Peto pairwise comparisons to the control.
Significant p-values are presented in bold at 2.5% level for trend tests and 5% level for pairwise comparisons.
: difference between the two controls is statistically significant at 5% level.
23 Nov 2009, 8:37

Table 5 - CAR0085 - Individual tumor incidence analysis - M No significant findings for appendix (continued)

Tumor by tissue/organ	Group Dose(µg/kg/BID)	1 0	2 0	3 40	4 200	5 1000
HARDERIAN GLAND	Examined tissues	60	60	58	59	60
Adenocarcinoma	Non lethal tumors	1	0	1	0	1
	Treated vs. Dual	0.4669				
	Treated vs. Ctrl1	0.6576				
	Treated vs. Ctrl2		0.3608			
Adenoma	Non lethal tumors	2	2	4	5	6
	Treated vs. Dual	0.0280				
	Treated vs. Ctrl1	0.0652				
	Treated vs. Ctrl2		0.0770			

Table 6 - CAR0085 - Combined tumor incidence analysis - M No significant findings for appendix

Tumor by tissue/organ	Group Dose(µg/kg/BID)	1 0	2 0	3 40	4 200	5 1000
HARDERIAN GLAND	Examined tissues	60	60	58	59	60
Adenoma/Adenocarcinoma	Non lethal tumors	3	2	5	5	7
	Treated vs. Dual	0.0292				
	Treated vs. Ctrl1	0.0913				
	Treated vs. Ctrl2		0.0562			

Methods

Doses and number/sex/group: see sponsor-generated table below

Dosages and number of animals per group

Group	Dosage (µg/kg) BID	Main study animals			Antibody animals			Toxicokinetic animals		
		No. / sex	Animal numbers		No. / sex	Animal numbers		No. / sex	Animal numbers	
			Male	Female		Male	Female		Male	Female
1	0	60	1-60	85-144	6	61-66	145-150	18	67-84	151-168
2	0	60	169–228	253–312	6	229-234	313-318	18	235-252	319-336
3	40	60	337–396	460–519	12	397-408	520-531	51	409-459	532-582
4	200	60	583–642	706–765	12	643-654	766-777	51	655-705	778-828
5	1000	60	829-888	952-1011	12	889-900	1012-1023	51	901-951	1024-1074

Frequency of dosing: Twice daily (approximately 8 hours apart)
Dose volume: 2.5 mL/kg
Route of administration: Subcutaneous
Formulation/Vehicle: AVE0010 in 50 mM sodium citrate, pH 5.3, then diluted to appropriate concentrations by adding 0.9% sodium chloride
Basis of dose selection: 3-month range-finding study
Species/Strain: Mouse/CD-1
Age: 6 to 7 weeks
Body weight: 24.3 - 34.1 g (males) and 19.4 - 30.6 (females)
Animal housing: Housed singly in Macrolon or transparent Polysulfon cages on soft wood granulate with standard room environment conditions
Paradigm for dietary restriction: None: Sniff R/M-H (V1534) ad libitum (standard diet)
Dual control employed: Yes
Interim sacrifice: No
Satellite groups: Yes (see above table)
Reason for amendment: Reporting of immunohistochemistry data from thyroid C-cell foci to assess for signs of proliferation (Ki-67)
Deviation from study protocol: No noteworthy deviations

Observations and Results

Mortality

Mortality - Males

Dose (µg/kg BID)	Vehicle Control 1	Vehicle Control 2	40	200	1000
Study Week	**Number of Deaths**				
0 - 50	2 (3.3%)	6 (10.0%)	6 (10.0%)	2 (3.3%)	8 (13.3%)
51 - 80	11 (18.3%)	10 (16.7%)	12 (20.0%)	16 (26.7%)	11 (18.3%)
81 - terminal sacrifice	14 (23.3%)	14 (23.3%)	13 (21.7%)	14 (23.3%)	13 (21.7%)
Total Early Deaths	27/60 (45.0%)	30/60 (50.0%)	31/60 (51.7%)	32/60 (53.3%)	32/60 (53.3%)
Surviving animals at terminal sacrifice	33/60 (55.0%)	30/60 (50.0%)	29/60 (48.3%)	28/60 (46.7%)	28/60 (46.7%)
Treated vs. pooled controls	p = 0.2089				
Treated vs. control 1	p = 0.1766				
Treated vs. control 2		p = 0.5744			
Summary of Animal Disposition					
Euthanized *in extremis*	1	2	3	3	2
Found dead	26	28	28	29	30
Terminal necropsy	33	30	29	28	28
Tumor-Related Deaths					
Total Early Deaths	27	30	31	32	32
Hemolymphoreticular system: lymphoma, malignant	4	4	2	1	6
Hemolymphoreticular system: sarcoma, histiocytic	1	-	1	2	1
Liver - hepatocellular carcinoma	-	1	1	-	1
Liver: hemangiosarcoma	-	-	-	-	1
Lung: bronchioloalviolar carcinoma	1	1	-	3	1
Trachea - carcinoma, bronchioloalviolar	-	-	-	-	1
Cecum: sarcoma	1	-	-	-	-
Bladder: transitional cell carcinoma	1	-	-	-	-

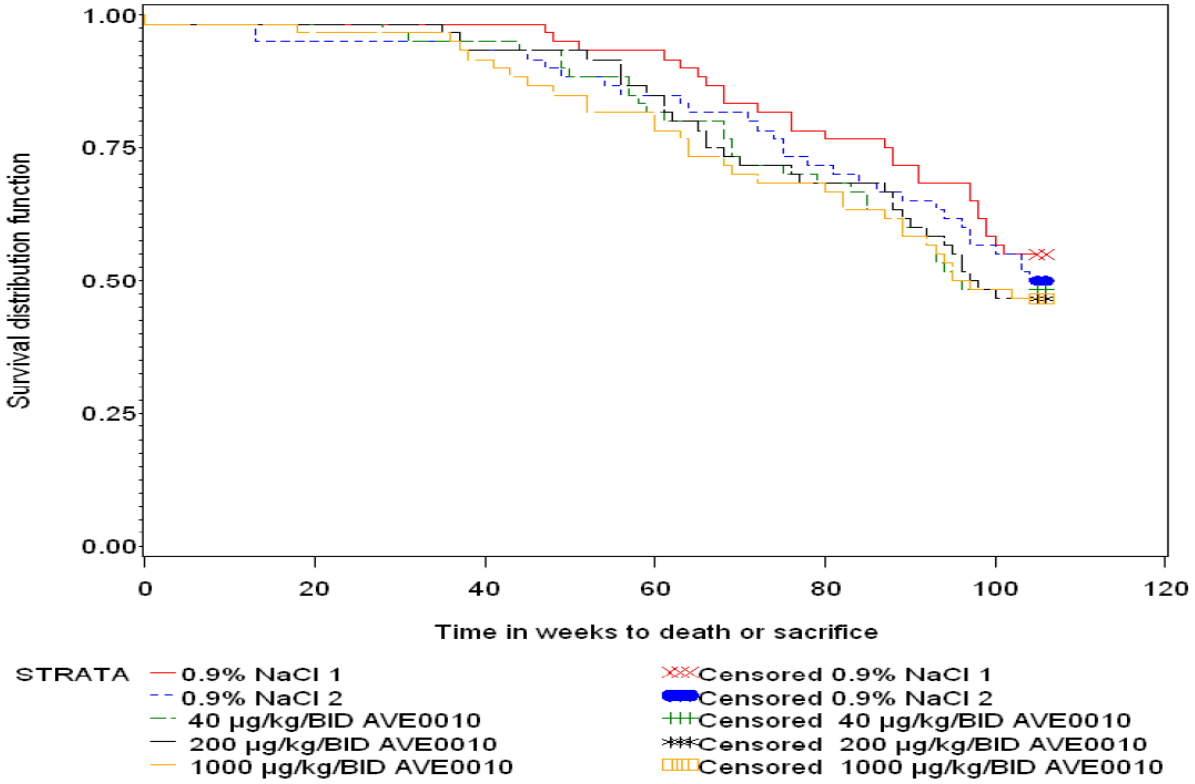
Dose (µg/kg BID)	Vehicle Control 1	Vehicle Control 2	40	200	1000
Non-tumor Related Deaths					
Amyloidosis, generalized	5	6	12	10	4
Body cavity - hemorrhage	-	-	-	-	1
Cecum - chronic inflammation	-	-	1	-	-
Heart - chronic inflammation	-	1	-	-	-
Heart - thromboembolus	-	1	-	-	-
Injection site - acute reaction	-	-	-	1	-
Kidney - nephropathy	1	1	1	-	-
Kidney - suppurative inflammation / necrosis	-	-	-	2	-
Kidney / pancreas - arteritis/periarteritis	-	1	-	-	-
Peritoneum - chronic inflammation	-	1	-	-	-
Prostate gland and/or **seminal vesicle** - suppurative inflammation	1	-	1	-	1
Skin/subcutis - chronic / suppurative inflammation	-	1	-	1	1
Not Evident	12	12	12	12	14

Mortality - Females

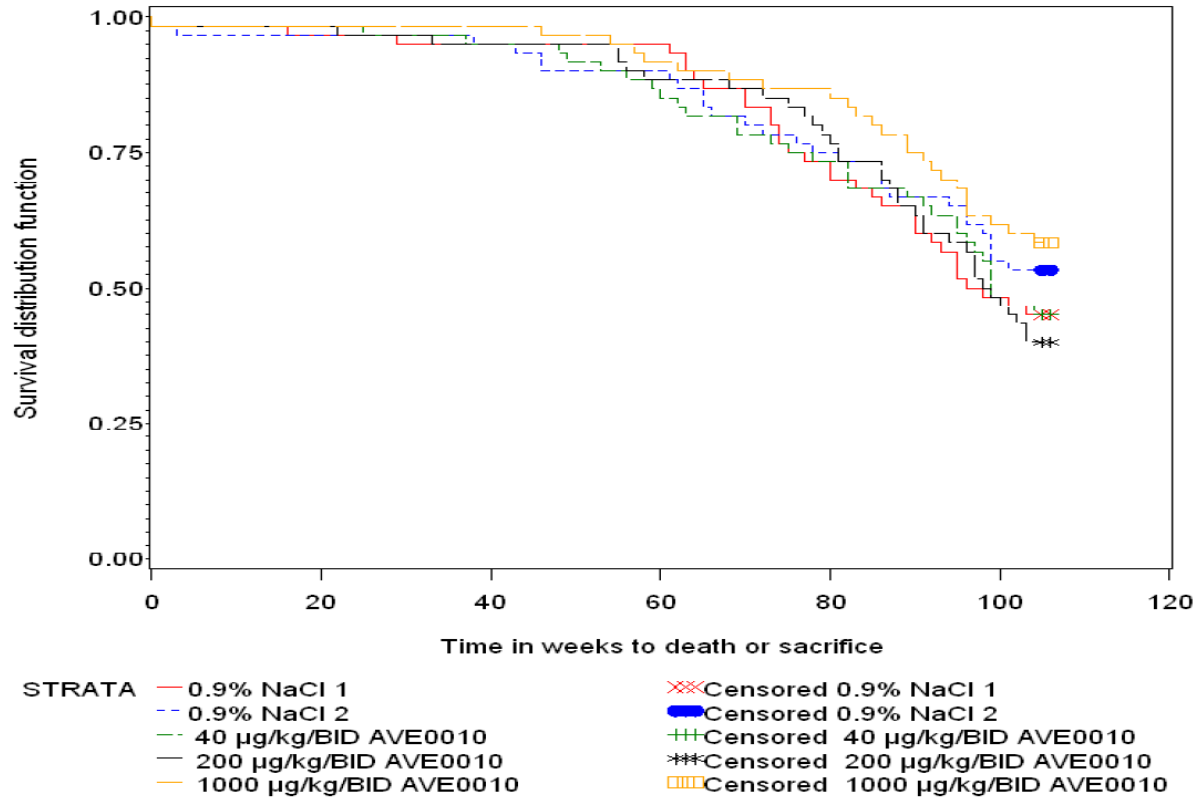
Dose (µg/kg BID)	Vehicle Control 1	Vehicle Control 2	40	200	1000
Study Week	**Number of Deaths**				
0 - 50	2 (3.3%)	4 (6.7%)	4 (6.7%)	2 (3.3%)	1 (1.7%)
51 - 80	14 (23.3%)	10 (16.7%)	11 (18.3%)	11 (18.3%)	7 (11.7%)
81 - terminal sacrifice	17 (28.3%)	14 (23.3%)	18 (30.0%)	23 (38.3%)	17 (28.3%)
Total Early Deaths	33/60 (55.0%)	28/60 (46.7%)	33/60 (55.0%)	36/60 (60.0%)	25/60 (41.7%)
Surviving animals at terminal sacrifice	27/60 (45.0%)	32/60 (53.3%)	27/60 (45.0%)	24/60 (40.0%)	35/60 (58.3%)
Treated vs. pooled controls	p = 0.5410				
Treated vs. control 1	p = 0.2213				
Treated vs. control 2		p = 0.5946			
Summary of Animal Disposition					
Euthanized *in extremis*	9	7	7	12	6
Found dead	24	21	26	24	19
Terminal necropsy	27	32	27	24	35
Tumor-Related Deaths					
Total Early Deaths	33	28	33	36	25
Hemolymphoreticular system: Lymphoma, malignant	15	6	11	12	2
Hemolymphoreticular system: Sarcoma, histiocytic	3	3	2	4	-
Kidney: fibrosarcoma	-	-	1	-	-
Liver: hemangiosarcoma	1	-	-	-	-
Lung: Bronchioloalviolar carcinoma	2	1	-	1	-
Mammary gland: adenocarcinoma	-	1	-	-	-
Ovary: malignant teratoma	-	-	-	1	-
Skin: keratoacanthoma	-	1	-	-	-
Spleen: hemangiosarcoma	1	-	-	-	-
Stomach - glandular: malignant neuroendocrine	-	-	-	-	1
Stomach -nonglandular: squamous cell carcinoma	-	-	1	-	-

Uterus: endometrial adenocarcinoma	-	-	-	-	1
Uterus: hemangioma	-	1	-	-	2
Uterus: hemangiosarcoma	-	1	1	-	-
Uterus: leiomyosarcoma	-	-	1	2	-
Uterus - cervix: hemangiosarcoma	-	-	1	-	-
Non-tumor Related Deaths					
Amyloidosis, generalized	6	6	5	8	8
Body cavity - hemorrhage	-	2	-	-	1
Heart - granulation tissue	-	-	1	-	-
Injection site - acute reaction	-	-	1	-	-
Kidney - nephropathy	-	1	2	-	-
Kidney - suppurative / chronic inflammation and/or necrosis	-	-	1	1	-
Lung - interstitial inflammation	-	-	-	-	1
Ovary - hemorrhage / abscess	-	-	-	1	2
Urinary bladder - inflammation	-	-	-	-	1
Uterus - hemorrhage / suppurative inflammation	-	-	-	-	2
Not Evident	5	5	5	6	4

Males



Females



Clinical Signs

(sponsor-generated table)

Text table 5 – Summary of main clinical signs

Daily Dose (µg/kg BID)	0 / 0*		40		200		1000	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Clinical Observations [number of animals with sign]								
Abdomen distended [C]	2 / 5	7 / 2	7	11	11	24	23	27
Cornea, epithelial opacity [A]	3 / 3	2 / 2	5	4	5	2	6	9
Coat unkempt [A]	3 / 4	10 / 4	9	10	6	8	9	6
Cold to touch [A]	1 / 0	6 / 5	6	5	4	9	3	7
Paleness; whole body [A]	4 / 2	9 / 7	6	9	2	12	3	11
Respiration laboured [A]	0 / 0	9 /5	2	8	3	10	1	5
Hair loss; neck dorsal [T]	2/ 0	1 / 2	1	3	1	5	0	3
Scab; neck dorsal [T]	6 / 8	2 / 6	5	6	3	7	4	5
Humane euthanasia	1 / 2	8 / 8	2	7	1	12	1	6
Moribund euthanasia	0 / 0	1 / 0	1	0	3	0	1	0

* Non-pooled controls 1 and 2 = 2 x 60 control mice/sex, compared to 60 treated mice/sex and group;
[T] = secondary to treatment procedures; [A] = age-related; [C] _ = test-article related;

Body Weights

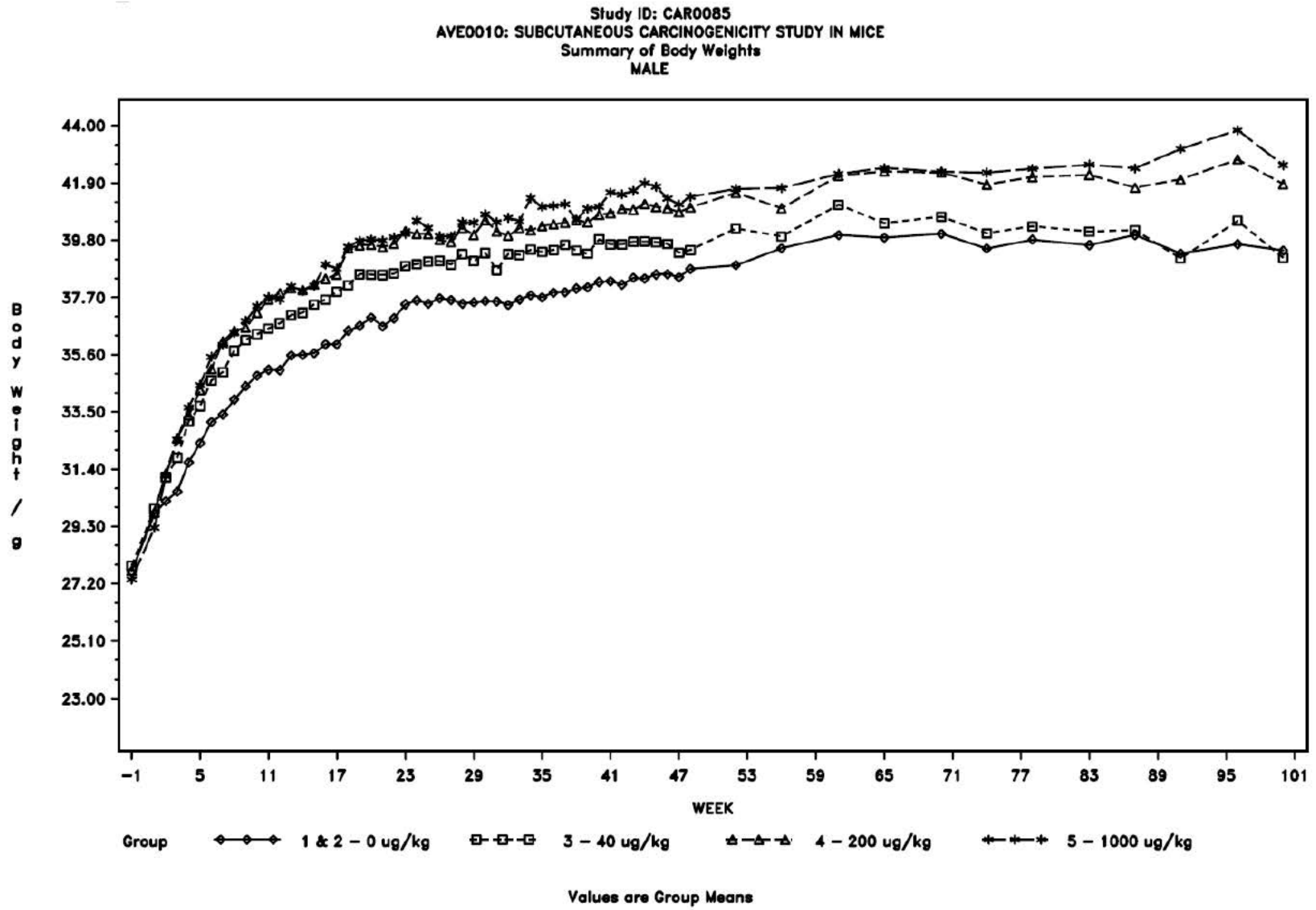
(sponsor-generated tables and figures)

Text table 6 – Effects on body weight development in male mice

Daily dose (µg/kg BID); Males	0 °	40	200	1000
Absolute Body Weight [g] & (%)[a]				
Week 1	29.77	+1	0	-2
Week 8	33.96	+5*	+7*	+7*
Week 13	35.58	+4*	+7*	+7*
Week 26	37.68	+4*	+6*	+6*
Week 52	38.89	+3*	+7*	+7*
Week 78	39.83	+1	+6*	+7*
Week 100	39.42	-1	+6*	+8*
Absolute body weight gain [g]; (%)[a]				
Week 1 to 2	0.46	+152*	+189*	+313*
Week 1 to 13	5.80	+23*	+42*	+53*
Week 1 to 26	7.90	+15*	+27*	+35*
Week 1 to 52	9.16	+12*	+27*	+34*
Week 1 to 78	10.06	+3	+23*	+30*
Week 1 to 100	9.96	-10	+23*	+33*

Text table 7 – Effects on body weight development in female mice

Daily dose (µg/kg BID); Females	0 °	40	200	1000
Absolute Body Weight [g] & (%)[a]				
Week 1	24.37	+1	-1	-2
Week 8	28.11	+5*	+7*	+9*
Week 13	29.32	+6*	+7*	+10*
Week 26	30.77	+7*	+9*	+8*
Week 52	32.37	+5*	+6*	+8*
Week 78	33.82	+2	+7*	+7*
Week 100	34.55	+4	+4	+7*
Absolute body weight gain [g]; (%)a				
Week 1 to 2	0.53	+75*	+193*	+205*
Week 1 to 13	4.98	+30*	+47*	+70*
Week 1 to 26	6.37	+31*	+48*	+47*
Week 1 to 52	7.99	+17*	+29*	+38*
Week 1 to 78	9.43	+7	+31*	+30*
Week 1 to 100	10.01	+13	+18*	+30*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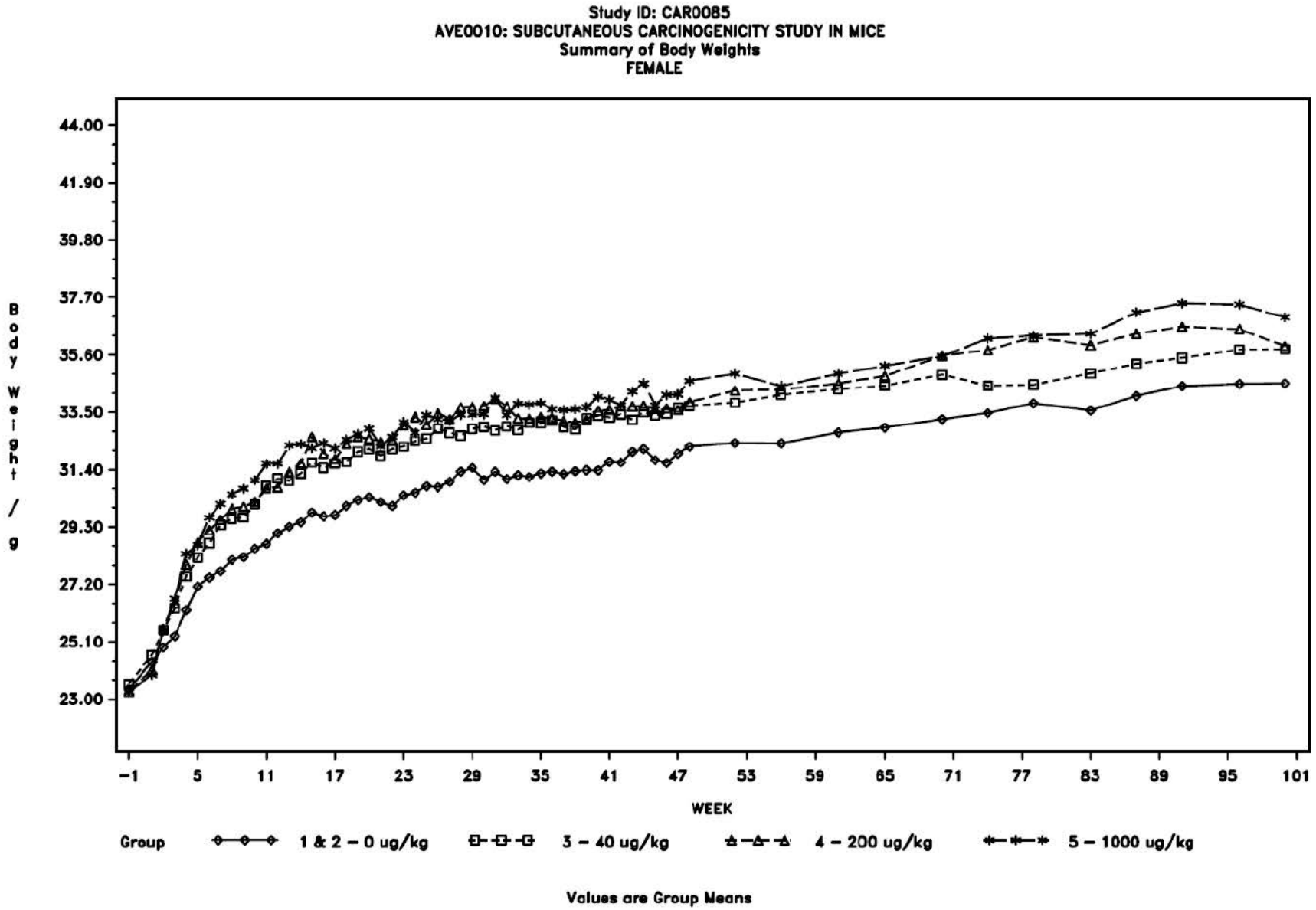
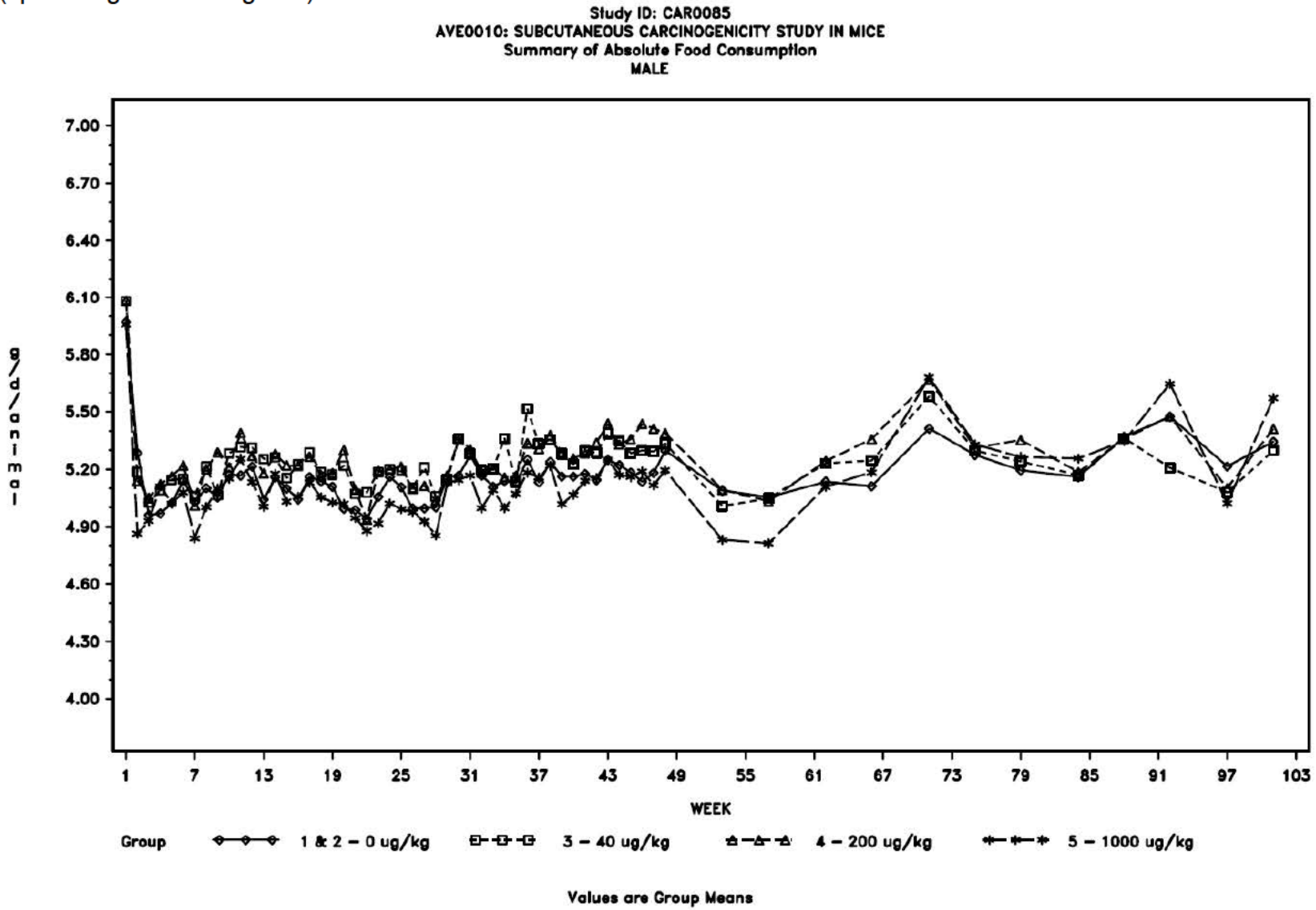
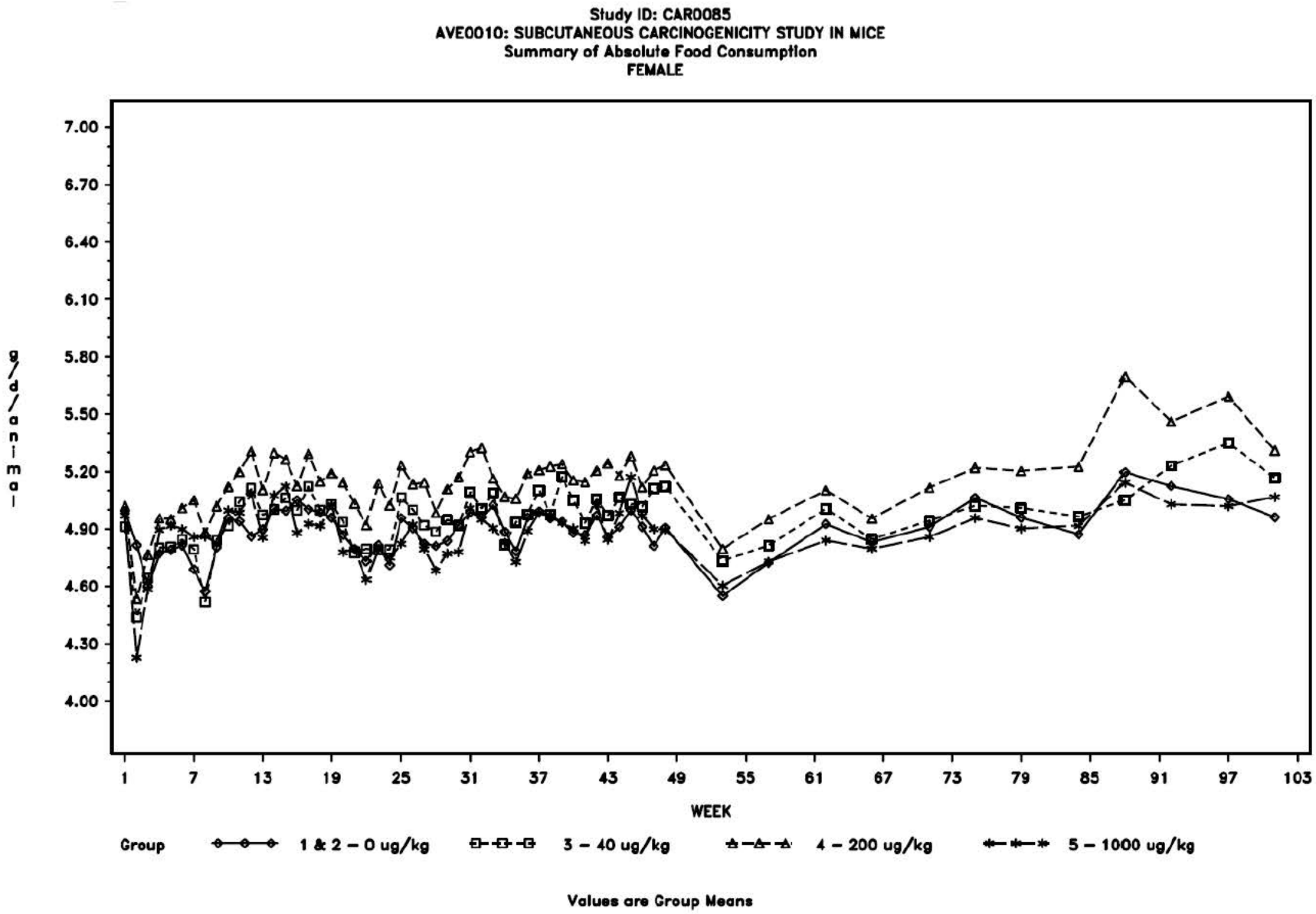
AVE0010: SUBCUTANEOUS CARCINOGENICITY STUDY IN MICE
Summary of Body Weights
MALE
Body Weight / g
44.00
41.90
39.80
37.70
35.60
33.50
31.40
29.30
27.20
25.10
23.00
-1
5
11
17
23
29
35
41
47
53
59
65
71
77
83
89
95
101
WEEK
Group
1 & 2 – 0 ug/kg
3 – 40 ug/kg
4 – 200 ug/kg
5 – 1000 ug/kg
Values are Group Means


Study ID: CAR0085
AVE0010: SUBCUTANEOUS CARCINOGENICITY STUDY IN MICE
Summary of Body Weights
FEMALE
Body Weight / g
44.00
41.90
39.80
37.70
35.60
33.50
31.40
29.30
27.20
25.10
23.00
-1
5
11
17
23
29
35
41
47
53
59
65
71
77
83
89
95
101
WEEK
Group
1 & 2 – 0 ug/kg
3 – 40 ug/kg
4 – 200 ug/kg
5 – 1000 ug/kg
Values are Group Means

Feed Consumption

(sponsor-generated figures)





Gross Pathology

Gender	Males					Females				
Dose (µg/kg BID)	Con 1	Con 2	40	200	1000	Con 1	Con 2	40	200	1000
Gall Bladder - Large	1	1	**5**	**5**	**4**	4	3	**16**	**16**	**24**
Thyroid Gland - Large	0	0	0	0	0	0	0	0	0	**1**

Histopathology

Peer Review: A pathology peer review was performed by Dr (b) (4). However, the peer review statement is located in the test facility archives rather than being included in the final study report.

Neoplastic

Gender	Males					Females				
Dose (µg/kg BID)	**Con 1**	**Con 2**	**40**	**200**	**1000**	**Con 1**	**Con 2**	**40**	**200**	**1000**
Thyroid Gland -										
C-cell Adenoma	0	0	**1**	**1**	**4**	0	0	0	0	**1**
C-cell hyperplasia	1	3	3	**8**	**4**	4	2	3	2	**11**
-Grade 1	1	2	2	6	1	3	2	2	2	2
-Grade 2	-	1	1	2	3	1	-	1	-	6
-Grade 3	-	-	-	-	-	-	-	-	-	2
-Grade 4	-	-	-	-	-	-	-	-	-	1
Harderian Gland										
-Adenoma	2	2	4	5	**6**	2	4	1	1	4
-Adenocarcinoma	1	0	1	0	1	1	-	1	-	-
-Total	3	2	5	5	**7**	3	4	2	1	4
Uterus - endometrium	NA	NA	NA	NA	NA					
Adenocarcinoma						0	0	0	**3**	**2**
Uterus - cervix	NA	NA	NA	NA	NA					
Squamous cell papilloma						0	0	0	**1**	**1**
Squamous cell hyperplasia						8	15	6	12	**24**
-Grade 1						-	1	1	1	1
-Grade 2						6	8	4	11	21
-Grade 3						2	6	-	-	2
-Grade 4						-	-	1	-	-
Squamous cell metaplasia						6	10	7	13	**15**
-Grade 1						-	-	2	4	4
-Grade 2						3	9	3	6	6
-Grade 3						3	1	2	3	5

Non Neoplastic

Gender	Males					Females				
Dose (µg/kg BID)	**Con 1**	**Con 2**	**40**	**200**	**1000**	**Con 1**	**Con 2**	**40**	**200**	**1000**
Adrenal Gland -medulla hyperplasia, focal	2	1	1	1	2	0	0	1	1	**3**
-Grade 1	-	-	-	-	1	-	-	-	-	-
-Grade 2	1	1	1	1	1	-	-	1	1	1
-Grade 3	1	-	-	-	-	-	-	-	-	2
Bone - Sternum fibro-osseous lesion	0	0	0	0	1	2	3	3	**7**	**7**
-Grade1	-	-	-	-	-	1	2	1	2	1
-Grade 2	-	-	-	-	-	1	1	2	3	6
-Grade 3	-	-	-	-	1	-	-	-	2	-
Gall Bladder Hyperplasia - epithelial cell, focal	1	0	0	1	1	1	1	0	0	**4**
-Grade 1	-	-	-	-	-	-	-	-	-	1
-Grade 2	1	-	-	1	1	1	1	-	-	3
Hyperplasia - epithelial cell, diffuse	0	1	0	1	2	0	0	**2**	**2**	**4**
-Grade 1	-	-	-	-	-	-	-	-	1	1
-Grade 2	-	1	-	1	1	-	-	2	-	3
-Grade 3	-	-	-	-	1	-	-	-	1	-

Gender	Males					Females				
Dose (µg/kg BID)	**Con 1**	**Con 2**	**40**	**200**	**1000**	**Con 1**	**Con 2**	**40**	**200**	**1000**
Hematopoiesis - extramedullary										
Liver	0	6	2	1	1	3	5	3	4	**11**
-Grade 1	-	1	-	-	-	1	2	2	2	3
-Grade 2	-	5	1	1	-	1	3	1	2	5
-Grade 3	-	-	1	-	1	1	-	-	-	3
Lymph node - mesenteric	0	0	0	0	0	0	0	**1**	**3**	**2**
-Grade 1	-	-	-	-	-	-	-	-	-	1
-Grade 2	-	-	-	-	-	-	-	-	1	1
-Grade 3	-	-	-	-	-	-	-	-	2	-
-Grade 4	-	-	-	-	-	-	-	1	-	-
Liver - hepatocyte hypertrophy, diffuse	0	0	0	**2**	**4**	0	0	0	0	0
-Grade 1	-	-	-	-	3	-	-	-	-	-
-Grade 2	-	-	-	1	1	-	-	-	-	-
-Grade 3	-	-	-	1	-	-	-	-	-	-
Liver - single cell necrosis	1	0	0	2	**3**	0	1	0	1	0
-Grade 1	-	-	-	1	3	-	1	-	-	-
-Grade 2	1	-	-	1	-	-	-	-	1	-
Pancreas - acinar cell hyperplasia	0	1	0	**3**	**3**	0	0	0	1	0
-Grade 1	-	-	-	1	-	-	-	-	-	-
-Grade 2	-	1	-	2	3	-	-	-	1	-
Pancreas - acinar cell degeneration -Grade 1	0	0	0	**2**	**1**	0	1	0	0	0

Gender	Males					Females				
Dose (µg/kg BID)	**Con 1**	**Con 2**	**40**	**200**	**1000**	**Con 1**	**Con 2**	**40**	**200**	**1000**
Parathyroid - mononuclear cell infiltrate	4	0	0	0	0	2	6	2	2	**11**
-Grade 1	3	-	-	-	-	-	2	1	1	3
-Grade 2	1	-	-	-	-	2	4	1	1	8
Parotid Gland - focus, basophilic hypertrophic	3	0	**57**	**56**	**53**	3	3	**57**	**58**	**58**
-Grade 1	3	-	4	2	4	1	3	2	2	3
-Grade 2	-	-	23	14	17	1	-	8	15	22
-Grade 3	-	-	17	22	9	1	-	15	10	8
-Grade 4	-	-	11	17	19	-	-	28	25	16
-Grade 5	-	-	2	1	4	-	-	4	6	9
Parotid Gland - mononucelar cell infiltrate	3	3	1	0	**6**	4	7	2	4	5
-Grade 1	2	1	1	-	4	1	6	2	3	2
-Grade 2	1	2	-	-	2	3	1	-	1	3
Preputial Gland - squamous cell hyperplasia -Grade 4	0	0	0	0	**1**	NA	NA	NA	NA	NA
Seminal Vesicle - abscess	0	0	0	**1**	**2**	NA	NA	NA	NA	NA
-Grade 3	-	-	-	-	2					
-Grade 4	-	-	-	1	-					

Immunohistochemistry

Animals from control and high-dose groups with minimal to moderate C-cell hyperplasia were selected for further microscopic investigation. Double staining was performed for calcitonin, to identify C-cells, and Ki-67, a marker for cell proliferation. Due to the deeper level of the cut of the paraffin-imbedded tissue for the new slides for immunohistochemistry, minimal to moderate hyperplastic foci were not present in the deeper levels of the thyroid gland. Only 3/10 animals showed hyperplastic foci in the next level. Ki-67-stained cells were not identified in these hyperplastic C-cell foci. A conclusion regarding the proliferative status of C-cells between control and treated animals could not be made from this examination because there were only 3 animals from the high-dose group available for evaluation of focal C-cell hyperplasia. A summary of the immunohistochemistry findings is presented in the sponsor-generated table below.

Table 1 - Immunhistochemical evaluation of thyroid gland

	Control					AVE0010				
	C1			C2		D3				
Animal No.	37	134	142	172	297	870	885	955	962	1010
Focal hyperplasia (original diagnosis)										
Grade	(1	(1	(1	(2	(1	(2	(2	2	(3	(3
IHC										
Focal hyperplasia	-	-	-	-	-	(P	(P	-	P	-
Ki67 positive C-cells	-	-	-	-	-	-	-	-	-	-

IHC = Immunhistochemistry; P = present; - = not present; (= unilateral finding;
Grade 1 = minimal; Grade 2 = mild; Grade 3 = moderate

Dose group: C1 = Control BID, C2 = Control BID; D3 = 1000 µg/kg BID AVE0010 (BID = twice daily)

Toxicokinetics

Exposure increased in a greater than dose-proportional manner. Exposures increased 23X from 40 to 200 µg/kg BID (5X increase in dose), 21X from 200 to 1000 µg/kg BID (5X increase in dose), and 480X from 40 to 1000 µg/kg BID (25X increase in dose). Exposures after 6 months in this study were 3-4X higher than the exposures noted in the 3-month range-finding study at 200 and 1000 µg/kg BID, respectively. Even though a majority of mice from the 3-month study were antibody positive, these data suggest that antibody positive animals may not have reached an exposure threshold by 3 months. The data suggest that the presence of anti-AVE0010 antibodies increases AUC values (see antibody results below). Note that because the antibody sample and TK sample measurements came from different subgroups, it is not possible to determine an exact correlation between antibody status and exposure.

Species	Sex	Dose (µg/kg BID)	Dose (µg/kg/day)	Cmax (ng/mL)[a] Day 176	AUC0-24h (ng.h/mL)[a] Day 176
Mouse (Crl CD-1)	male	40	80	44.5	67.3
		200	400	1050	1970
		1000	2000	4540	20000
	female	40	80	33.7	51.2
		200	400	349	706
		1000	2000	5700	36700

a Values are rounded to 3 significant figures.

Anti-AVE0010 Antibody Analysis

Table 1 - Numbers of the two categories of antibody status in the plasma samples

Day 185

	male			female		
	negative	positive	total	negative	positive	total
Control	12	0	12	12	0	12
low dose	8	4	12	5	6	11
mid dose	1	10	11	4	8	12
high dose	1	10	11	0	12	12

Stability and Homogeneity

Prepared formulations of AVE0010 in sterile isotonic saline (0.9% NaCl) were assessed at several time points throughout the study. Analyses confirmed that the concentrations of AVE0010 were within the limits specified for solutions, 90% to 110% of the nominal concentration(s). At one time point, all concentrations, including back up samples, were in the range of 70% to 72%. This was considered a minor incidence without compromising the integrity and validity of this study.

Study title: Subcutaneous carcinogenicity study in rats, Amendment 1

Study number:	CAR0084
Study report location:	Module 4.2.3.4.1
Conducting laboratory:	Sanofi-Aventis Deutschland GmbH, Mainzer Landstrasse 500, Hattersheim, Germany
Date of study initiation:	Not stated in study report
First day of dosing:	21 Aug 2006 (males), 22 Aug 2006 (females)
GLP compliance:	Yes, except: The bioanalysis phases for the determination of neutralization, antibody-cross reactivity and antibody titer bioanalysis assays were conducted "in the spirit of GLP"
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010 #PPL-AVE100501A (until 31 Oct 2007) - 98.5% to 100.5% (b) (4) pure #B002 (01 Nov 2007 until 06 Aug 2008) - 100.8% #B004 (07 Aug 2008 until study end) - 98.6%
CAC concurrence:	The Committee conditionally concurred with the sponsor's proposed doses, which were based on the AUC exposure ratio in rats relative to that in humans. Concurrence was contingent upon demonstration that the anti-AVE0010 antibodies are not neutralizing in rodents and that the clinical dose does not change such that the ratio is no longer greater than 25-fold. Otherwise the 2-year studies may be considered to be inadequate. Development of a bioassay in a rodent species was recommended to address the neutralization as well as determination of cross reactivity of anti-AVE0010 antibodies to endogenous GLP-1 in humans and rodents.

Key Study Findings

- There were no treatment-related effects on mortality. There were no noteworthy differences in deaths due to tumors or non-tumor causes between control and treated animals.
- Treatment-related clinical signs included an increased incidence in distended abdomens and increased salivation for all treated groups.
- A dose-related decrease in body weight was observed throughout the study. The effect on body weight was somewhat correlated with decreased food consumption.
- Macroscopically, a slight increase in the number of treated females with a large thyroid was observed. Microscopically, thyroid c-cell hyperplasia and tumors were observed, which may have correlated with the increased organ size. Small increases

in females with bone fractures and mid- and high-dose males with small adrenal glands were observed. No microscopic correlate was noted.

- 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 (7 versus 4 and 2 for each for the two control groups). The increase for the high dose was statistically significant for trend ($p = 0.0158$) and by pair-wise analysis ($p = 0.0431$) when compared with Control Group 2. However, the increase was not statistically significant when compared with Control Group 1 and the increase was also not statistically significant versus either of the control groups when adenomas and carcinomas were added together.
- There were no definitive treatment-related microscopic findings outside of the thyroid. Some treated groups were noted as having a small number of animals with a microscopic finding that was not observed in the control groups or having a slightly higher incidence than control groups. However, because of the low incidence or small difference from controls, a relationship to treatment cannot be concluded. These findings were observed at the **injection site** (hemorrhage - all treated groups), **adipose tissue** (necrosis, grade 3/4 - MD and HD males), **kidney** (transitional cell hyperplasia [all treated male groups], mineralization [all treated male groups], calculus [HD males], and/or neutrophil infiltrate [MD and HD males]), **mammary gland** (focal hyperplasia with or without atypia - HD females), and **lung** (hemorrhage - all male groups)
- Exposures on Day 359 increased in a dose-proportional manner between 40 and 200 µg/kg BID; however, exposures were not meaningfully different between the 200 and 1000 µg/kg BID for male or female groups. A large increase in exposure occurred for all dose groups between Day 4 and Day 86. This was likely due to the development of ADAs that appear to decrease the clearance of AVE0010. Exposure was slightly higher on Day 86 compared with Day 359. ADAs were detected in a large proportion of all AVE0010-treated animals (88% to 100%), which did not appear to be dose related. 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.
- The NOEL for thyroid c-cell tumors was not identified (<40 µg/kg BID). The clinical exposure margin based on the low dose used in this study is 1,028X for QD dosing and 182X for BID dosing. No other tumor types were determined to be related to AVE0010 treatment.

Adequacy of Carcinogenicity Study

The design of the study was conditionally approved by the ECAC prior to study initiation. The ECAC noted that it should be determined whether ADAs had neutralizing activity. Cross-reactivity and neutralization assessments of the ADAs showed that the

ADAs did not react with endogenous GLP-1 or glucagon or have neutralizing activity. Additionally, the pharmacodynamic effect of decreased body weight was noted throughout the study. Because the high-dose was based on a 25-fold clinical exposure margin rather than a maximum tolerated dose or maximum feasible dose, the ECAC also noted that the study may not be considered adequate if the exposure margin falls below 25 fold. Based on available PK data, the exposure margin between the high-dose tested in this study and the exposure at the highest anticipated clinical dose is approximately 960X.

There was an apparent contamination of control vehicle with AVE0010 or misdosing of the control group on one or more occasions. The sponsor presented rationale as to why they feel that this protocol deviation did compromise the integrity or interpretability of this study, which is presented in the bulleted text below.

- Formulation analysis throughout the entire study identified only one sample with AVE0010 contamination in the control vehicle.

- Apart from transient effects in weeks 42/43 and 50/52, in-life data confirmed normal body weight development and food consumption in both control group 1 and control group 2 animals during 2 years of the study with consistent differences to the effects observed in treated group animals.

- Extensive procedural precautions (treatment, sampling, labeling, shipment and analyses procedures) were in place to preclude misdosing, sample mix-up and accidental cross contamination (control and dose group procedures were always separated and treatment of vehicle control groups commenced first, while frozen AVE0010 dosing vials were thawed). Intensive investigations failed to reveal an explanation for an accidental AVE0010 contamination in the control groups.

- Any changes in environmental housing conditions as potential cause for isolated effects on food consumption and body weight restricted to control animals could be excluded.

- Finally, clinical signs, survival rates as well as non-neoplastic and neoplastic lesions observed in the control groups were in the expected range for rats of this strain and age.

Based on the apparent transient nature of this dosing error and the fact that the control animal data appear to be within the expected ranges, the dosing errors do not appear to have compromised the integrity of this study. After considering all of the information, this carcinogenicity study was considered to be adequately conducted.

Appropriateness of Test Model

The test model was found to be appropriate.

Evaluation of Tumor Findings

A summary of the sponsor's statistical analysis for thyroid c-cell tumors is shown in the sponsor-generated tables below. **A statistically significant increase in c-cell adenomas and combined adenomas plus carcinomas was observed for all treated males and females**. An increase in thyroid c-cell carcinomas was not statistically significant. No other tumor types were found to be increased by statistical analysis. (Statistical analyses were not verified by our statistical group; ECAC members did not feel that an internal statistical analysis was needed based on the clear positive signal for thyroid c-cell tumors and lack of signal for other tumor types).

Table 3 - CAR0084 - Individual tumor incidence analysis - M Significant findings

Tumor by tissue/organ	Group Dose(µg/kg/BID)	1 0	2 0	3 40	4 200	5 1000
THYROID GLAND	Examined tissues	57	56	59	58	60
Adenoma: C-cell	Non lethal tumors	12	14	34	24	37
	Treated vs. Dual	<.0001		<.0001	<.0001	<.0001
	Treated vs. Ctrl1	<.0001		<.0001	0.0036	<.0001
	Treated vs. Ctrl2		<.0001	<.0001	0.0061	<.0001

The p-values under the controls are from upper-tailed Peto trend tests.
The p-values under each treated group are from upper-tailed Peto pairwise comparisons to the control.
Significant p-values are presented in bold at 2.5% level for trend tests and 5% level for pairwise comparisons.
: difference between the two controls is statistically significant at 5% level.
15 Dec 2009, 14:55

Table 4 - CAR0084 - Combined tumor incidence analysis - M Significant findings

Tumor by tissue/organ	Group Dose(µg/kg/BID)	1 0	2 0	3 40	4 200	5 1000
THYROID GLAND	Examined tissues	57	56	59	58	60
Adenoma/Carcinoma: C-Cell	Non lethal tumors	12	14	34	25	38
	Treated vs. Dual	<.0001		<.0001	<.0001	<.0001
	Treated vs. Ctrl1	<.0001		<.0001	0.0023	<.0001
	Treated vs. Ctrl2		<.0001	<.0001	0.0038	<.0001

The p-values under the controls are from upper-tailed Peto trend tests.
The p-values under each treated group are from upper-tailed Peto pairwise comparisons to the control.
Significant p-values are presented in bold at 2.5% level for trend tests and 5% level for pairwise comparisons.
: difference between the two controls is statistically significant at 5% level.
15 Dec 2009, 14:55

Table 5 - CAR0084 - Individual tumor incidence analysis - F Significant findings

Tumor by tissue/organ	Group Dose(µg/kg/BID)	1 0	2 0	3 40	4 200	5 1000
THYROID GLAND	Examined tissues	57	53	60	54	59
Adenoma: C-cell	Non lethal tumors	10	7	20	30	26
	Treated vs. Dual	**<.0001**		**0.0002**	**<.0001**	**<.0001**
	Treated vs. Ctrl1	**0.0003**		**0.0126**	**<.0001**	**0.0017**
	Treated vs. Ctrl2		**0.0005**	**0.0145**	**<.0001**	**0.0026**

The p-values under the controls are from upper-tailed Peto trend tests.
The p-values under each treated group are from upper-tailed Peto pairwise comparisons to the control.
Significant p-values are presented in bold at 2.5% level for trend tests and 5% level for pairwise comparisons.
: difference between the two controls is statistically significant at 5% level.
15 Dec 2009, 14:55

Table 6 - CAR0084 - Combined tumor incidence analysis - F Significant findings

Tumor by tissue/organ	Group Dose(µg/kg/BID)	1 0	2 0	3 40	4 200	5 1000
THYROID GLAND	Examined tissues	57	53	60	54	59
Adenoma/Carcinoma: C-Cell	Non lethal tumors	10	7	20	30	28
	Lethal tumors	0	0	0	1	0
	Treated vs. Dual	**<.0001**		**0.0001**	**<.0001**	**<.0001**
	Treated vs. Ctrl1	**<.0001**		**0.0121**	**<.0001**	**0.0005**
	Treated vs. Ctrl2		**0.0001**	**0.0161**	**<.0001**	**0.0010**

The p-values under the controls are from upper-tailed Peto trend tests.
The p-values under each treated group are from upper-tailed Peto pairwise comparisons to the control.
Significant p-values are presented in bold at 2.5% level for trend tests and 5% level for pairwise comparisons.
: difference between the two controls is statistically significant at 5% level.
15 Dec 2009, 14:55

Methods

Doses and Number/Sex/Group: (see sponsor-generated table below)

Group	Dosage (µg/kg BID)	Main study animals			Toxicokinetic animals		
		Number/ sex	Animal numbers		Number/ sex	Animal numbers	
			Male	Female		Male	Female
1	0	60	1 - 60	70 - 129	9	61 - 69	130 - 138
2	0	60	139 - 198	208 - 267	9	199 - 207	268 - 276
3	40	60	277 - 336	355 - 414	18	337 - 354	415 - 432
4	200	60	433 - 492	511 - 570	18	493 - 510	571 – 588
5	1000	60	589 - 648	667 - 726	18	649 - 666	727 - 744

Frequency of dosing: Twice daily (~8 hours apart)
Dose volume: 0.5 mL/kg
Route of administration: Subcutaneous (rotation of 4 injection sites)

Formulation/Vehicle:	AVE0010 in 50 mM sodium citrate, pH 5.3, then diluted to appropriate concentrations by adding 0.9% sodium chloride
Basis of dose selection:	3-month range-finding study
Species/Strain:	Rat / Sprague-Dawley
Age:	6 to 7 weeks
Weight:	145 to 200 g (males), 130 to 182 g (females)
Animal housing:	In groups of three animals in transparent Polysulfon cages (type IV) on soft wood granulate in an air conditioned room
Paradigm for dietary restriction:	No dietary restriction; ssniff R/M-H (V1534)* pellets ad libitum
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Yes, see above table
Reason for amendment:	Reporting of immunohistochemistry data from thyroid C-cell foci to assess for signs of proliferation (Ki-67)
Deviation from study protocol:	Control group animals may have received test article around Week 42 and again around Week 51.

Observations and Results

Mortality

Mortality - Males

Dose (µg/kg BID)	Vehicle Control 1	Vehicle Control 2	40	200	1000
Study Week	**Number of Deaths**				
0 - 50	3 (5.0%)	1 (1.7%)	3 (5.0%)	3 (5.0%)	2 (3.3%)
51 - 80	10 (16.7%)	9 (15.0%)	9 (15.0%)	22 (36.7%)	14 (23.3%)
81 - terminal sacrifice	15 (25.0%)	26 (43.3%)	20 (33.3%)	12 (20.0%)	19 (31.7%)
Total Early Deaths	28/60 (46.7%)	36/60 (60.0%)	32/60 (53.3%)	37/60 (61.7%)	35/60 (58.3%)
Surviving animals at terminal sacrifice	32/60 (53.3%)	24/60 (40.0%)	28/60 (46.7%)	23/60 (38.3%)	25/60 (41.7%)
Treated vs. pooled controls	p = 0.1717				
Treated vs. control 1	p = 0.0739				
Treated vs. control 2		p = 0.5110			

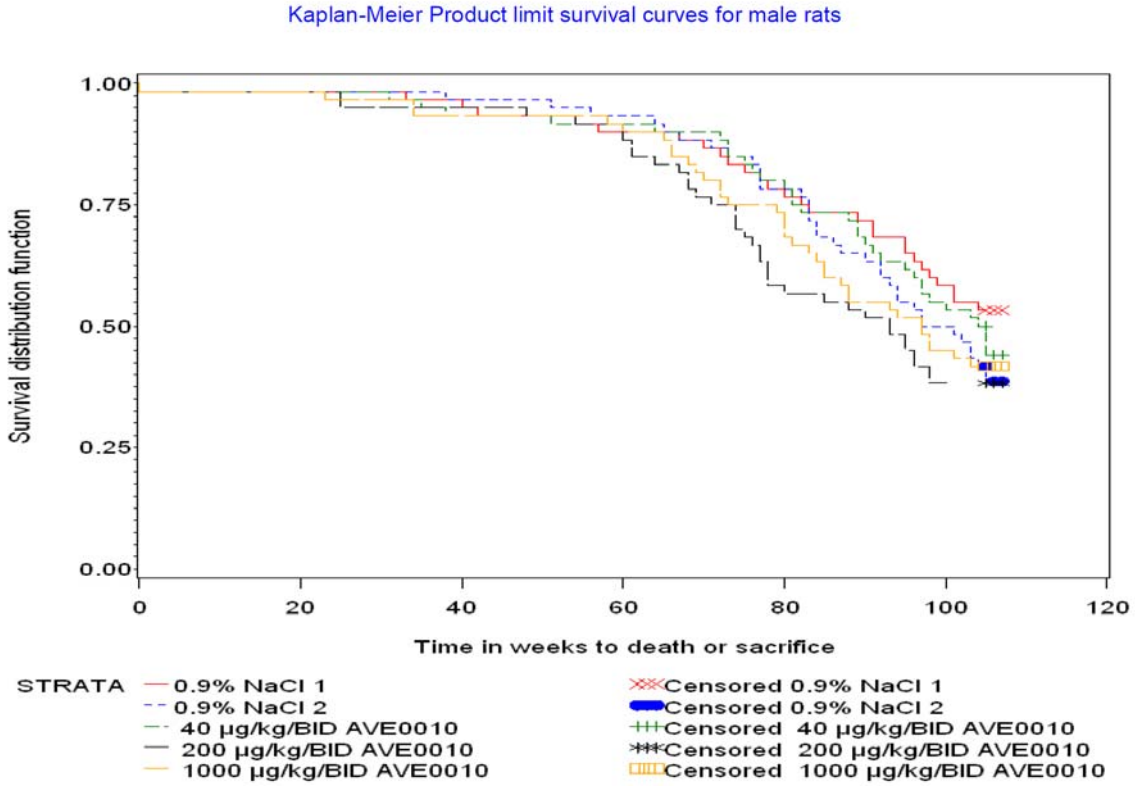
Dose (µg/kg BID)	Vehicle Control 1	Vehicle Control 2	40	200	1000
Summary of Animal Disposition					
Euthanized *in extremis*	16	19	14	25	24
Found dead	12	17	18	12	11
Terminal necropsy	32	24	28	23	25
Tumor-Related Deaths					
Total Early Deaths	28	36	32	37	35
Adrenal gland - medulla: pheochromocytoma, malignant	1	-	-	-	-
Brain - cerebrum: granular cell, malignant	1	-	-	-	-
Brain - cerebrum: astrocytoma, benign	1	-	-	1	-
Brain - cerebrum: astrocytoma, malignant	-	-	1	-	-
Brain - cerebrum : reticulosis, malignant	-	1	-	-	-
Bone: osteosarcoma	-	1	-	1	-
Heart: mesothelioma, malignant	1	-	-	-	-
Hemolymphoreticular system: sarcoma, histiocytic	1	2	1	1	-
Hemolymphoreticular system: lymphoma, malignant	-	-	-	1	1
Hemolymphoreticular system: leukemia, granulocytic	-	-	-	1	-
Kidney - sarcoma	1	-	-	-	-
Liver: hepatocyte carcinoma	-	1	1	-	-
Mammary gland: adenocarcinoma	-	1	-	-	-
Pancreas: islet cell carcinoma	-	-	1	-	-
Pituitary: adenoma, pars distalis	9	16	12	21	23
Pituitary: neuroblastoma, malignant	1	-	-	-	-
Prostate gland: adenocarcinoma	-	-	1	1	-

Dose (µg/kg BID)	Vehicle Control 1	Vehicle Control 2	40	200	1000
Skin/subcutis : fibrosarcoma	1	-	-	-	1
Skin/subcutis : schwannoma, malignant	-	-	-	1	-
Non-tumor Related Deaths					
Kidney: necrosis, papilla	-	-	-	1	-
Kidney: degeneration/ regeneration: tubule	-	1	-	-	-
Lung: hemorrhage	-	-	1	-	1
Lung: chronic inflammation	-	-	1	-	-
Lung: inflammation, aspiration	-	-	-	1	-
Not Evident	11	13	13	7	9

Mortality - Females

Dose (µg/kg BID)	Vehicle Control 1	Vehicle Control 2	40	200	1000
Study Week	**Number of Deaths**				
0 - 50	3 (5.0%)	2 (3.3%)	3 (5.0%)	3 (5.0%)	0 (0.0%)
51 - 80	12 (20.0%)	19 (31.7%)	17 (28.3%)	14 (23.3%)	15 (25.0%)
81 - terminal sacrifice	21 (35.0%)	24 (40.0%)	24 (40.0%)	19 (31.7%)	18 (30.0%)
Total Early Deaths	36/60 (60.0%)	**45**/60 (75.0%)	44/60 (73.3%)	36/60 (60.0%)	**33**/60 (55.0%)
Surviving animals at terminal sacrifice	24/60 (40.0%)	15/60 (25.0%)	16/60 (26.7%)	24/60 (40.0%)	27/60 (45.0%)
Treated vs. pooled controls	p = 0.2364				
Treated vs. control 1	p = 0.7846				
Treated vs. control 2		**p = 0.0204**	p = 0.8179	p = 0.1715	**p = 0.0371**
Summary of Animal Disposition					
Euthanized *in extremis*	20	25	18	21	24
Found dead	16	20	26	15	9
Terminal necropsy	24	15	16	24	27
Tumor-Related Deaths					
Total Early Deaths	36	45	44	36	33
Brain - cerebrum: astrocytoma, malignant	-	-	1	-	-

Hemolymphoreticular system: Sarcoma, histiocytic	-	1	-	1	2
Kidney: transitional cell carcinoma	-	-	1	-	-
Liver: hepatocyte carcinoma	-	-	-	-	-
Lung: Bronchioloalviolar carcinoma	-	-	-	-	-
Mammary gland: adenocarcinoma	9	5	4	7	2
Osteosarcoma: metastastasis in lung	-	1	-	-	-
Pituitary: adenoma, pars distalis	12	22	15	9	17
Peritoneum: mesothelioma, malignant	1	-	-	-	-
Spinal cord - cervical: oligodendroglioma, malignant	1	-	-	-	-
Thyroid: c-cell carcinoma	-	-	-	1	-
Uterus: schwannoma, malignant	-	-	-	-	1
Non-tumor Related Deaths					
Lung: hemorrhage	-	-	1	-	-
Pituitary: hyperplasia, distalis	-	-	1	-	-
Not Evident	13	15	21	18	11
Not indicated in sponsor summary or individual animal data	-	1	-	-	-



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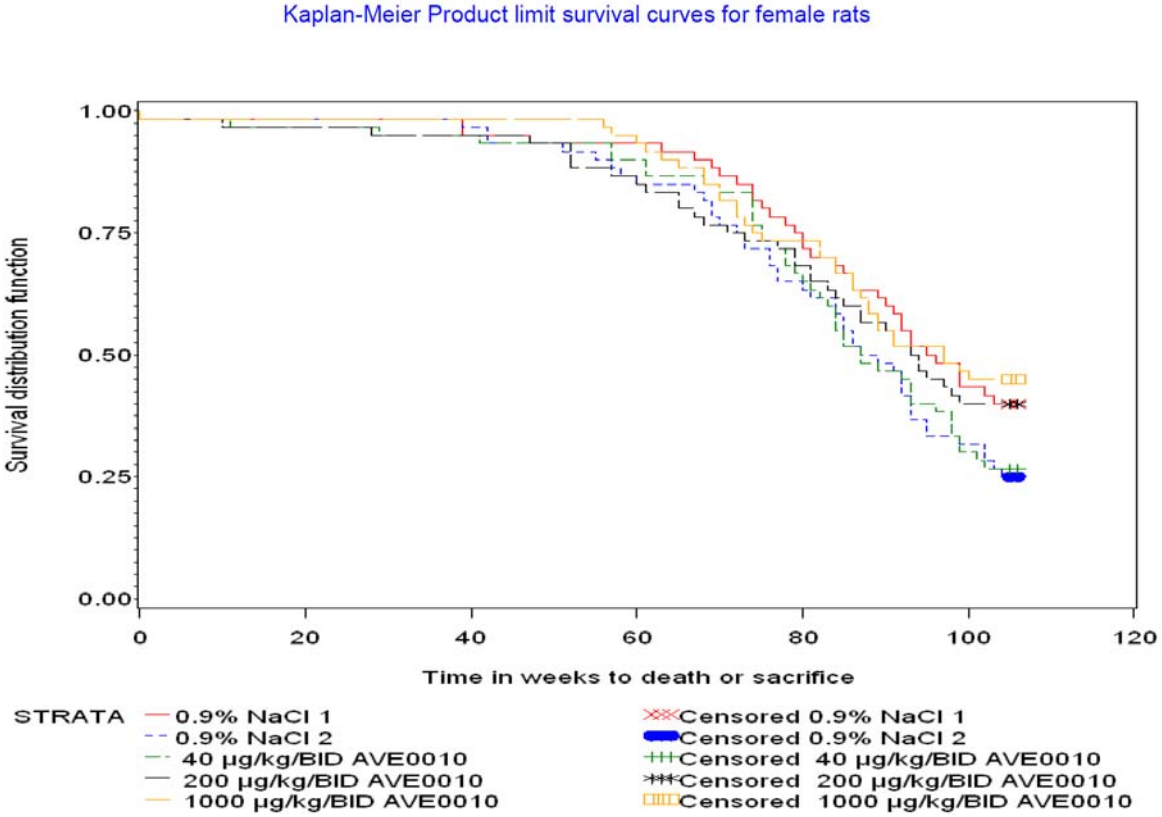

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Clinical Signs

(sponsor-generated table)

Text table 10 – Summary of compound-related clinical signs

Daily Dose (µg/kg BID)	**0***		**40**		**200**		**1000**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Number of animals on study	120	120	60	60	60	60	60	60
Clinical Observations [No. of animals affected]								
Salivation, marked	-	-	3	1	2	5	2	3
Salivation, mild	-	-	1	18	6	16	8	24
Salivation, moderate	1	-	39	38	46	38	51	52
Abdomen distended, mild	-	-	1	9	5	6	6	6
Abdomen distended, moderate	-	1	4	2	4	2	3	6
Abdomen distended, marked	-	1	-	-	-	-	-	-
Humane euthanasia	25	38	10	12	17	18	15	17
Moribund euthanasia	10	7	4	6	8	3	9	7

* Control groups 1 and 2 were pooled

Body Weights (sponsor-generated tables)

Text table 11 – Effects on body weight and body weight gain in male rats

Daily dose (µg/kg BID); Males	0 °	40	200	1000
Absolute Body Weight [g] & (%)[a]				
Week 2	229.1	-5*	-7*	-10*
Week 8	449.1	-10*	-10*	-11*
Week 13	533.8	-9*	-11*	-12*
Week 26	646.5	-9*	-12*	-13*
Week 41	711.5	-10*	-13*	-14*
Week **43**	**683.2**	**-5***	**-8***	**-9***
Week 50	734.4	-9*	-12*	-13*
Week **52**	**702.0**	**-4***	**-7***	**-8***
Week 65	779.8	-10*	-13*	-14*
Week 78	798.7	-11*	-12*	-16*
Week 91	800.8	-12*	-14*	-15*
Week 104	771.2	-11*	-13*	-17*
Absolute body weight gain [g]; (%)[a]				
Day 1 to 92	372.7	-13	-16	-16
Day 1 to 183	482.3	-13	-17	-16
Day 1 to 351	531.6	-12	-16	-16
Day 1 to 540	628.0	-14	-15	-19
Day 1 to 722	599.8	-15	-17	-21
Average weekly body weight change [g/animal/week]; (%)[a]				
Week 1 to 41	13.19	-13*	-17*	-17*
Week **42 to 43**	**-13.50**	na #	na #	na #
Week 44 to 50	3.43	-47*	-52*	-38*
Week **51 to 52**	**-16.2**	na #	na #	na #
Week 1 to 104	5.78	-15*	-16*	-21*

° 1&2 = control groups 1 and 2 were pooled
na # = not applicable; bw gains were negative for controls in week 42 to 43 vs. positive or almost unchanged bw gains for treated groups

[a] For controls, group means are shown. For treated groups, percent differences from controls are shown.
* Statistical significance (where applicable) is based on actual data (not on the percent differences)

Text table 12 - Effects on body weight and body weight gain in female rats

Daily dose (µg/kg BID); Females	0 °	40	200	1000
Absolute Body Weight [g] & (%)[a]				
Week 2	181.1	+1	-1	0
Week 8	256.6	-2*	-3*	-3*
Week 13	280.5	-2*	-6*	-4*
Week 26	323.6	-10*	-13*	-13*
Week 41	353.5	-12*	-16*	-15*
Week **43**	**342.1**	**-8***	**-13***	**-12***
Week 50	376.4	-14*	-18*	-18*
Week **51**	**364.7**	**-10***	**-15***	**-16***
Week 65	421.8	-15*	-21*	-20*
Week 78	444.8	-16*	-21*	-21*
Week 91	464.2	-15*	-23*	-20*
Week 104	463.9	-11*	-18*	-18*
Absolute body weight gain [g]; (%)[a]				
Day 1 to 92	125.6	-5	-12	-10
Day 1 to 183	160.6	-15	-21	-21
Day 1 to 351	206.4	-17	-25	-26
Day 1 to 540	286.5	-24	-31	-32
Day 1 to 722	307.1	-17	-27	-27
Weekly body weight changes [g/animal/week]; (%)[a]				
Week 1 to 41	4.75	-20*	-26*	-25*
Week **42 to 43**	**-6.40**	na #	na #	na #
Week 44 to 50	3.27	-60*	-73*	-72*
Week **50 to 51**	**-5.85**	na #	na #	na #
Week 1 to 104	2.94	-15*	-26*	-26*

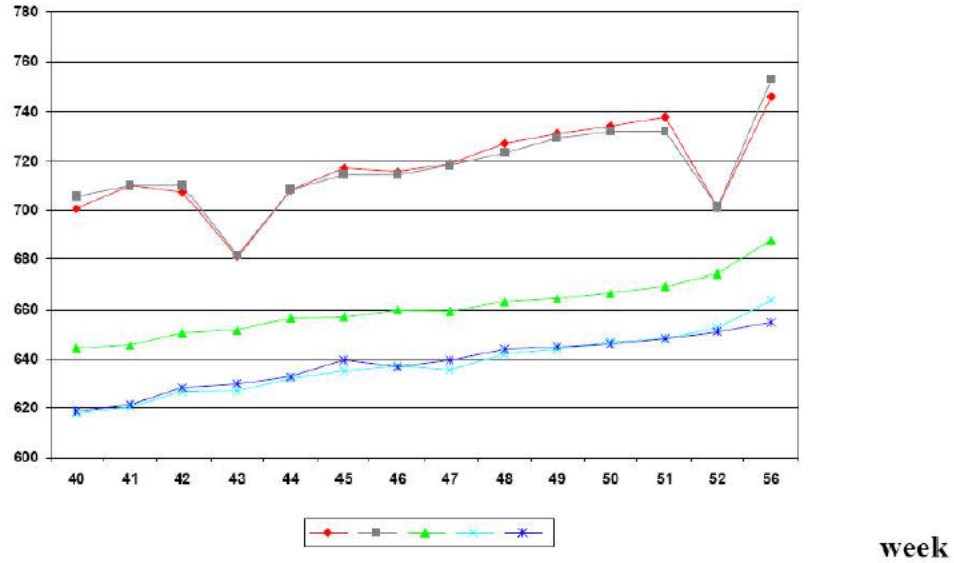
° 1&2 = control groups 1 and 2 were pooled
na # = not applicable; bw gains were negative for controls in week 42 to 43 vs. positive or almost unchanged bw gains for treated groups
[a] For controls, group means are shown. For treated groups, percent differences from controls are shown.
* Statistical significance (where applicable) is based on actual data (not on the percent differences)

Potential Dosing Error for Control Groups

Two events of distinct, transient losses in body weights were observed for control group males and females in Week 42/43 intervals and again about 10 weeks later (Week 51/52 for males and 50/51 for females). Sponsor-generated figures that show this weight loss graphically are presented below.

Text table 13 - Temporary effects on body weight in main control males in week 42/43 and week 51/52 intervals

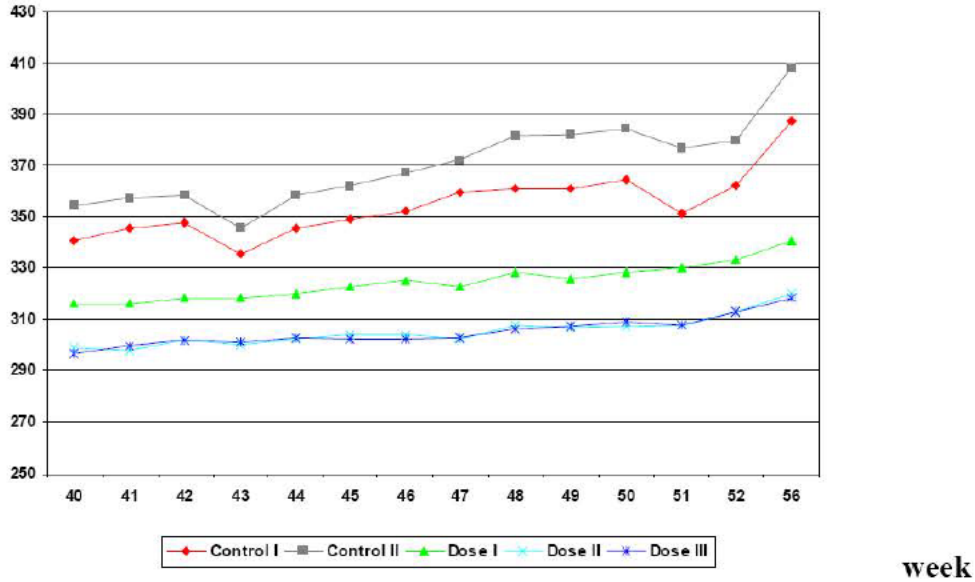
g



Control - control 2 - Dose I (40 µg/kg BID) - Dose II (200 µg/kg BID) - Dose III (1000 µg/kg BID)

Text table 14 - Temporary effects on body weight in main control females in week 42/43 and 50/51 intervals

g



Feed Consumption

(sponsor-generated tables)

Text table 15 – Effects on absolute food consumption in male rats

Daily dose (µg/kg BID); Males	0 °	40	200	1000
Average Daily Food Consumption				
[g/animal/day] & (%)[a]				
Week 2	23.98	-19*	-22*	-26*
Week 8	28.28	-10*	-11*	-11*
Week 13	27.66	-7*	-10*	-7*
Week 26	28.75	-9*	-10*	-9*
Week 41	30.46	-10*	-10*	-10*
Week **43**	**25.96**	**+7***	**+4***	**+6***
Week 50	30.29	-9*	-10*	-8*
Week **52**	**25.19**	**+8***	**+7***	**+9***
Week 66	29.61	-6*	-12*	-9*
Week 79	30.47	-10*	-11*	-12*
Week 92	29.94	-9*	-13*	-9*
Week 105	29.21	-11	-2	-13*

Text table 16 – Effects on absolute food consumption in female rats

Daily dose (µg/kg BID); Females	0 °	40	200	1000
Average Daily Food Consumption				
[g/animal/day] & (%)[a]				
Week 2	17.91	-13*	-18*	-18*
Week 8	18.41	-4	-1	-5*
Week 13	18.18	-3*	-6*	-7*
Week 26	19.66	-12*	-14*	-13*
Week 41	18.62	-2	-3	-1
Week **43**	**15.21**	**+22***	**+21***	**+19***
Week 50	20.55	-10*	-7*	-10*
Week **52**	**17.74**	**+3**	**+6**	**+4**
Week 66	19.62	-3	+2	-1
Week 79	21.89	-4	-9*	-9*
Week 92	20.31	-2	-8*	0
Week 105	22.83	+4*	+1*	-8

° 1&2 = ccontrol groups 1 and 2 were pooled

[a] For controls, group means are shown. For treated groups, percent differences from controls are shown.
* Statistical significance is based on actual data (not on the percent differences)

Gross Pathology

Gender	Males					Females				
Dose (µg/kg BID)	Con 1	Con 2	40	200	1000	Con 1	Con 2	40	200	1000
Adrenal Gland - Large	7	4	3	**2**	**1**	12	9	6	18	9
Adrenal Gland - Small	-	-	-	**1**	**1**	-	1	-	-	-
Bone - Fracture	-	-	-	-	-	-	-	**1**	**3**	**1**
Injection site - focus/area, red	-	-	**1**	-	**3**	-	-	**2**	**1**	**2**
Pituitary Gland - Large	19	21	21	25	**31**	22	33	30	20	28
Thyroid Gland - Large	2	1	**8**	**3**	**5**	-	1	**3**	**2**	**5**

Histopathology

Peer Review: A pathology peer review was performed by Dr. (b) (4). However, the peer review statement is located in the test facility archives rather than being included in the final study report.

Neoplastic

Gender	Males					Females				
Dose (µg/kg BID)	**Con 1**	**Con 2**	**40**	**200**	**1000**	**Con 1**	**Con 2**	**40**	**200**	**1000**
Thyroid Gland:										
C-cell adenoma	12	14	**34**	**24**	**37**	10	7	20	30	26
C-cell carcinoma	0	0	0	3	1	0	0	0	1	2
Total†	12	14	**34**	**25**	**38**	10	7	**20**	**31***	**28**
C-cell hyperplasia	20	17	**26**	**29**	**30**	21	12	**28**	**25**	**33**
-Grade 1	6	6	6	6	3	6	3	5	3	6
-Grade 2	3	6	9	9	10	10	5	8	10	10
-Grade 3	4	3	8	5	9	2	-	4	3	4
-Grade 4	6	3	3	8	3	1	2	8	5	9
-Grade 5	1	1	-	1	5	2	2	3	4	4
Lung: metastasis, thyroid c-cell	0	0	0	2	0	0	0	0	1	2
Lymph node - mandibular: metastasis, thyroid c-cell	0	0	0	0	0	0	0	0	1	0
Pancreas: islet cell										
-Adenoma	4	2	2	5	**7**	1	1	2	1	2
-Carcinoma	1	0	2	0	0	0	0	0	0	0
-Total†	5	2	4	5	**7**	1	1	2	1	2

Gender	Males					Females				
Dose (µg/kg BID)	**Con 1**	**Con 2**	**40**	**200**	**1000**	**Con 1**	**Con 2**	**40**	**200**	**1000**
Adipose tissue:										
-Lipoma	0	0	1	1	1	0	0	0	0	0
-Liposarcoma	0	0	0	0	0	0	0	1	0	0
Injection site 2:										
-Fibroma	0	0	1	0	1	0	0	0	0	0
Injection site 3:										
-Fibroma	0	0	0	0	1	0	0	0	0	0
Skin/subcutis:										
basal cell, malignant	0	0	0	0	2	0	0	0	0	0
squamous cell carcinoma	0	0	0	0	0	0	0	0	1	1
Uterus: schwannoma, malignant	NA	NA	NA	NA	NA	0	0	0	0	2
Vagina: schwannoma, malignant	NA	NA	NA	NA	NA	0	0	0	0	1

†Animals having both an adenoma and carcinoma were only counted once for the calculation of total tumors.
*Sponsor table has the number of combined tumors for mid-dose females as 30; however the animal that was noted as having a c-cell carcinoma (animal 527) did not have an adenoma, so it appears that the sum should be 31.

Non Neoplastic

Gender	Males					Females				
Dose (µg/kg BID)	**Con 1**	**Con 2**	**40**	**200**	**1000**	**Con 1**	**Con 2**	**40**	**200**	**1000**
Adipose tissue: necrosis	0	0	0	1	1	0	0	0	0	0
-Grade 3	-	-	-	-	1	-	-	-	-	-
-Grade 4	-	-	-	1	-	-	-	-	-	-
Injection site 1: hemorrhage	0	0	0	0	1	0	0	0	0	1
-Grade 1	-	-	-	-	-	-	-	-	-	1
-Grade 3	-	-	-	-	1	-	-	-	-	-
Injection site 2: hemorrhage -Grade 1	0	0	1	0	0	0	0	0	0	0
Injection site 3: hemorrhage	0	0	0	1	2	0	1	3	0	3
-Grade 1	-	-	-	1	1	-	-	2	-	2
-Grade 2	-	-	-	-	1	-	1	1	-	1
Injection site 4: hemorrhage -Grade 1	0	0	0	0	0	0	0	1	0	0
Kidney: hyperplasia, transitional cell, pelvis	17	20	**28**	**27**	**32**	42	41	44	40	37
-Grade 1	17	15	27	23	28	39	33	28	30	29
-Grade 2	-	4	1	3	4	3	8	15	9	8
-Grade 3	-	1	-	1	-	-	-	1	1	-
mineralization, pelvis	15	14	**23**	**24**	**26**	50	43	44	43	35
-Grade 1	6	8	9	14	11	28	24	28	35	31
-Grade 2	7	4	8	5	12	18	17	14	6	4
-Grade 3	2	2	5	4	3	4	2	2	2	-
-Grade 4	-	-	1	1	-	-	-	-	-	-
calculus, pelvis	0	4	5	2	**9**	3	3	2	1	4
-Grade 1	-	-	-	-	1	-	1	-	-	1
-Grade 2	-	-	1	1	3	2	2	-	-	1
-Grade 3	-	4	4	1	4	1	-	2	1	2
-Grade 4	-	-	-	-	1	-	-	-	-	-

Gender	Males					Females				
Dose (µg/kg BID)	**Con 1**	**Con 2**	**40**	**200**	**1000**	**Con 1**	**Con 2**	**40**	**200**	**1000**
infiltrate, neutrophil	9	9	9	**16**	**18**	6	10	11	7	11
-Grade 1	9	8	9	13	15	6	10	10	7	11
-Grade 2	-	1	-	1	3	-	-	1	-	-
-Grade 3	-	-	-	2	-	-	-	-	-	-
Lung: hemorrhage	1	1	**5**	**3**	**5**	2	7	10	8	2
-Grade 1	1	-	3	3	4	1	5	5	3	-
-Grade 2	-	1	1	-	-	1	2	3	2	2
-Grade 3	-	-	-	-	-	-	-	2	3	-
-Grade 4	-	-	1	-	1	-	-	-	-	-
Lymph node: mandibular, dilation, sinus	0	0	0	1	**3**	0	1	0	2	0
-Grade 1	-	-	-	-	-	-	1	-	-	-
-Grade 2	-	-	-	-	1	-	-	-	1	-
-Grade 3	-	-	-	-	-	-	-	-	1	-
-Grade 4	-	-	-	1	2	-	-	-	-	-
Mammary gland: hyperplasia, focal	NA	NA	NA	NA	NA	1	0	1	0	**4**
-Grade 1						-	-	1	-	3
-Grade 2						-	-	-	-	1
-Grade 3						1	-	-	-	-
hyperplasia, with atypia, focal	NA	NA	NA	NA	NA	4	3	2	4	**7**
-Grade 1						2	2	-	4	4
-Grade 2						2	1	2	-	2
-Grade 3						-	-	-	-	1

Text table 3 - Historical control data from the Registry of Industrial Toxicology Animal-data (RITA) from 2009 based 38 studies with overall 2039 male and 1945 female rats

Rat	Mean in %		Range in %	
	male	female	male	female
C-cell hyperplasia	10	12.9	1.4-38.0	1.5-35.6
C-cell adenoma	13.2	12.5	3.3-66	1.8-54
C-cell carcinoma	2.2	1.6	0-20	0-8

Text table 4 - Historical control data from the supplier [redacted] (b) (4) (2004) based on 30 or 31 studies with overall 2141 male or 2343 female Sprague Dawley rats, respectively

Rat	Mean in %		Range in %	
	male	female	male	female
C-cell adenoma	7.85	7.21	0-14.77	0-16.67
C-cell carcinoma	1.40	0.85	0-14.81	0.-11.43

Text table 5 - Study period 6 to 12 months (Day 187 – 372) of treatment

	Males					Females				
µg/kg BID	0	0	40	200	1000	0	0	40	200	1000
No. of intercurrent deaths	4	2	4	3	1	2	4	2	3	-
Thyroid glands examined	4	2	4	3	1	2	4	2	3	-
C-cell hyperplasia	-	-	1	1	1	1	-	1	-	-
C-cell adenoma	1	-	-	-	-	-	-	-	-	-
C-cell carcinoma	-	-	-	-	-	-	-	-	-	-

Text table 6 - Study period 12 to 18 (Day 373 – 558) months of treatment

	Males					Females				
µg/kg BID	0	0	40	200	1000	0	0	40	200	1000
No. of intercurrent deaths	9	8	8	21	14	11	15	17	13	15
Thyroid glands examined	8	7	8	20	14	10	14	17	10	15
C-cell hyperplasia	2	1	5	10	9	4	-	6	5	9
C-cell adenoma	2	1	3	4	6	1	-	3	2	5
C-cell carcinoma	-	-	-	1	-	-	-	-	-	-
C-cell hyperplasia %	25	14	63	50	64	40	-	35	50	60
C-cell adenoma %	25	14	38	20	43	10	-	18	20	33
C-cell carcinoma %	-	-	-	5	-	-	-	-	-	-

Text table 7 – Study period 18 to 24 (Day 559 – 744) months of treatment

	Males					Females				
µg/kg BID	0	0	40	200	1000	0	0	40	200	1000
No. of intercurrent deaths	15	26	20	12	19	22	26	24	19	18
Thyroid glands examined	13	23	19	12	19	21	21	24	16	17
C-cell hyperplasia	6	4	3	6	9	8	8	10	7	8
C-cell adenoma	5	6	10	6	14	4	3	6	10	10
C-cell carcinoma	-	-	-	1	1	-	-	-	1	-
C-cell hyperplasia %	46	17	16	50	47	38	38	42	44	47
C-cell adenoma %	39	26	53	50	74	19	14	25	63	59
C-cell carcinoma %	-	-	-	8	5	-	-	-	6	-

Text table 8 - Terminal sacrifice after 2 years of treatment

	Males					Females				
µg/kg BID	0	0	40	200	1000	0	0	40	200	1000
No. of intercurrent deaths	32	24	28	23	25	24	15	16	24	27
Thyroid glands examined	32	24	28	23	25	24	14	16	24	27
C-cell hyperplasia	12	12	17	12	11	8	4	11	13	16
C-cell adenoma	4	7	21	14	17	5	4	11	18	11
C-cell carcinoma	-	-	-	1	-	-	-	-	-	2
C-cell hyperplasia %	38	50	61	52	44	33	29	69	54	59
C-cell adenoma %	13	29	75	61	68	21	29	69	75	41
C-cell carcinoma %	-	-	-	4	-	-	-	-	-	7

(Text tables 3-8 above are from the sponsor's submission)

Immunohistochemistry

Animals from control and high-dose groups with moderate to severe C-cell hyperplasia were selected for further microscopic investigation. Double staining was performed for calcitonin, to identify C-cells, and Ki-67, a marker for cell proliferation. Due to the deeper level of the cut of the paraffin-imbedded tissue for the new slides for immunohistochemistry, minimal to moderate hyperplastic foci were not present in the deeper levels of the thyroid gland. Focal hyperplasia diagnosed in the original H&E slide was confirmed in most animals in the calcitonin/Ki-67 double stained slides. Any inconsistencies between the H&E and IHC slides were caused by the different tissue cut levels of these slides. In the hyperplastic foci, several C-cells were stained by Ki67, showing a proliferative status of these C-cells. Overall, there was no difference in the occurrence of Ki—67 positive C-cells between control animals with C-cell hyperplasia and animals treated with AVE0010 at a dose of 1000 µg/kg BID. A

summary of the immunohistochemistry findings is presented in the sponsor-generated table below.

Table 1 - Immunhistochemical evaluation of thyroid gland

	Control						AVE0010					
	C1		C2				D3					
Animal No.	56	75	143	195	238	245	593	610	629	684	702	713
Focal hyperplasia (original diagnosis)												
Grade	(4	5	(5	(3	(5	4	5	3	(5	4	5	4
IHC												
Focal hyperplasia	(P	(P	(P	P	(P	(P	-	P	P	P	P	P
Ki67 positive C-cells												
Grade	-	-	(2	3	(2	1	-	2	3	2	2	1

IHC = Immunhistochemistry; P = present; - = not present; (= unilateral finding
Grade 1 = minimal; Grade 2 = mild; Grade 3 = moderate; Grade 4 = marked; Grade 5 = severe
Dose group: C1 = Control BID, C2 = Control BID; D3 = 1000 µg/kg BID AVE0010 (BID = twice daily)

Toxicokinetics

(sponsor-generated table)

Table 1 - Compiled table of toxicokinetic parameters

Sex	Dose (µg/kg/day)	Day	t_{max} (h)	C_{max}[c] (ng/mL)	AUC_{0-24}[c] (ng.h/mL)	AUC/Dose[c]	R_{ac} AUC vs. Day 4	R_{ac} AUC vs. Day 86	R_{AUC} female/male
Male	80	4	0.17	26.2	66.7	0.834			
		86	16.00	1310	15500	194	233		
		359	8.00	531	8290	104	124	0.53	
	400	4	0.33	122	242	0.606			
		86	0.33	3130	53100	133	219		
		359	0.33	2190	39400	98.4	163	0.74	
	2000	4	0.17	274	863	0.431			
		86	0.17	1910	20400	10.2	24		
		359	3.00	5740	45700	22.9	53	2.24	
Female	80	4	0.33	47.7	69.5	0.869			1.04
		86	16.00	860	8340	104	120		0.54
		359	3.00	683	6620	82.8	95	0.79	0.80
	400	4	0.33	157	268	0.671			1.11
		86	0.17	1930	31200	77.9	116		0.59
		359	3.00	1810	31700	79.3	118	1.02	0.81
	2000	4	0.17	301	823	0.412			0.95
		86	16.00	1900	14100	7.03	17		0.69
		359	8.00	2980	32800	16.4	40	2.34	0.72

Stability and Homogeneity

Sample analysis for determination of the concentration of dosing preparations showed that concentrations were within the specified limits of 90% to 110% of nominal on all but two occasions. The concentration range of the first set of

samples for the 40 µg/kg dose was 83% to 91% of nominal. Later in the study, the concentration range of samples for the 200 and 1000 µg/kg dose was 44% to 87% of nominal. It was confirmed that AVE0010 was absent from the vehicle control sample, except for sample 57 A-C and backup 57 A-C, for which a concentration of 0.0047 mg/mL was measured. These minor dosing formulation deviations did not compromise the integrity or validity of this study.

Anti-AVE0010 Antibody Assessment

(sponsor-generated tables)

Table 1 - Numbers of the two categories of antibody status in the plasma samples

Day 93

	male			female		
	negative	positive	total	negative	positive	total
Control	18	0	18	17	1	18
low dose	1	17	18	0	18	18
mid dose	0	18	18	0	18	18
high dose	0	18	18	0	18	18

Day 366

	male			female		
	negative	positive	total	negative	positive	total
Control	15	3	18	1	15	16
low dose	1	15	16	0	13	13
mid dose	1	17	18	1	17	18
high dose	2	15	17	1	15	16

Table 2 – Results of anti-AVE0010 Titer determination and quantification of the Biologically active concentration of AVE0010 in a cellular assay

(b) (4) Pool Number	Dose kg/BID	Day	Sex	Antibody titer -ELISA, (b) (4) provided by sponsor [pg/mL]	Number of freeze/thaw at (b) (4)	Sample dilution factor	Biological active AVE0010 concentration cell assay (b) (4) -[pg/mL]	Mean titer per sex	SD	Mean BAC per sex	SD
1	DOSE0	4	male	negative	2	1	0				
2	DOSE0	4	male	negative	2	1	0				
3	DOSE0	4	male	negative	2	1	0			0,0	0,00
4	DOSE0	4	female	negative	2	1	0				
5	DOSE0	4	female	negative	2	1	0				
6	DOSE0	4	female	negative	2	1	0			0,0	0,00
	mean						**0**				
	SD						**0**				
7	DOSE40	4	male	negative	2	4	1635				
8	DOSE40	4	male	negative	2	4	1306				
9	DOSE40	4	male	negative	2	4	1335			1425	182,08
10	DOSE40	4	female	negative	2	32	3946				
11	DOSE40	4	female	negative	2	8	2131				
12	DOSE40	4	female	negative	2	8	2274			2784	1009,29
	mean						**2104**				
	SD						**987**				
13	DOSE200	4	male	negative	2	32	6053				
14	DOSE200	4	male	negative	2	32	4261				
15	DOSE200	4	male	1,88	2	32	3730			4682	1217,32
16	DOSE200	4	female	negative	2	32	6411				
17	DOSE200	4	female	negative	2	32	8327				
18	DOSE200	4	female	negative	2	32	8231			7656	1079,78
	mean						**6169**				
	SD						**1927**				
19	DOSE1000	4	male	negative	2	32	5647				
20	DOSE1000	4	male	negative	3	64	17220				
21	DOSE1000	4	male	negative	2	32	13846			12238	5951,56
22	DOSE1000	4	female	negative	3	64	25730				
23	DOSE1000	4	female	negative	3	64	25445				
24	DOSE1000	4	female	4,01	2	32	19536			23570	3497,11
	mean						**17904**				
	SD						**7589**				

[redacted] Pool Number	Dose kg/BID	Day	Sex	Antibody titer -ELISA, [redacted] provided by sponsor [pg/mL]	Number of freeze/thaw at [redacted]	Sample dilution factor	Biological active AVE0010 concentration cell assay [redacted]-[pg/mL]	Mean titer per sex	SD	Mean BAC per sex	SD
25	DOSE0	86	male	negative	2	1	0				
26	DOSE0	86	male	negative	2	1	0				
27	DOSE0	86	male	1.50	2	1	0	0		0	
28	DOSE0	86	female	2.25	2	1	0				
29	DOSE0	86	female	2.96	2	1	0				
30	DOSE0	86	female	3.57	2	1	0	2.92	0.66	0	0
	mean			**1.74**			**0**				
	SD			**1.45**			**0**				
31	DOSE40	86	male	2947	1	1	577				
32	DOSE40	86	male	2899	5*	640†	127319				
33	DOSE40	86	male	718	5*	640†	288411	2188	1273	138769	144258
34	DOSE40	86	female	2758	1	1	60				
35	DOSE40	86	female	12359	1	1	0				
36	DOSE40	86	female	2938	1	1	312	6018	5492	124	165
	mean			**4103**			**69447**				
	SD			**4137**			**118705**				
37	DOSE200	86	male	1509	5	8 000†	708860				
38	DOSE200	86	male	1451	5	8 000†	652500				
39	DOSE200	86	male	624	5	8 000†	1076558	1194	495	812640	230291
40	DOSE200	86	female	2877	5	8 000†	669986				
41	DOSE200	86	female	2539	5	8 000†	1239334				
42	DOSE200	86	female	2910	5	8 000†	1111645	2775	205	1006988	298754
	mean			**1985**			**909814**				
	SD			**930**			**261241**				
43	DOSE1000	86	male	1077	4*	640†	367892				
44	DOSE1000	86	male	535	4	8 000†	739747				
45	DOSE1000	86	male	2705	4	8 000†	1590270	1439	1129	899303	626615
46	DOSE1000	86	female	2720	4	8 000†	1418808				
47	DOSE1000	86	female	411	4*	640†	256265				
48	DOSE1000	86	female	593	4	640†	768330	1241	1284	814468	582643
	mean			**1340**			**856885**				
	SD			**1087**			**543145**				

(b) (4) Pool Number	Dose kg/BID	Day	Sex	Antibody titer -ELISA, (b) (4) provided by sponsor [pg/mL]	Number of freeze/thaw at (b) (4)	Sample dilution factor	Biological active AVE0010 concentration cell assay (b) (4) [pg/mL]	Mean titer per sex	SD	Mean BAC per sex	SD
49	DOSE0	359	male	2.94	2	1	0				
50	DOSE0	359	male	3.82	2	1	0				
51	DOSE0	359	male	22.0	2	1	0	10	11	0	0
52	DOSE0	359	female	2823	2	1	0				
53	DOSE0	359	female	5502	2	1	0				
54	DOSE0	359	female	19158	2	1	0	9161	8760	0	0
	mean			**4585**			**0**				
	SD			**7471**			**0**				
55	DOSE40	359	male	1972	1	1	339				
56	DOSE40	359	male	469	5	640†	129743				
57	DOSE40	359	male	467	1	1	425	969	868	43502	74687
58	DOSE40	359	female	6719	1	1	0				
59	DOSE40	359	female	9703	1	1	53				
60	DOSE40	359	female	2769	1	1	348	6397	3478	133	187
	mean			**3683**			**21818**				
	SD			**3739**			**52872**				
61	DOSE200	359	male	1875	5*	640†	503007				
62	DOSE200	359	male	485	5*	640†	212368				
63	DOSE200	359	male	291	5*	640†	409724	884	864	375033	148393
64	DOSE200	359	female	2478	5	8 000†	733498				
65	DOSE200	359	female	2049	5*	640†	492234				
66	DOSE200	359	female	3011	5*	640†	269864	2513	482	498532	231881
	mean			**1698**			**436782**				
	SD			**1090**			**186792**				
67	DOSE1000	359	male	2472	4	8 000†	969947				
68	DOSE1000	359	male	121	4	8 000†	997607				
69	DOSE1000	359	male	617	4	8 000†	942998	1070	1239	970184	27305
70	DOSE1000	359	female	1638	4	8 000†	2309948				
71	DOSE1000	359	female	106	4*	640†	36959				
72	DOSE1000	359	female	2778	4	8 000†	1604033	1507	1340	1316980	1163366
	mean			**1289**			**1143582**				
	SD			**1179**			**760096**				

Note: LLOQ set at 40 pg/ml and ULOQ set at 800 pg/ml
* Interpolated results from a run failing because of Run QCs.
† See tab "Results for repeated runs" in final results.
For BAC: < LLOQ was set as 0,00

Histopathology Inventory for IND #62,724

Study Type	Carcinogenicity Studies	
Study Number	CAR0085	CAR0084
Species	MOUSE	RAT
Adrenals	X	X
Aorta	X	X
Bone Marrow (in bone sections)	X	X
Brain	X	X
Cecum	X	X
Clitoral gland	X	X
Colon	X	X
Duodenum	X	X
Epididymides	X	X
Esophagus	X	X
Eyes with optic nerve	X	X
Femur with bone marrow and femorotibial joint	X	X
Gall bladder	X	
Gross lesions	X	X
Harderian gland	X	X
Heart	X	X
Injection sites	X	X
Ileum	X	X
Jejunum	X	X
Kidneys	X	X
Lachrymal gland	X	X
Larynx		
Liver	X	X
Lung	X	X
Lymph nodes, submandibular	X	X
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity (level III)	X	X
Ovaries with oviduct	X	X
Pancreas	X	X
Peyer's patches	X	X
Pharynx		
Pituitary	X	X
Preputial gland	X	X
Prostate	X	X
Rectum	X	X
Salivary gland (parotid, mand bular, and sublingual)	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle (diaphragm and quadriceps femoris)	X	X
Skin	X	X
Spinal cord (cervical, thoracic, lumbar)	X	X
Spleen	X	X
Sternum with bone marrow	X	X
Stomach	X	X
Testes	X	X
Thymus	X	X
Thyroid + parathyroid	X	X
Tongue	X	X
Trachea	X	X
Ureters	X	X
Urinary bladder	X	X
Uterus + cervix	X	X
Vagina	X	X
Zymbal gland (collected by not assessed)		

X, histopathology performed

9 Reproductive and Developmental Toxicology

Dose Range-Finding Studies

Study	Species	Treatment	Findings
DSE2003-1923 Lot #1022/3330 Segment 2 range finding GLP	Rat /Sprague-Dawley 6 pregnant females/group	0, 0.14, 2, and 4 mg/kg/d Subcutaneous Twice daily GD6-17; necropsy on GD20	• Long lasting hypoactivity and piloerection at all dose levels. • Decreased FC and BW gain at all doses. • Decreased embryofetal death (early resorption or dead fetus) at ≥2 mg/kg/d. • Decreased fetal BW, crown-rump length, and placental weight at all dose levels. • No macroscopic malformations were observed; visceral and skeletal assessments were not conducted. • The NOAEL for maternal and embryofetal toxicity is <0.14 mg/kg/d.
DSE2003-1924 Lot #1022/3330 Segment 2 range finding GLP	Rabbit / Himalayan 6 pregnant females/group	0, 0.08, 0.4, and 2 mg/kg/d Subcutaneous Twice daily GD6-18; necropsy on GD29	• Hypoactivity, piloerection, and lack of appetite seen at all dose levels. • Decreased FC and BW at all dose levels. • Two premature deliveries at HD on GD24; preceded by lack of appetite, diarrhea, and bristling coat for 3 days. • No effect on resorptions, live litter sizes, or placental weights. • Slightly reduced fetal BW at 2 mg/kg/d. • At LD, 1 fetus showed thoracogastroschisis and related other visceral and skeletal malformations. This was considered to be treatment related as anomalies of a similar type were also observed in few main study fetuses. • The NOAEL for maternal and embryofetal toxicity is <0.08 mg/kg/d.
DPP0025 Segment 3 range finding GLP	Rat /Sprague-Dawley 6 pregnant females/group	0, 0.005, 0.07, and 1 mg/kg/d Subcutaneous Twice daily GD6-LcD3; necropsy on LcD4	• Decreased motor activity and piloerection at all dose levels. • Initial decreased FC and BW loss followed by ↓ BW gain at all dose levels. • Pregnancies, gestation length, and births were not affected by treatment. • Nursing behavior and suckling of pups was slightly decreased and increased cannibalization at all dose levels. • Pup mortality slightly higher at 1 mg/kg/d. • 1 LD pup had multiple skeletal malformations of shoulder girdle and long bones of forelimbs and wavy ribs; 3 HD pups had sternebrae anomalies. 2 HD pups showed necrosis of tail tip. • The NOAEL for maternal and embryofetal toxicity is <0.005 mg/kg/d.

BW = body weight; FC = food consumption; GD = gestation day; GLP = Good Laboratory Practice; HD = high dose; LD = low dose; LcD = lactation day.

9.1 Fertility and Early Embryonic Development

Study title: AVE0010: Subcutaneous Study of Fertility and Early Embryonic Development in the Rat

Study number:	DSE 2004-0550, Amendment 1 (Sanofi) (b) (4)
Study report location:	Module 4.2.3.5.1
Conducting laboratory:	(b) (4)
Date of study initiation:	27 April 2004 (males) and 11 May 2004 (females)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, batch #PPL-AVE100401, and 95.3% pure

Key Study Findings

- Minor treatment-related effects were noted in males and females that included subdued behavior after dosing, a dose-related reduction in male body weight gain in the pre-mating period, and a dose-related reduction in male and female food consumption during the pre-mating period and early gestation.
- There were no adverse effects on mating, fertility, or early embryonic development at the dose levels tested.

Methods

Doses:	0, 4, 58, and 828 µg/kg/day, divided into 2 daily doses
Frequency of dosing:	Twice daily, 8 hours apart
Dose volume:	0.5 mL/kg/dose
Route of administration:	Subcutaneous
Formulation/Vehicle:	Stock solution: 50 mM sodium citrate, pH 5.3 Control and diluting solution: 0.9% NaCl
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	24/sex/group
Satellite groups:	None
Study design:	Males were treated for 4 weeks before pairing with females from the same dose group, throughout the pairing period, and until the day before necropsy after completion of the female necropsies. Females were treated for 2 weeks before being paired with males from the same dose group and continued throughout pairing and until Day 6 of gestation, inclusive. On Day 13 of gestation, females were killed, examined macroscopically, and the uterine contents examined.

Reason for report amendment: The report issue date was incorrect. The correct date is January 2005, not 2004.
Deviation from study protocol: There were no deviations from the protocol that impacted the integrity of the study.

Observations and Results

Mortality (twice daily)

All animals survived until the scheduled kill.

Clinical Signs (immediately, 1, 2, and 4 hours after first dose and immediately after second dose; detailed observations on body weight measurement days)

In males, subdued behavior was noted 0.5 hours after dosing in all males from Week 3 (LD group) and from Week 2 (MD and HD groups). These signs persisted for up to 1 hour is some animals on some occasions.

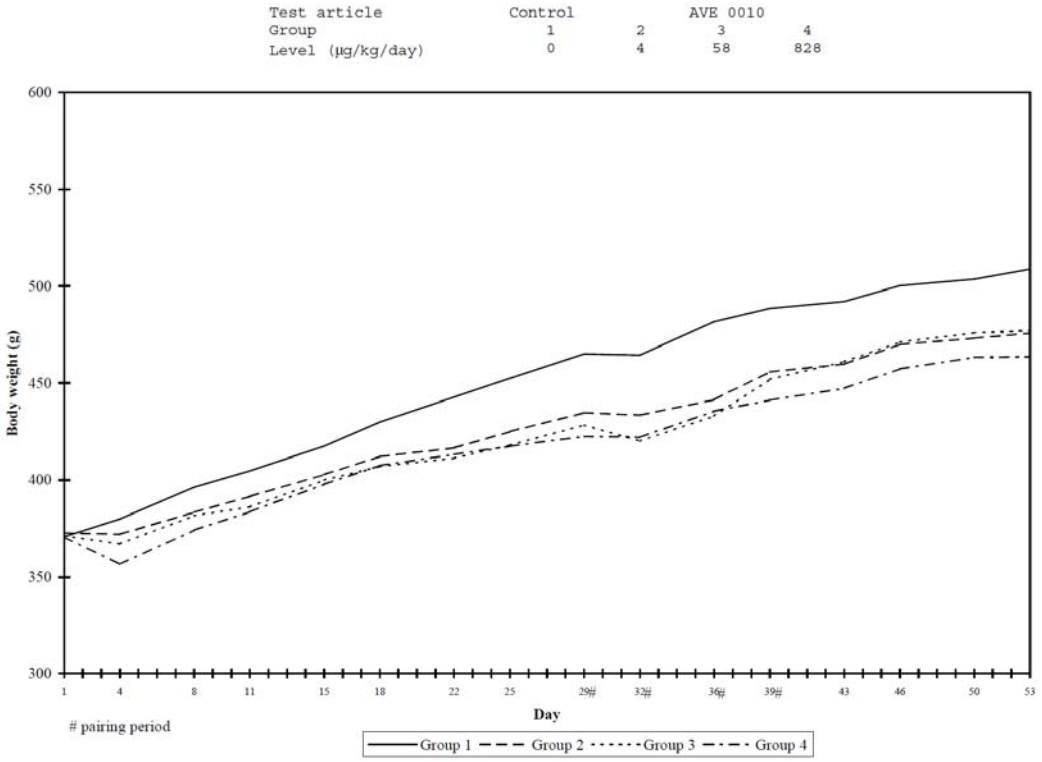
If females, subdued behavior was noted at 0.5 hours after dosing throughout the 2 weeks of the pre-pairing period in all treated groups and also was occasionally noted during the gestation period. As with males, these signs persisted for up to 1 hour after dosing.

Body Weight (twice weekly [males]; twice weekly prior to mating and until confirmation of mating and on Days 0, 3, 6, 10, and 13 of gestation [females])

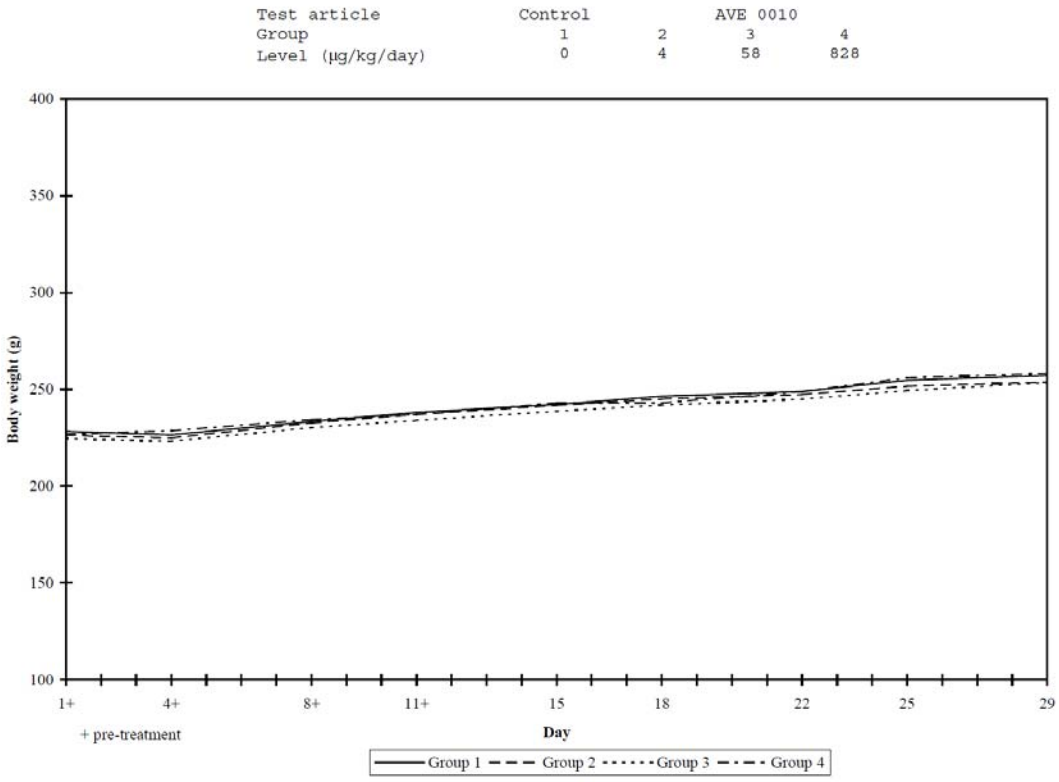
Initial treatment-related body weight loss followed by decreased body weight gain was observed in males as shown in the sponsor-generated figure below. The effect on body weight gain correlated with decreases in food intake.

A meaningful effect on female mean body weights was not observed during the pre-pairing or gestation periods, even though a slight decrease in food consumption was observed (sponsor-generated table).

Males



Females: pre-pairing



Feed Consumption: (same intervals as body weights prior to pairing [males and females] and during gestation [females only])

A treatment-related decrease in food consumption was observed in males for the first 3 days, after which time all treated groups had a similar decrease compared with the control group.

A similar decrease in food consumption was observed in females during the 2-week pre-mating period and first 6 days of gestation, with the greatest effect noted during the first 3 days of treatment.

Toxicokinetics: (not conducted)

Dosing Solution Analysis: (beginning, middle, and end of dosing period)

Results of the dose formulation analysis for the beginning and middle of the study showed that concentrations were higher than the target range (90% to 110% of nominal) due to leakage of the vessels used to store the dose formulation samples. The vessels used for formulation collection at the end of the study were changed to prevent leakage. These later samples showed that the formulations at the end of the dosing period were within the range of 93.1% and 108.7% of nominal. The leakage of formulation samples is not felt to impact the interpretability of the study results.

Necropsy: (Caesarian section on Day 13 of gestation [females] and Week 9 [males])

Fertility parameters:

All animals mated and there was no meaningful difference in the time to mating between groups. There was no statistically significant difference in mating, fertility, or fecundity indices compared with the control group.

There were no statistically significant differences in the number of corpora lutea, number of implantations, number of embryos, pre-implantation loss, or post-implantation loss compared with control.

Macroscopic evaluation:

There were no apparent treatment related macroscopic findings in males or females.

9.2 Embryonic Fetal Development

Study title: AVE0010: Subcutaneous embryo-fetal toxicity study in rats

Study no.:	DSE 2004-0551
Study report location:	Module 4.2.3.5.2
Conducting laboratory:	Aventis Pharma Deutschland GmbH, Test Facility Kastengrund, 65926 Frankfurt am Main, Germany
Date of study initiation:	23 June 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, lot # (b) (4), and 84.4% pure

Key Study Findings

- Maternal effects included decreased motor activity, sleepiness, decreased reactivity, piloerection, reduced food consumption and an initial dose-dependent decrease in body weights at all dose levels.
- Fetuses showed a trend towards retarded growth and retarded ossification in the two lower dose groups, and fetal growth and skeletal ossification were significantly retarded at the HD. A single case of fetal malformations was observed for each dose level: one microphthalmia (0.005 mg/kg), one anophthalmia and one diaphragmatic hernia (one fetus each at 0.07 mg/kg) and similar multiple skeletal malformations in one retarded fetus of each dose group. All malformation data exceed the historical ranges and mean values.
- The NOAEL for maternal and developmental toxicity of AVE0010 is <0.005 mg/kg/d, the lowest dose tested.

Methods

Species/Strain:	Rat/Sprague-Dawley
Frequency of dosing:	Twice daily, approximately 8 hours apart
Dose volume:	0.5 mL/kg/dose
Route of administration:	Subcutaneous
Formulation/Vehicle:	0.9% NaCl

Study Design: (sponsor-generated table)

Group number	Dose level (mg/kg/day)	Mated study animals		Toxicokinetic animals	
		Mated	Pregnant	Mated	Pregnant
1	0	32*	20	3	2
2	0.005	30*	21	15	11
3	0.07	30*	21	15	12
4	1	30*	16	15	10

Pregnant females were treated from GD 6 through GD 17. Cesarean sections occurred on GD 20.

Study protocol deviations: The study director certified that no unforeseen circumstances were observed that might have affected the quality or integrity of the study.

Observations and Results

Mortality: (twice daily on weekdays and once daily on weekends)

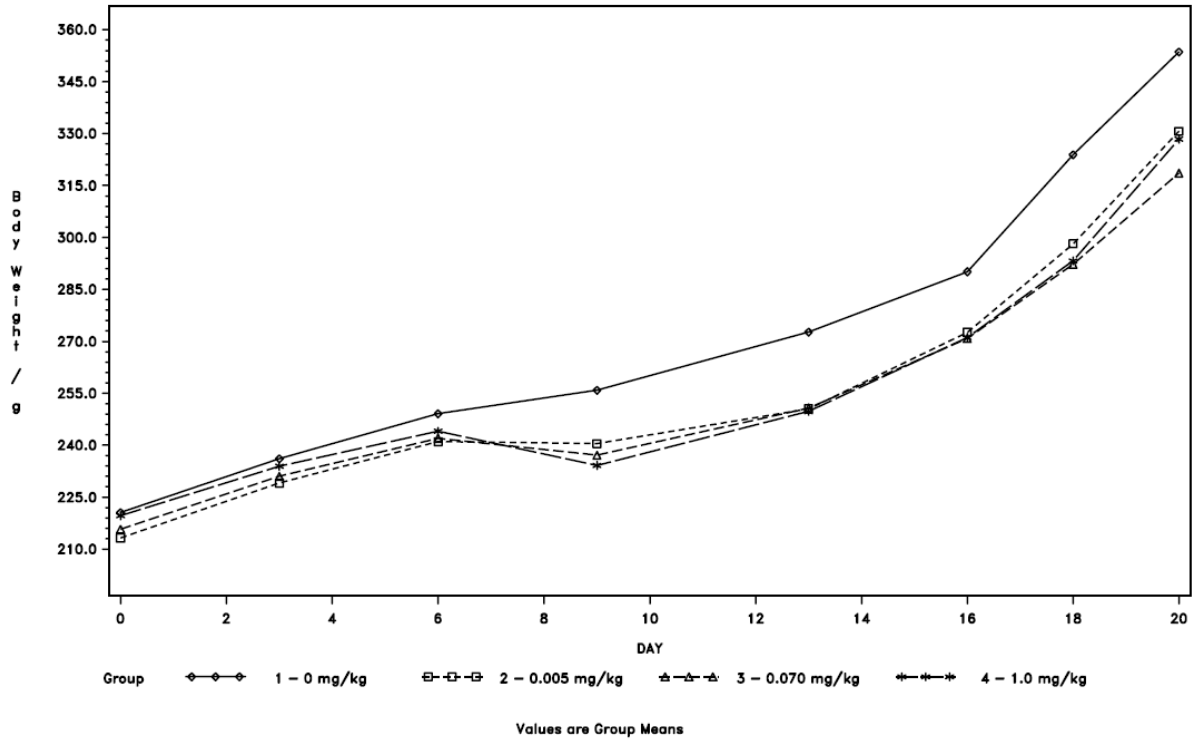
There were no mortalities

Clinical Signs: (twice daily on weekdays and once daily on weekends)

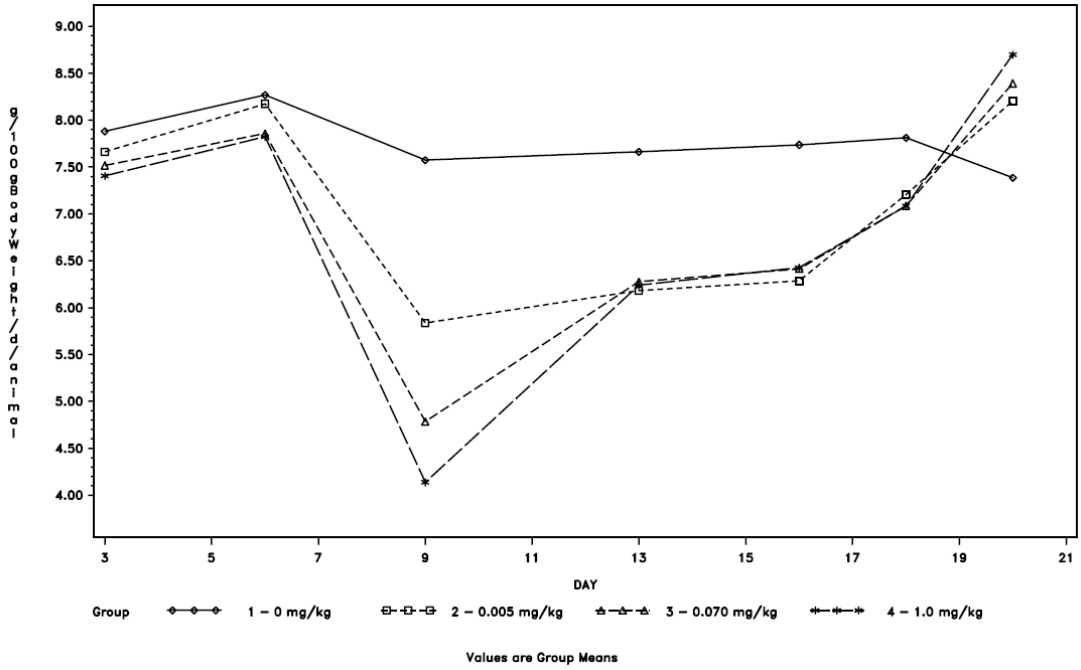
Decreased motor activity in connection with sleepiness and decreased reactivity were observed in all animals of all treated groups from the first day to the last day of dosing. These effects started approximately 15 minutes after dosing and continued after the pm administration, although the clinical signs were not observed in the morning of the next day prior to treatment. Severity of symptoms appeared to be slightly dose dependent.

Body Weight: (GD 0, 3, 6, 9, 13, 16, 18, and 20)

Treatment resulted in an initial body weight loss between GD 6 and 9, after which time body weight gain was similar to controls. For the MD and HD groups, mean body weights were approximately 6% less than control weight on GD16 and up to 10% less on GD18 and GD20. As shown in the sponsor-generated figure below, body weight effects correlated with decreased food consumption, a known pharmacological effect of this drug class.



Feed Consumption: (GDs 0-3, 3-6, 6-9, 9-13, 13-16, 16-18, and 18-20)



Toxicokinetics: (GD 12/13 at 0.25, 1, 3, and 8 hours after the first dose and 0.25, 1, 3, 8, or 16 hours after the second dose)

Values following the two doses on Day 7 of the treatment period					
Dose mg/kg per day		AVE0010			
		C_{max} [ng/mL]	t_{max} [hr]	C_{24hr} [ng/mL]	$AUC_{(0-24hr)}$ [ng*hr/mL]
0.005		3.5	0.25	< 0.05	3.9
0.07		18.8	0.25	< 0.05	49.4
1.0		150.8	0.25	0.06	581.4

Dosing Solution Analysis

A sample of 3 mL of each formulation (including controls) was taken after the last a.m. injection on the first and the last day of administration in the main study and on day 1 and 7 of administration in the TK part of the study. The results of all concentration analyses met the acceptance criteria of 90% to 110% of the nominal concentrations.

Necropsy

No compound-related macroscopic changes were observed in any of the dosed animals.

Cesarean Section Data

There were no treatment-related effects on the number of corpora lutea, number of implantation sites, pre-implantation loss, post-implantation loss, or number of live fetuses, or uterus weight.

Offspring

Fetuses from the high-dose group were statistically significantly smaller, both with regard to body weight and crown-rump length (combined genders only). The sponsor-generated table below summarizes the fetal weights.

Effects on fetal weight

Parameter	Control	0.005 mg/kg	0.07 mg/kg	1 mg/kg
Fetuses / litter	14.0 ± 2.9	14.0 ± 2.0	12.5 ± 4.2	14.6 ± 1.8
Mean fetal body weight	3.41 ± 0.43	3.28 ± 0.27	3.35 ± 0.34	3.06 ± 0.14*
Number of live fetuses weighing less than 3 g	10.0 [a] 55 [b]	19.7 [a] 47.6 [b]	15.9 [a] 47.6 [b]	37.3 [a] 100 [b]
Number of live fetuses weighing less than 2.5 g	1.07 [a] 15 [b]	3.40 [a] 33.3 [b]	2.65 [a] 28.6 [b]	2.58 [a] 25.0 [b]

* significantly lower than control a = % of total number of live fetuses b = % litters affected

Morphological examination showed unilateral microphthalmia and bilateral anophthalmia in one LD and one MD group fetus, respectively, and one diaphragmatic hernia in another MD group fetus. There was a very small fetus in each of the LD and MD groups (2.6 g and 1.9 g respectively); the MD fetus also showed general edema. One HD fetus weighing 1.6 g showed incomplete ossification or no ossification of the majority of bones. In addition to decreased ossification, these latter three fetuses also exhibited similar multiple skeletal malformations and minor skeletal anomalies. Typically, all or almost all long bones of the fore and hindlimbs were short and bent up to 90° at the locus of weakest ossification, i.e., the middle of these bones. Also noted in these three fetuses were bent and short scapulae, bent scapular spine, and misshapen clavicle. The pelvic girdle of the MD fetus was bent on both sides.

Although these findings did not occur in a dose-related manner, all of these findings were considered by the study director to be related to treatment based on the very rare occurrence of these malformations in the historical control database from the laboratory. In a total of 5350 control fetuses from 39 studies, there were three cases of microphthalmia and once case of anophthalmia. No diaphragmatic hernia was observed in these historical controls. Other skeletal effects were noted (see sponsor-

generated table below) at all dose levels, although the effect was most noteworthy at the high dose. These skeletal effects may have resulted from the treatment-related effects on maternal food consumption and body weight. Other developmental malformations occurred as single findings and were observed with a similar frequency as the concurrent control group or in historical controls, and therefore were not considered to be treatment related.

Effects on skeletal development (sponsor-generated table)

Parameter		Control	0.005 mg/kg	0.07 mg/kg	1 mg/kg
Skull bones: incomplete or not ossified	% fetuses % litters	0.7 5.0	3.3 19.0	1.5 9.5	3.3 25.0
Metacarpal 5 unossified	% fetuses % litters	3.5 20	7.8 33.3	6.7 33.3	15.7* 56.3
Sternebra: incomplete or not ossified	% fetuses % litters	20.3 75.0	34.6 76.2	35.6 76.2	57.9* 93.8
Caudal Vert. Centra: ossification of less than 4.	% fetuses % litters	19.6 60.0	34.0 76.2	36.3 66.7	61.2* 93.8

* Significantly higher than control

A dose-related decrease in the incidence of short or full supernumerary 14th rib was also observed (15.4%, 13.7%, 8.1%, and 4.1% in the control, LD, MD, and HD groups, respectively).

Study title: AVE0010: Subcutaneous embryo-fetal toxicity study in rabbits

Study no.:	2004-0552
Study report location:	Module 4.2.3.5.2
Conducting laboratory:	Aventis Pharma Deutschland GmbH Drug Safety Evaluation Mainzer Landstrasse 500 65795 Hattersheim, Germany
Date of study initiation:	9 June 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, batch # (b) (4), 84.4% pure

Key Study Findings

- Maternal toxicity consisted of dose-dependent decreases in mean body weight associated with reduced food consumption, decreased feces, reduced motor activity, and piloerection at all three dose levels.
- A statistically significant increase in post-implantation loss occurred for the 0.5 mg/kg/day dose group.
- There were 2 fetuses each from the 0.005 and 0.05 mg/kg/d groups and 1 fetus from the 0.5 mg/kg/d group with multiple visceral and skeletal malformations. Other malformations in addition to the ones observed in these 5 fetuses included absent or small gallbladder, cardiac ventricular septum defect, and anomalies of the sternebrae. Treatment-related minor defects included lung abnormalities, hyperflexion of forepaws, supernumerary 13th rib, and ossification of less than 15 caudal vertebral centers.
- TK results showed that AUC increased in a dose-proportional manner.
- The NOAEL was considered to be <0.005 mg/kg/d for both maternal and developmental toxicity in the rabbit.

Methods

Species/Strain:	Rabbit / Chbb: HM (SPF) (b) (4)
Doses:	0, 0.005, 0.05, and 0.5 mg/kg/day
Frequency of dosing:	Twice daily at an interval of approximately 8 hours
Dose volume:	2.5 mL/kg
Route of administration:	Subcutaneous
Formulation/Vehicle:	0.9% NaCl
Number/Sex/Group:	20 pregnant females/group
TK groups:	3 (control) or 9 pregnant females/group
Study design:	Dosing occurred from GD6 through GD18; main study animals were euthanized and necropsied on GD29.
Study protocol deviations:	The study director attested that there were no unforeseen circumstances that may have affected the quality or integrity of the study.

Observations and Results

Mortality (twice daily on weekdays and once daily on weekends)

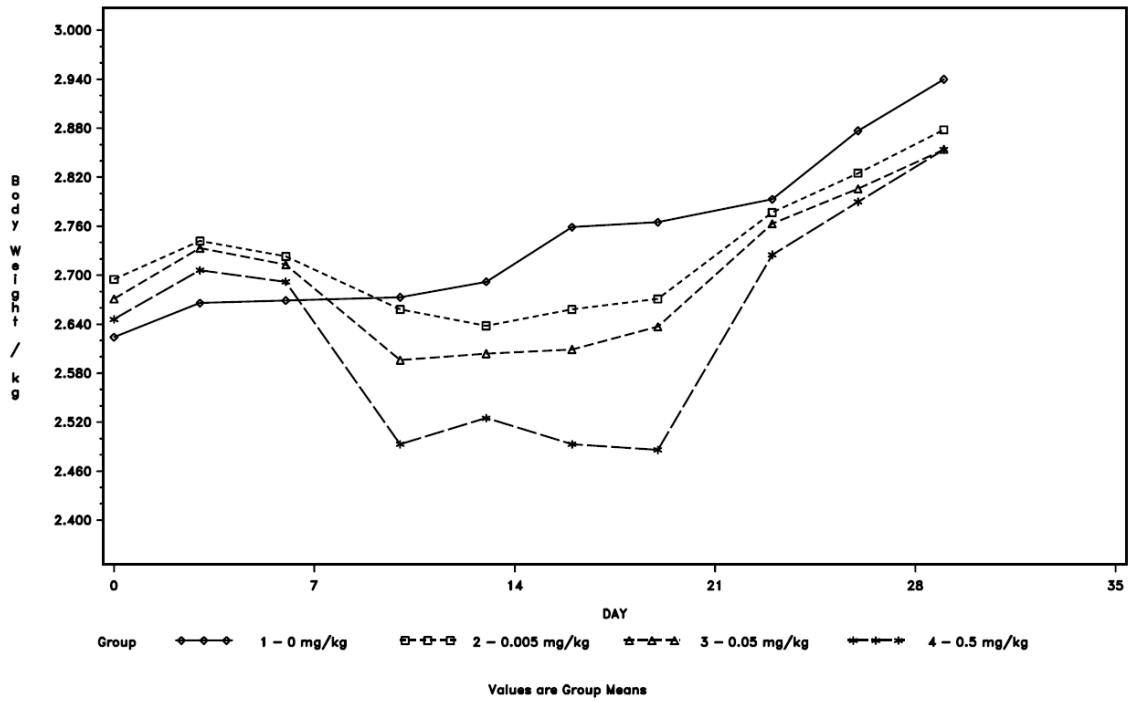
One MD female delivered prematurely, and therefore was euthanized early. There were no other unscheduled deaths.

Clinical Signs (twice daily on weekdays and once daily on weekends)

Reduced feces, decreased food consumption, decreased motor activity, and piloerection were noted in all treatment groups during the dosing period.

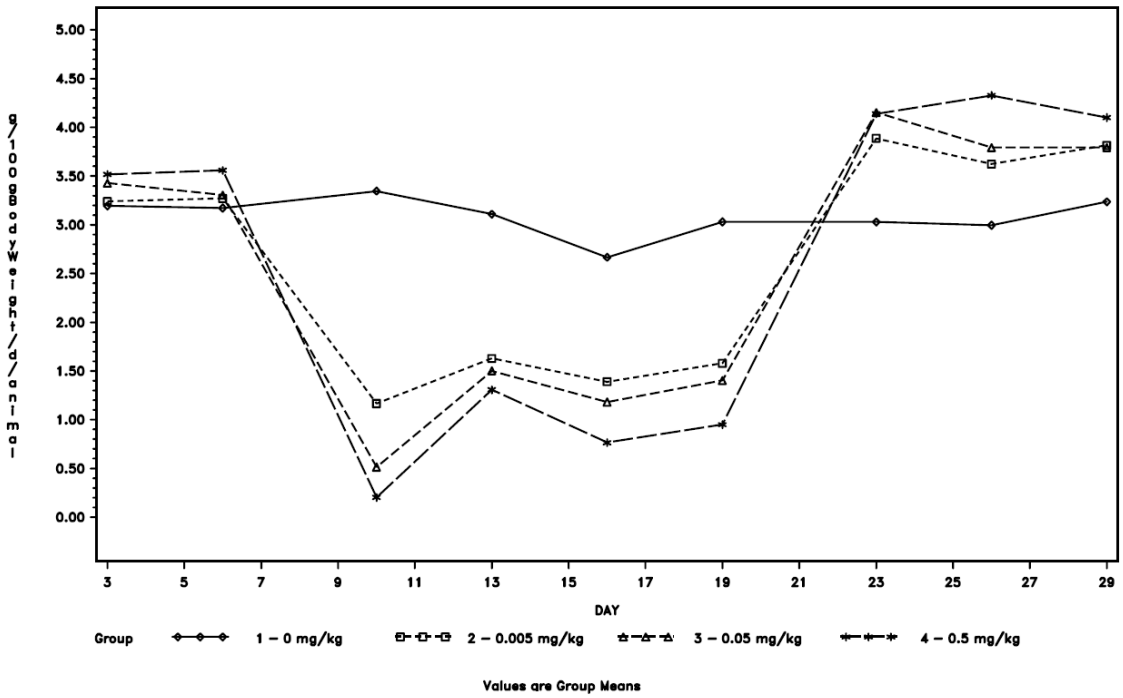
Body Weight (GD 0, 3, 6, 10, 13, 16, 19, 23, 26, and 29)

As shown in the sponsor-generated figure below, treatment resulted in body weight loss for all dose groups. Body weights were statistically significantly different from control starting at Day 10 (HD), Day 13 (MD), and Day 16 (LD). On Day 19, mean body weights for the LD, MD, and HD groups were 3%, 5%, 10% less than control weights, respectively. No significant differences in mean body weight were observed after GD19.



Feed Consumption (GDs 0-3, 3-6, 6-10, 10-13, 13-16, 16-19, 19-23, 23-26, and 26-29)

As shown in the sponsor-generated figure below, mean food consumption was statistically significantly reduced during the dosing period (GD6-19). Once dosing ended, there was a statistically significant increase in food consumption for all dose groups (GD19-29).



Toxicokinetics

Samples were taken at 0.25, 1, 3, and 8 hours after the a.m. dose and 0.25, 3, 8, and 16 hours after the p.m. dose on GD 12/13. Control animals were sampled 15 minutes after dosing.

Values following the two doses on day 7					
Dose mg/kg per day		AVE0010			
		C_{max} [ng/mL]	t_{max} [hr]	C_{24hr} [ng/mL]	$AUC_{(0-24hr)}$ [ng*hr/mL]
0.005		4.14	1.00	0.05	27.2
0.05		48.83	1.00	0.15	292.5
0.5		499.70	3.00	1.43	3899.2

(Sponsor-generated table)

Dosing Solution Analysis

A sample of 3 mL of each formulation (including controls) was taken after the last a.m. injection on the first and the last day of administration in the main part of the study and on Day 1 and 8 of administration in the TK part of the study. The results of all concentration analyses were found to be within the acceptance criteria (90% to 110% of nominal).

Necropsy

There were no treatment-related macroscopic findings.

Pregnancy Data

One MD female delivered on Day 29 prior to scheduled necropsy. This doe had 8 corpora lutea, 6 implantation sites, and 6 dead fetuses; 4 were found on the fecal tray and 2 were still in the uterus. Another MD female had total post-implantation loss. This doe had 3 corpora lutea and only 1 implantation site that was absorbed early. These incidences were considered to be spontaneous and are consistent with corresponding background data. The data from these two does were not included in the sponsor-generated table below.

A dose-related increase in total post-implantation loss was noted, with statistical significance occurring at the HD. This resulted in a statistically significant decrease in the absolute number of live fetuses and number of live fetuses compared with the number of implantation sites at the HD level.

Summary of Female Cesarean Section Data (Excluding Females with Total Litter Loss)

Dose mg/kg		Corpora Lutea	-Preimplantation- -Loss- Absolute (c)	-Preimplantation- -Loss- %Corpora Lutea	Implant Sites (c)	Postimplantation Loss Resorptions Early	Postimplantation Loss Resorptions Late	Postimplantation Loss Dead Fetuses	Postimplantation Loss Absolute Total (i)	Postimplantation Loss %Implantations	Live Fetuses Absolute (i)	Live Fetuses %Implantations
Group 1	MEAN	8.1	0.1	1.3	8.0	0.4	0.0	0.0	0.37	4.4	7.6	95.6
0	STD	1.5	0.3	4.0	1.6	0.8	0.0	0.0	0.76	8.8	1.6	8.8
	MEDIAN	8.0	0.0	0.0	8.0	0.0	0.0	0.0	0.00	0.0	7.0	100.0
	N	19	19	19	19	19	19	19	19	19	19	19
Group 2	MEAN	8.2	1.1	13.8	7.1	0.4	0.2	0.0	0.58	8.8	6.5	91.2
0.005	STD	2.2	1.8	23.6	2.9	0.7	0.5	0.0	0.77	16.2	2.8	16.2
	MEDIAN	9.0	0.0	0.0	8.0	0.0	0.0	0.0	0.00	0.0	7.0	100.0
	N	19	19	19	19	19	19	19	19	19	19	19
Group 3	MEAN	8.2	1.0	13.0	7.2	0.6	0.1	0.0	0.63 NS	7.1	6.6 NS	92.9
0.05	STD	2.0	1.3	20.8	2.4	1.0	0.3	0.0	0.96	10.1	2.0	10.1
	MEDIAN	8.0	1.0	9.5	7.0	0.0	0.0	0.0	0.00	0.0	7.0	100.0
	N	16	16	16	16	16	16	16	16	16	16	16
Group 4	MEAN	8.7 NS	0.5 NS	6.5	8.2 NS	1.1	0.1	0.2	1.35 *	18.8	6.8 *	81.2
0.5	STD	1.9	1.1	14.5	2.4	1.9	0.3	0.4	1.90	23.4	3.1	23.4
	MEDIAN	8.5	0.0	0.0	8.0	0.0	0.0	0.0	1.00	12.7	7.5	87.3
	N	20	20	20	20	20	20	20	20	20	20	20

Offspring

There were no treatment-related effects on mean crown-rump length, mean live fetal weight, or mean placental weight (see sponsor-generated table below).

There were two fetuses with multiple malformations in each of the LD and MD groups and a single fetus from the HD group with multiple malformations. A summary of major malformations is shown in the table below.

With regard to major skeletal malformations, there was a trend for an increased number of fetuses with anomalies of sternebrae in the MD group and a statistically significant increase in such anomalies in the HD group. Two fetuses from the LD group showed aplasia of the 4th rib and a short 1st rib, respectively and two additional LD fetuses had fused 15th and 16th caudal vertebral centers.

These major defects were considered to be treatment related. A summary of major malformations and minor defects are presented in the sponsor-generated tables below.

Summary of Major Malformations

Dose Group (mg/kg/day)	Animal Number	Major Malformation
0.005	202 R04	- Low weight (10.3 g) - Thorocogastroschisis with short, partly rudimentary trunk (rudimentary part of trunk not covered by the skin, thoracic cavity and abdominal cavity not formed), and amelia of both forelimbs. - Multiple skeletal malformations mainly on the skull, trunk (all ribs and sternebrae absent and thoracic vertebral column rudimentary) and on shoulder girdle.
	213 R04	- Spina bifida with dorsad opened lumbar vertebral arches - Skeletal anomalies on head (small and large holes in frontals and parietals, respectively), fused interparietal and supraoccipital bones and fused sternebrae.
0.05	229 L01	- Markedly stunted growth (3.0 g) - Developed only head, neck, and part of viscera. The trunk, body cavities and shoulder and pelvic girdles with fore and hindlimbs were not formed. - Bilaterally open eyes with bilateral aphakia, protruding tongue, misshapen heart, malpositioned main arterial vessels and absence of several organs. - Malformed bones of head and rudimentary cervical vertebral column; all other parts of the skeleton absent.
	235 R02	- Low weight (15.3 g) - Exencephaly, protruding tongue, short trunk with thorocogastroschisis, amelia of both forelimbs, and adactyly on both hindpaws. - The neck and thoracic part of the trunk were rudimentary, partly not covered by skin and thoroacic cavity not formed. - Microphthalmia and aphakia of the right eye. - Absence of both subclavian arteries, malposition of main arteries and absence of diaphragm. - Multiple skeletal malformations of the head, trunk (mainly rudimentary cervical vertebral column, absence of part of the thoracic vertebrae and ribs, and all sternebrae) and on the shoulder girdle and paws of the hindlimbs.
	230 R01	- Aplasia of the gallbladder*

Dose Group (mg/kg/day)	Animal Number	Major Malformation
0.5	252 L01	- Omphalocele and fused sternebrae, supernumerary full 13th ribs on both sides, and less than 15 ossified caudal vertebral centra.
	238 L01	- Cardiac ventricular septum defect
	245 R06	- Aplasia of the gallbladder*
	256 R01	- Aplasia of the gallbladder*

*Three other fetuses from the MD and HD group had small gall bladder.

Summary of Female Cesarean Section Data (Excluding Females with Total Litter Loss)

Dose mg/kg		-------Live Fetuses------- Total (i)	Percent Males	---Crown-Rump Length (mm)--- Male	Female	Total	----Mean Live Fetal Wt (g)---- Male (f)	Female (f)	Total (f)	Placental Wt (g)
Group 1	MEAN	7.6	46.2	94.6	94.0	94.4	40.20	39.67	40.01	4.95
0	STD	1.6	18.7	3.4	3.3	3.0	3.45	2.92	2.83	0.61
	MEDIAN	7.0	50.0	95.6	94.3	94.7	40.33	39.67	40.07	4.80
	N	19	19	19	19	19	19	19	19	19
Group 2	MEAN	6.5	53.6	95.3	95.1	95.6	41.16	39.91	41.04	5.31
0.005	STD	2.8	27.4	4.5	3.8	4.1	4.81	4.61	4.69	1.16
	MEDIAN	7.0	55.6	95.3	95.3	95.7	40.86	38.97	39.44	5.13
	N	19	19	18	17	19	18	17	19	19
Group 3	MEAN	6.6 NS	50.6	96.2	95.7	95.2	40.07	38.86	39.28	5.02
0.05	STD	2.0	31.1	2.7	3.4	3.4	3.33	3.15	2.95	0.71
	MEDIAN	7.0	50.0	96.8	96.5	95.0	40.11	39.54	39.25	4.82
	N	16	16	13	14	15	14	14	16	16
Group 4	MEAN	6.8 *	45.2 NS	95.6	96.0	95.8 NS	40.71 NS	40.53 NS	40.35 NS	5.11 NS
0.5	STD	3.1	21.0	2.3	3.1	2.6	3.47	3.38	3.15	0.62
	MEDIAN	7.5	50.0	95.3	95.3	95.5	40.91	40.55	40.67	5.14
	N	20	20	20	19	20	20	19	20	20

REPORT: EXTERNAL EXAM TYPE: EXTERNAL	CLASSIFICATION	GROUP 1 0 mg/kg NO	%	GROUP 2 0.005 mg/kg NO	%	GROUP 3 0.05 mg/kg NO	%	GROUP 4 0.5 mg/kg NO	%
NUMBER OF FETUSES EXAMINED		145		122		103		135	
NUMBER OF LITTERS EXAMINED		19		19		16		20	
FOREPAWS/DIGITS									
PAW - HYPERFLEXION	MIN	1	0.7	8	6.6 *	2	1.9	5	3.7
		1	5.3	8	42.1	1	6.3	4	20.0
FETUSES WITHOUT ABNORMALITIES	(NE)	144	99.3	114	93.4	101	98.1	130	96.3
LITTERS WITHOUT ABNORMALITIES		18	94.7	11	57.9	15	93.8	16	80.0

REPORT: VISCERAL EXAM TYPE: FIXED HEART AND/OR HEAD	CLASSIFICATION	GROUP 1 0 mg/kg		GROUP 2 0.005 mg/kg		GROUP 3 0.05 mg/kg		GROUP 4 0.5 mg/kg	
		NO	%	NO	%	NO	%	NO	%
NUMBER OF FETUSES EXAMINED		145		122		103		135	
NUMBER OF LITTERS EXAMINED		19		19		16		20	
HEART									
VENTRICULAR SEPTUM - SEPTUM DEFECT	MAL	0	0.0	0	0.0	0	0.0	1	0.7
		0	0.0	0	0.0	0	0.0	1	5.0
FETUSES WITHOUT ABNORMALITIES	(NE)	145	100.0	122	100.0	103	100.0	134	99.3
LITTERS WITHOUT ABNORMALITIES		19	100.0	19	100.0	16	100.0	19	95.0

EXAM TYPE: FRESH VISCERAL	CLASSIFICATION	GROUP 1 0 mg/kg		GROUP 2 0.005 mg/kg		GROUP 3 0.05 mg/kg		GROUP 4 0.5 mg/kg	
		NO	%	NO	%	NO	%	NO	%
LUNG									
LUNG - ABNORMALITIES	MIN	9	6.2	12	9.8	17	16.5	18	13.3
		6	31.6	6	31.6	5	31.3	9	45.0
GALLBLADDER - ABSENT	MAL	0	0.0	0	0.0	1	1.0	2	1.5
		0	0.0	0	0.0	1	6.3	2	10.0
GALLBLADDER - SMALL	MIN	0	0.0	0	0.0	1	1.0	2	1.5
		0	0.0	0	0.0	1	6.3	2	10.0
STOMACH - ABNORMALITIES	MIN	1	0.7	4	3.3	1	1.0	2	1.5
		1	5.3	3	15.8	1	6.3	1	5.0
FETUSES WITHOUT ABNORMALITIES	(NE)	122	84.1	94	77.0	74	71.8	101	74.8
LITTERS WITHOUT ABNORMALITIES		8	42.1	9	47.4	4	25.0	4	20.0

REPORT: SKELETAL EXAM TYPE: SKELETAL	CLASSIFICATION	GROUP 1 0 mg/kg		GROUP 2 0.005 mg/kg		GROUP 3 0.05 mg/kg		GROUP 4 0.5 mg/kg	
		NO	%	NO	%	NO	%	NO	%
NUMBER OF FETUSES EXAMINED		144		122		103		135	
NUMBER OF LITTERS EXAMINED		19		19		16		20	
SKULL									
SKULL - SPLITTING OF BONE	MIN	0	0.0	5	4.1 *	5	4.9 *	0	0.0
		0	0.0	4	21.1	4	25.0	0	0.0
SKULL - SUTURAL BONE	MIN	5	3.5	10	8.2	5	4.9	4	3.0
		3	15.8	4	21.1	4	25.0	3	15.0
SKULL - MALFORMATION	MAL	0	0.0	0	0.0	0	0.0	0	0.0
		0	0.0	0	0.0	0	0.0	0	0.0
SKULL - HOLE, SMALL	MIN	3	2.1	1	0.8	1	1.0	1	0.7
		3	15.8	1	5.3	1	6.3	1	5.0
PARIETAL - ADDITIONAL SUTURE	MIN	1	0.7	7	5.7	2	1.9	1	0.7
		1	5.3	5	26.3	2	12.5	1	5.0

STERNEBRAE											
STERNEBRA - ABNORMALITIES	MAL	12	8.3	14	11.5	19	18.4	32	23.7	*	
		8	42.1	7	36.8	7	43.8	13	65.0		
STERNEBRA - UNOSSIFIED OR INCOMPLETE OSSIFICATION	OSS	57	39.6	36	29.5	25	24.3	39	28.9		
		15	78.9	14	73.7	10	62.5	13	65.0		
RIBS											
RIB - AT 7TH CERVICAL ARCH - SHORT	MIN	7	4.9	2	1.6	1	1.0	1	0.7		
		4	21.1	1	5.3	1	6.3	1	5.0		
RIB - ABNORMALITIES	MAL	0	0.0	2	1.6	0	0.0	0	0.0		
		0	0.0	2	10.5	0	0.0	0	0.0		
RIB - 13TH RIB - SUPERNUMERARY - SHORT OR FULL	MIN	1	0.7	2	1.6	0	0.0	7	5.2	*	
		1	5.3	2	10.5	0	0.0	7	35.0		
CAUDAL VERTEBRAE											
CAUDAL VERT.CENTRA - ABNORMALITIES	MAL	0	0.0	2	1.6	0	0.0	0	0.0		
		0	0.0	1	5.3	0	0.0	0	0.0		
CAUDAL VERT.CENTRA - UNOSSIFIED OR INCOMPLETE OSSIFICATION	OSS	3	2.1	3	2.5	2	1.9	4	3.0		
		3	15.8	3	15.8	2	12.5	3	15.0		
CAUDAL VERTEBRA - ABNORMALITIES	MAL	0	0.0	0	0.0	0	0.0	0	0.0		
		0	0.0	0	0.0	0	0.0	0	0.0		
CAUDAL VERT.CENTRA - OSSIFICATION OF LESS THAN 15	OSS	17	11.8	35	28.7	*	23	22.3	*	40	29.6	*
		10	52.6	11	57.9		12	75.0		15	75.0	
FETUSES WITHOUT ABNORMALITIES	(NE)	57	39.6	39	32.0	41	39.8	43	31.9		
LITTERS WITHOUT ABNORMALITIES		1	5.3	1	5.3	1	6.3	0	0.0		

Study title: AVE0010: Subcutaneous embryo-fetal toxicity study in rabbits

Study no.:	2005-1086
Study report location:	Module 4.2.3.5.2
Conducting laboratory:	Aventis Pharma Deutschland GmbH Drug Safety Evaluation Safety Science and Quality Services (SSQS) Mainzer Landstrasse 500 65795 Hattersheim, Germany
Date of study initiation:	2 November 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, batch # (b) (4), 84.4% pure

Key Study Findings

- A lower dose range of 0.3, 2 and 5 µg/kg/d was tested in this study because a NOAEL was not identified for either maternal or embryofetal toxicity in the previous study (2004-0552).
- Decreased motor activity and piloerection were noted at 2 and 5 µg/kg/d treated dams during the dosing period.
- Mean body weights were dose-dependently decreased in dams at ≥2 µg/kg/d during the dosing period, which correlated with decreased food and water consumption. Minimal effects on food consumption and body weight were noted at 0.3 µg/kg/d.
- One early delivery occurred at 2 µg/kg/d and one total implantation loss and one nearly total implantation loss were seen in 2 females at 2 µg/kg/d; these findings were not considered to be treatment related because similar findings were not observed at 5 µg/kg/d and were within the historical control range. There was no apparent effect on embryofetal survival.
- Treatment-related malformations were not observed. A slight increase in some developmental variations was observed at ≥ 2 µg/kg/d, including skull (small hole, splitting of bone, and additional suture of parietal bone), 13th rib (supernumerary - short or full), central caudal vertebrae (ossification of less than 15), and forepaw (hyperflexion).
- The NOAEL for maternal toxicity is considered to be 0.3 µg/kg/d based on decreased body weight and clinical signs at ≥2 µg/kg/d. Because the skeletal variations observed at ≥2 µg/kg/d cannot be ruled out as being related to the test article, the NOAEL for embryofetal developmental toxicity is considered to be 0.3 µg/kg/d. It should be noted that the study director concluded that the skeletal variations were not treatment related and placed the NOAEL at 5 µg/kg/d.

Methods

Species/Strain:	Rabbit / Chbb: HM(SPF) (b) (4)
Doses:	0, 0.3, 2, and 5 µg/kg/day
Frequency of dosing:	Twice daily separated by ~8 hours
Dose volume:	37.5 µL/kg/dose
Route of administration:	Subcutaneous
Formulation/Vehicle:	0.9% NaCl
Number/Group:	20 mated females
Satellite groups:	3 (control) or 9 mated females/group
Study design:	Mated females were treated from GD6 through GD18 with necropsy occurring on GD29
Protocol deviations:	Fetal skeletons for 15 of 18 and 6 of 17 litters at 0.3 and 2 µg/kg/d, respectively, could not be examined completely because of technical issues. This may have impacted the ability to definitively determine whether a treatment-related effect on skeletal variations occurred at 2 µg/kg/d.

Observations and Results

Mortality / Abortion: (twice daily, or once daily on weekends and holidays during non-dosing period)

There were no treatment-related mortalities. One MD group rabbit delivered prematurely on GD27 and was euthanized the same day.

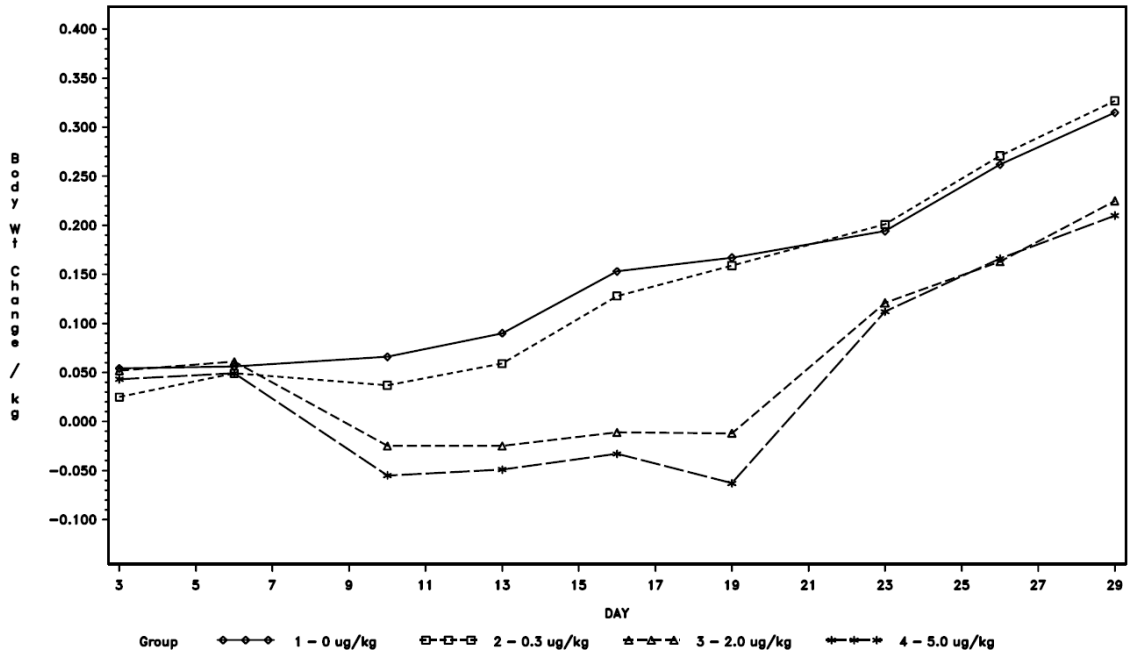
Clinical Signs: (twice daily, or once daily on weekends and holidays during non-dosing period)

No clinical signs were noted at the LD. At the MD and HD, decreased motor activity and piloerection were observed from the first day of treatment through the end of the treatment period. Signs were still present at the p.m. dosing but were no longer seen by morning.

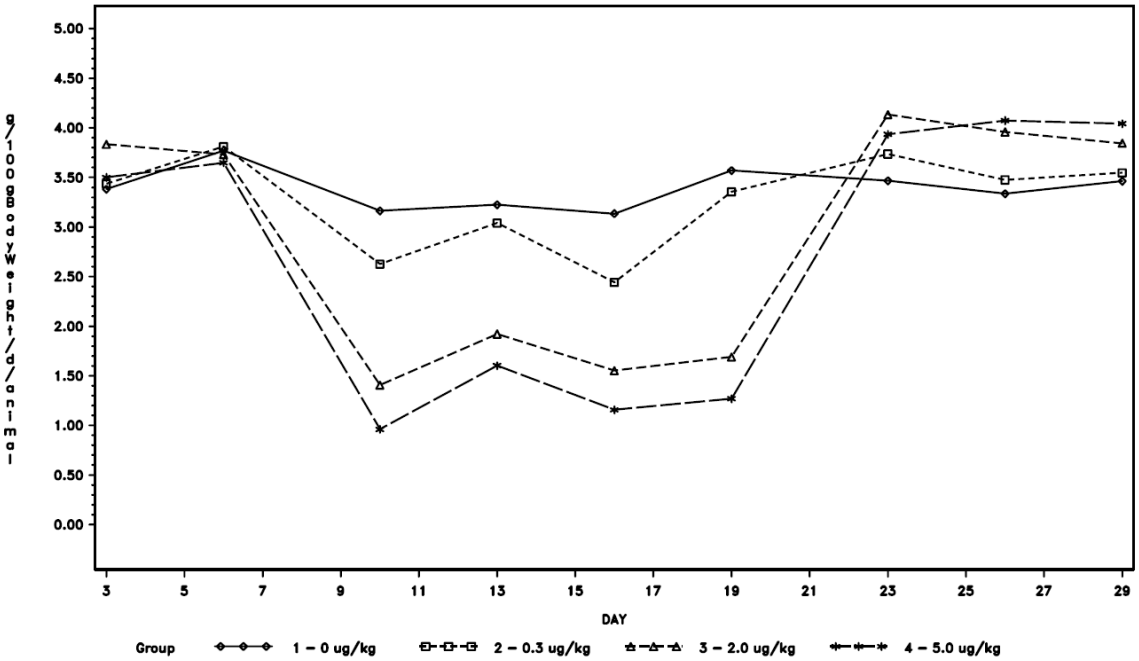
No clinical signs were noted prior to early delivery of the MD doe on GD27. Two rabbits from the MD group had red discolored bedding on the fecal tray from GD21 to 29 or GD22 to 26. These rabbits had either total implantation loss or had an increased number of conceptuses undergoing resorption at cesarean section, respectively.

Body Weight: (GDs 0, 3, 6, 10, 13, 16, 19, 23, 26, 29)

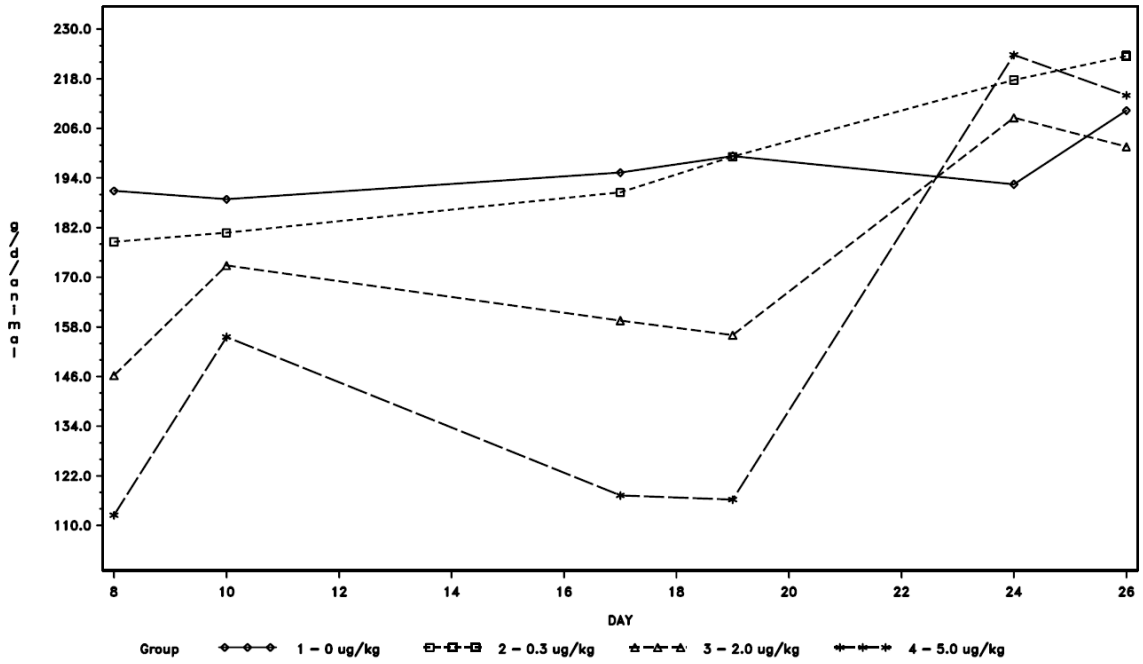
As shown in the sponsor-generated figure below, initiation of treatment at 2 and 5 µg/kg/day resulted in an initial body weight loss followed by decreased body weight gain. Once treatment ended, mean body weights rebounded for the MD and HD groups. A very slight decrease in mean body weight was noted between GD6 and 10 for the 0.3 µg/kg/day group.



Food Consumption: (GDs 0-3, 3-6, 6-10, 10-13, 13-16, 16-19, 19-23, 23-26, and 26-29)



Water Consumption: (GDs 6-8, 8-10, 15-17, 17-19, 22-24, and 24-26)



Toxicokinetics: (GD12 at 0.25, 1, 3, 8 hours after a.m. dosing and 0.25, 1, 3, 8, and 16 hours after p.m. dosing; 3 animals/time point)

Values following the two doses on day 7 of treatment (gestation day 12)

Dose µg/kg/day	C_{max} [ng/mL]	t_{max} [hr]	C_{24hr} [ng/mL]	$AUC_{(0-24hr)}$ [ng*hr/mL]
0.3	0.263	0.25	< 0.040	0.929
2	2.22	1.0	0.479	16.0
5	8.43	1.0	0.044	41.5

Dosing Solution Analysis

Test article was not detected in the vehicle control formulations. Most sampled formulations were below the acceptance criteria of 90% to 110% of nominal. The LD formulations ranged between 63% and 76%, the MD formulations ranged between 73% and 86%, and the HD formulations ranged between 77% and 91%. This deviation is not expected to effect the interpretation of the study results, as TK data are available for the actual administered doses.

Necropsy

There were treatment-related macroscopic findings.

Cesarean Section Data

One MD female had a total litter loss and another MD female had a near complete litter loss, resulting in a higher post-implantation loss percentage at the MD level (see sponsor-generated table below). A similar effect was not observed at the HD. There were no treatment-related effects on sex ratio, fetal crown-rump length, fetal weight, or placental weight.

Summary of Female Cesarean Section Data (Including Females with Total Litter Loss)

Dose ug/kg		Corpora Lutea	-Preimplantation- Loss- Absolute (c)	Loss- %Corpora Lutea	Implant Sites (c)	Postimplantation Loss Absolute Resorptions Early	Resorptions Late	Dead Fetuses	Total (i)	%Implantations	Live Fetuses Absolute (i)	%Implantations
Group 1 0	MEAN	7.3	0.6	6.8	6.7	0.3	0.0	0.2	0.45	6.7	6.2	93.3
	STD	1.8	1.4	14.4	1.7	0.6	0.0	0.4	0.60	9.4	1.7	9.4
	MEDIAN	7.0	0.0	0.0	7.0	0.0	0.0	0.0	0.00	0.0	6.0	100.0
	N	20	20	20	20	20	20	20	20	20	20	20
Group 2 0.3	MEAN	7.4	0.6	6.5	6.9	0.2	0.0	0.1	0.28	4.6	6.6	95.4
	STD	2.1	1.3	14.0	2.0	0.5	0.0	0.2	0.57	9.8	2.1	9.8
	MEDIAN	7.0	0.0	0.0	7.0	0.0	0.0	0.0	0.00	0.0	7.0	100.0
	N	18	18	18	18	18	18	18	18	18	18	18
Group 3 2.0	MEAN	8.1	0.6	7.9	7.4	1.4	0.1	0.1	1.56	20.5	5.9	79.5
	STD	1.3	1.1	14.4	1.8	2.4	0.2	0.2	2.41	29.6	2.7	29.6
	MEDIAN	8.0	0.0	0.0	7.5	1.0	0.0	0.0	1.00	11.8	6.5	88.2
	N	18	18	18	18	18	18	18	18	18	18	18
Group 4 5.0	MEAN	7.1 NS	0.6 NS	8.1	6.5 NS	0.3	0.1	0.0	0.37 NS	5.0	6.1 NS	95.0
	STD	1.9	1.1	14.1	1.9	0.6	0.2	0.0	0.60	7.9	1.8	7.9
	MEDIAN	7.0	0.0	0.0	7.0	0.0	0.0	0.0	0.00	0.0	7.0	100.0
	N	19	19	19	19	19	19	19	19	19	19	19

Offspring (Malformations, Variations, etc.)

A summary of skeletal malformations and minor anomalies is presented in the sponsor-generated tables below. Note that 15 of 18 and 6 of 17 litters at the 0.3 and 2 µg/kg/d groups were not completely evaluated for skeletal defects due to inadvertent exaggerated clearing with potassium hydroxide. For these fetuses, parts of the skeletons (head, vertebral column, sternebrae, and shoulder and pelvic girdle including long bones of the fore and hind limbs) could be evaluated. There were no apparent treatment-related effects on visceral organ development.

Few malformations were observed. When present, the specific malformation typically occurred as a single incidence and was often also observed in the concurrent control group or was within or slightly outside of the historical control range.

There were some potential treatment-related minor defects observed in the MD and HD groups, which included the skull (small hole, splitting of bone, and additional suture of parietal bone), 13th rib (supernumerary, short or full), central caudal vertebrae (ossification of less than 15), and forepaw (hyperflexion). These findings were either statistically significantly higher than the concurrent control group or were outside of the historical control range; however the findings did not always occur in a dose-related manner. Because of treatment-related findings observed at ≥5 µg/kg/d in Study 2004-0552, these skeletal variations cannot be ruled out as being possibly related to the test

article. Given the treatment-related effects on maternal food and water consumption at the MD and HD levels, it is possible that these minor variations were related to diminished maternal nutrition and body weight loss.

REPORT: EXTERNAL EXAM TYPE: EXTERNAL	CLASSIFICATION	GROUP 1 0 ug/kg NO	%	GROUP 2 0.3 ug/kg NO	%	GROUP 3 2.0 ug/kg NO	%	GROUP 4 5.0 ug/kg NO	%
NUMBER OF FETUSES EXAMINED		123		119		106		116	
NUMBER OF LITTERS EXAMINED		20		18		17		19	
FOREPAWS/DIGITS									
PAW - HYPERFLEXION	MIN	1	0.8	0	0.0	4	3.8	3	2.6
		1	5.0	0	0.0	2	11.8	1	5.3

REPORT: SKELETAL EXAM TYPE: SKELETAL	CLASSIFICATION	GROUP 1 0 ug/kg NO	%	GROUP 2 0.3 ug/kg NO	%	GROUP 3 2.0 ug/kg NO	%	GROUP 4 5.0 ug/kg NO	%
NUMBER OF FETUSES EXAMINED		123		17		58		116	
NUMBER OF LITTERS EXAMINED		20		3		11		19	
SKULL									
SKULL - HOLE, SMALL	MIN	0	0.0	0	0.0	3	5.2 *	3	2.6 *
		0	0.0	0	0.0	3	27.3	3	15.8
SKULL - SPLITTING OF BONE	MIN	2	1.6	0	0.0	0	0.0	7	6.0
		2	10.0	0	0.0	0	0.0	6	31.6
SKULL - SUTURAL BONE	MIN	2	1.6	3	17.6 M	1	1.7	2	1.7
		1	5.0	1	33.3	1	9.1	2	10.5
FRONTAL - ADDITIONAL SUTURE	MIN	0	0.0	1	5.9 M	0	0.0	0	0.0
		0	0.0	1	33.3	0	0.0	0	0.0
PARIETAL - ADDITIONAL SUTURE	MIN	0	0.0	0	0.0	5	8.6 *	2	1.7
		0	0.0	0	0.0	3	27.3	2	10.5
RIBS									
RIB - ANOMALIES	MAL	0	0.0	0	0.0	0	0.0	1	0.9
		0	0.0	0	0.0	0	0.0	1	5.3
RIB - 13TH RIB - SUPERNUMERARY - SHORT OR FULL	MIN	2	1.6	0	0.0	3	5.2	4	3.4
		2	10.0	0	0.0	2	18.2	3	15.8
VERTEBRAL COLUMN									
THORACIC VERTEBRAE - SCOLIOSIS	MAL	0	0.0	0	0.0	0	0.0	1	0.9
		0	0.0	0	0.0	0	0.0	1	5.3
CAUDAL VERT.CENTRA - OSSIFICATION OF LESS THAN 15	OSS	23	18.7	2	11.8 M	14	24.1	33	28.4
		12	60.0	1	33.3	6	54.5	13	68.4
THORACIC VERT.ARCH - ANOMALIES	MAL	0	0.0	0	0.0	0	0.0	1	0.9
		0	0.0	0	0.0	0	0.0	1	5.3
FETUSES WITHOUT ABNORMALITIES	(NE)	62	50.4	11	64.7	19	32.8	38	32.8
LITTERS WITHOUT ABNORMALITIES		1	5.0	1	33.3	0	0.0	0	0.0

Historical Background Data (based on 25 studies, 3,153 fetuses, and 474 litters)

	CLASSIFICATION	FOETUSES NO	%	TWO-SIDED TOLERANCE RANGE OF HISTORICAL GROUP SIZE 19 FOETUSES %
SKULL				
SKULL - SPLITTING OF BONE	MINOR DEFECT	38	1.2	0.0 - 3.6
SKULL - PERFORATION - SMALL	MINOR DEFECT	45	1.4	0.0 - 3.6
SKULL - EPACTAL BONE	MINOR DEFECT	114	3.6	0.0 - 7.6
FRONTAL BONE - FISSURE		0	0.0	0.0 - 0.0
PARIETAL BONE - FISSURE	MINOR DEFECT	26	0.8	0.0 - 2.4
VERTEBRAL COLUMN				
VERTEBRAL COLUMN - SCOLIOSIS	MAJOR DEFECT	2	0.1	0.0 - 0.5
CERVICAL VERT.ARCH				
CERVICAL VERT.ARCH - ABNORMALITIES	MINOR DEFECT	2	0.1	0.0 - 0.5
THORACIC VERT.ARCH				
THORACIC VERT.ARCH - ABNORMALITIES	MINOR DEFECT	3	0.1	0.0 - 0.6
RIB				
RIB - ABNORMALITIES	MINOR DEFECT	7	0.2	0.0 - 1.2
EXTRA RIB - AT 7TH CERVICAL VERTEBRA	VARIATION	96	3.0	0.0 - 7.0
EXTRA RIB - AT 13TH THORACIC VERTEBRA	VARIATION	50	1.6	0.0 - 4.4
FOREPAW				
TOES - SHORTENED	MAJOR DEFECT	1	0.0	0.0 - 0.3
CARPAL REGION - DEFLECTED	MINOR DEFECT	42	1.3	0.0 - 4.2

9.3 Prenatal and Postnatal Development

Study title: AVE0010: Subcutaneous pre- and postnatal developmental toxicity study in rats

Study number:	DPN0327
Study report location:	Module 4.2.3.5.3
Conducting laboratory:	Sanofi-Aventis Preclinical & Research Biostatistics 371, Rue du Professeur, Joseph Blayac 34184 Montpeller Cedex 04, Germany
Date of study initiation:	28 August 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, Lot # PPL-AVE100501A, and 88.4% pure

Key Study Findings

- Treatment with AVE0010 to pregnant and lactating rats at doses of 0.004, 0.04 and 0.4 mg/kg/d caused initial body weight loss that correlated with decreased food consumption and a decrease in motor activity (sleepiness and decreased reactivity).
- Treatment-related effects on the F1 generation included:
  1). A slight increase in pup mortality from PND0 to 21 at the HD (11.9% vs. 3.8% for control).
  2). A slight decrease in suckling of male pups at ≥0.04 mg/kg/d during the middle of the lactation period, resulting in a slight downward trend for male mean body weight during this period of lactation.
  3). Abnormal findings on tails of several pups at 0.4 mg/kg/d.
  4). Two cases of multiple skeletal malformations of long bones and ribs in two growth retarded dead pups at 0.4 mg/kg/d.
  5). 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).
- F1 reproductive parameters were not affected.
- The NOAEL for F_0 maternal rats was considered to be <0.004 mg/kg/d based on adverse effects on body weight. The NOAEL for the F_1 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.

Methods

Species/Strain:	Rat / Sprague-Dawley
Doses:	0, 0.004, 0.04, and 0.4 mg/kg/day
Frequency of dosing:	Twice daily approximately 8 hours apart
Dose volume:	2 x 0.5 mL/kg
Route of administration:	Subcutaneous

Formulation/Vehicle:	0.9% NaCl
Number/Sex/Group:	24 pregnant females
Satellite groups:	None
Study design:	F_0 dams were treated from GD6 through PND20. After lactation, F_1 groups were culled to 24 pups/sex and then followed for physical development, neurological development, and reproductive ability.
Protocol deviations:	The study director declared that there were no deviations from the protocol, SOPs, or GLP regulations that would have significantly impacted the validity or interpretation of the data.

Observations and Results

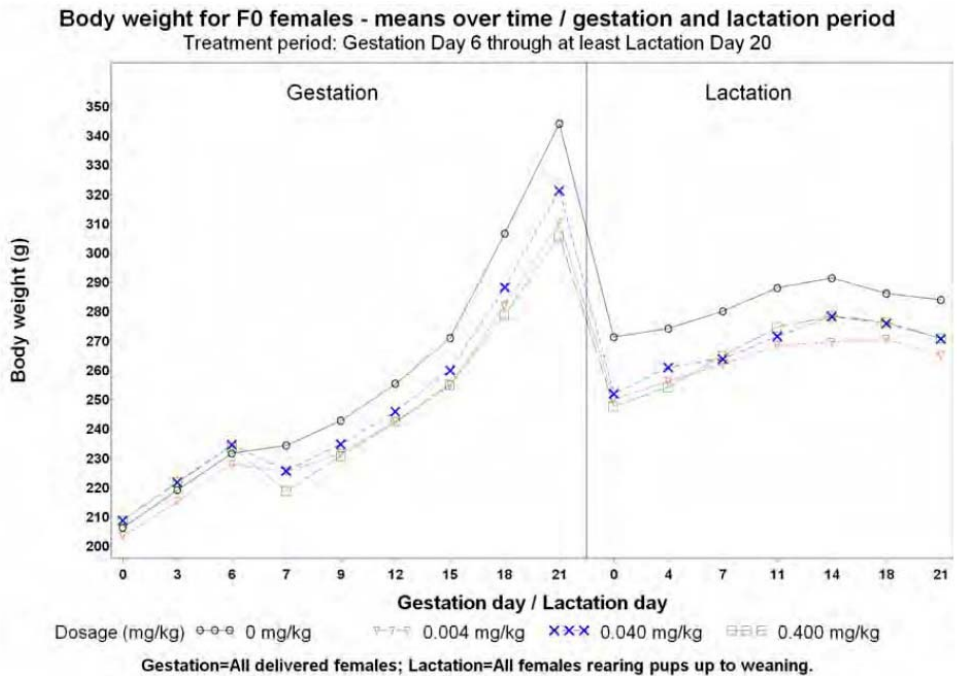
F_0 Dams

Survival: (at least twice daily)
There were no maternal deaths.

Clinical signs: (at least twice daily)
Decreased motor activity including sleepiness and decreased reactivity was observed during most of the day in all animals of the three dose groups during the entire treatment period (GD6 to PND20). Piloerection was also noted for most treated animals.

Body weight: (GD0, 3, 6, 7, 9, 12, 15, 18, 21, and 25 [GD25 only if animal did not deliver], and on LD0, 4, 7, 11, 14, 18, and 21)

Upon the initiation of treatment, a dose-related decrease in body weight was observed. Mean body weight gain was similar to control for all dose groups between GD7 and GD15. A slight decrease in mean BW gain was observed between GD15 and GD21. Mean BW gain was similar to controls during the lactation period, although the weight of treated dams remained lower than controls.



Feed consumption: (GD0, 3, 6, 7, 9, 12, 15, 18, and 21, and on LD0, 4, 7, 11, 14, 18, and 21)
Decreased mean food consumption was observed for all treated groups, especially between GD6 and GD9, which correlate with the effects on body weight.

Pregnancy data:
The parturition index values, 100%, 95.7%, 85.0%, and 95.5% for control, LD, MD, and HD groups, were slightly lower for treated groups, but lacked statistical significance. There were no apparent treatment-related effects on gestation length, post-implantation loss, male to female ratio, or live birth index (sponsor-generated table).

Group	AVE0010 (mg/kg)		Gestation length	Implantations	Postimplantation loss	Litter size	Percentage of males	Live birth index
1	0	Mean	22.2	11.8	0.2	10.2	49.2	98.6
		Std	0.49	5.08	0.13	5.00	16.77	4.21
		N	18	18	18	18	18	18
2	0.004	Mean	22.6 NS	10.0	0.3 NS	8.2	59.0 NS	88.9 NS
		Std	0.66	4.48	0.27	4.47	24.32	23.58
		N	22	22	22	22	22	22
3	0.040	Mean	22.4 NS	11.7	0.1 NS	10.4	44.8 NS	97.2 NS
		Std	0.56	4.58	0.19	4.37	16.98	8.21
		N	17	17	17	17	17	17
4	0.400	Mean	22.6 NS	9.5	0.3 NS	7.6	49.7 NS	89.8 NS
		Std	0.34	4.51	0.15	3.89	21.66	15.41
		N	21	21	21	21	21	21

N: delivered F0 females
Implantations: sum by delivered females of the right and left implantation sites
Postimplantation loss by female: ((total number of implants) - (total number of born-alive pups)) / (total number of implants)
Litter size: total number of pups
Percentage of males by female: 100 * (total number of males) / (total number of pups)
Live birth index: 100 * (number of pups born alive) / (total number of pups)
* = significant trend ($p \leq 0.05$) through indicated dose level
NS = no significant trend ($p > 0.05$) through indicated dose level
NT = not tested

Necropsy observation:
There were no treatment-related macroscopic observations noted in F_0 dams.

Toxicokinetics:
Not conducted

Dosing solution analysis:
Two low dose samples were 68% to 76% of nominal, which was below the acceptance criteria. This was not felt to affect the interpretability of the data. The MD and HD formulations were between 93% and 105% of the nominal concentration.

F_1 Generation

Survival: (once daily)
Pup mortality from LD0 to LD4 (including dead pups at birth) was 17 (9.4%), 5 (2.8%), and 17 (10.6%) at ascending doses compared with 7 (3.8%) for the control group. From LD4 to LD21 there were three deaths, one at MD and 2 at HD. Considering all deaths together, pup mortality was considered to be slightly increased by the test article at the HD.

Clinical signs: (at least once daily)
Suckling insufficiency was observed for MD and HD males (see food consumption). There were no other treatment-related clinical signs noted.

Body weight: (pre-weaning: Days 0, 4, 7, 11, 14, and 21; Post-weaning: once weekly; F1 gestation: GD0, 4, 8, 11, and 14)

The mean body weights of pups at birth were comparable in all dose groups. During LD4 to LD14, there was a slight downward trend for body weight gain of male pups at 0.04 and 0.4 mg/kg/d and during LD7 to LD11 for the males at 0.004 mg/kg/d. This trend was not observed for the female pups. The effects on the BW of male pups at the MD and HD levels correlated with insufficient suckling and were considered to be attributed to the test article.

Overall mean body weights of males from dosed dams were minimally lower during post-weaning days 0 to 77 compared with controls (-7.7%, -7.8%, and -6.4% in ascending dose order). A similar overall trend was not observed for F1 females, although mean body weights of MD and HD females were slightly lower during the sexual maturation landmark evaluations (-6% of control weights).

Feed consumption: (post-weaning: once weekly until day of cohabitation; F1 gestation: GD0, 4, 8, 11, and 14)
The number of litters in which pups were suckled insufficiently and the number of pups affected per litter was increased slightly at the MD and HD, but without clear dose dependency. This effect was most pronounced during the middle of the 21-day lactation period and was considered to be secondary to treatment-related maternal malnutrition.

No compound-related effects on food consumption were noted from post-weaning through the end of the study, including F1 females during pregnancy.

Macroscopic examination:
Several pups from the HD group showed abnormal tails such as necrotic or shortened tail tip, or wavy, bent, or deformed tail.

Morphological examination: (conducted on culled pups on PND4 and pups found dead during the lactation period)
Two cases of multiple skeletal malformations were observed in animals found dead at birth in the HD group consisting mainly of bent and shortened long bones of the fore and hindlimbs and wavy ribs, for which an indirect compound-related effect (malnutrition of dams) could not be excluded. A summary of relevant morphological findings is shown in the sponsor-generated tables below.

SUMMARY AND INTERGROUP COMPARISON OF MORPHOLOGICAL FINDINGS IN LIVE FETUSES

REPORT: SKELETAL EXAM TYPE: SKELETAL	CLASSIFICATION	GROUP 1 0 mg/kg NO	%	GROUP 2 0.004 mg/kg NO	%	GROUP 3 0.04 mg/kg NO	%	GROUP 4 0.4 mg/kg NO	%
NUMBER OF FETUSES EXAMINED		55		40		55		24	
NUMBER OF LITTERS EXAMINED		12		12		12		10	
SKULL									
SKULL - SUTURAL BONE	MIN	1	1.8	1	2.5	0	0.0	3	12.5
		1	8.3	1	8.3	0	0.0	3	30.0
FOREPAWS									
PHALANX - INCOMPLETE OSSIFICATION	OSS	0	0.0	0	0.0	0	0.0	1	4.2
		0	0.0	0	0.0	0	0.0	1	10.0
STERNEBRAE									
STERNEBRA - UNOSSIFIED OR INCOMPLETE OSSIFICATION	OSS	0	0.0	0	0.0	1	1.8	0	0.0
		0	0.0	0	0.0	1	8.3	0	0.0

SUMMARY OF MORPHOLOGICAL FINDINGS IN DEAD FETUSES

REPORT: VISCERAL EXAM TYPE: FRESH VISCERAL	CLASSIFICATION	GROUP 1 0 mg/kg NO	%	GROUP 2 0.004 mg/kg NO	%	GROUP 3 0.04 mg/kg NO	%	GROUP 4 0.4 mg/kg NO	%
NUMBER OF FETUSES EXAMINED		4		10		4		13	
NUMBER OF LITTERS EXAMINED		2		7		3		8	
GENERAL									
GENERAL - INCOMPLETE EXAMINATION DUE TO AUTOLYSIS		1	25.0	2	20.0	2	50.0	9	69.2
		1	50.0	2	28.6	2	66.7	6	75.0

REPORT: SKELETAL EXAM TYPE: SKELETAL	CLASSIFICATION	GROUP 1 0 mg/kg		GROUP 2 0.004 mg/kg		GROUP 3 0.04 mg/kg		GROUP 4 0.4 mg/kg	
		NO	%	NO	%	NO	%	NO	%
NUMBER OF FETUSES EXAMINED		4		10		4		13	
NUMBER OF LITTERS EXAMINED		2		7		3		8	
SHOULDER GIRDLE									
SPINA SCAPULA - BENT	MIN	0	0.0	0	0.0	0	0.0	2	15.4
		0	0.0	0	0.0	0	0.0	2	25.0
SCAPULA - BENT	MAL	0	0.0	0	0.0	0	0.0	2	15.4
		0	0.0	0	0.0	0	0.0	2	25.0
SCAPULA - SHORT	MAL	0	0.0	0	0.0	0	0.0	1	7.7
		0	0.0	0	0.0	0	0.0	1	12.5
CLAVICLE - BENT	MAL	0	0.0	0	0.0	0	0.0	2	15.4
		0	0.0	0	0.0	0	0.0	2	25.0
FORELIMBS									
HUMERUS - BENT	MAL	0	0.0	0	0.0	0	0.0	2	15.4
		0	0.0	0	0.0	0	0.0	2	25.0
ULNA - BENT	MAL	0	0.0	0	0.0	0	0.0	2	15.4
		0	0.0	0	0.0	0	0.0	2	25.0
RADIUS - BENT	MAL	0	0.0	0	0.0	0	0.0	2	15.4
		0	0.0	0	0.0	0	0.0	2	25.0
FOREPAWS									
PHALANX - INCOMPLETE OSSIFICATION	OSS	4	100.0	8	80.0	3	75.0	12	92.3
		2	100.0	5	71.4	3	100.0	8	100.0
STERNEBRAE									
STERNEBRA - UNOSSIFIED OR INCOMPLETE OSSIFICATION	OSS	0	0.0	0	0.0	0	0.0	2	15.4
		0	0.0	0	0.0	0	0.0	2	25.0
RIBS									
RIB - ANOMALIES	MAL	0	0.0	1	10.0	0	0.0	0	0.0
		0	0.0	1	14.3	0	0.0	0	0.0
RIB - WAVY AND/OR THICKENED	MIN	0	0.0	0	0.0	0	0.0	2	15.4
		0	0.0	0	0.0	0	0.0	2	25.0
RIB - 14TH RIB - SUPERNUMERARY - SHORT OR FULL	MIN	0	0.0	0	0.0	0	0.0	1	7.7
		0	0.0	0	0.0	0	0.0	1	12.5

Physical development landmarks:

Pinna separation: No difference from control was observed (1.6 days at HD vs. 1.8 days for control).

Coat growth: A slight, statistically significant (HD only) delay in the initiation of coat growth was observed for MD (4.6 days) and HD (4.5 days) animals compared with controls (3.8 days).

Incisor eruption: No difference from control was observed (9.7 days at HD vs. 9.5 days for control).

Eye opening: No difference from control was observed (14.5 days at HD vs. 14.5 days for control).

Vaginal opening: Despite a lower mean body weight, 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).

Balanopreputial separation: Despite a lower mean body weight, a very slight but statistically significant decrease in the time to balanopreputial separation was observed for HD males (34.2 days) compared with control males (34.8 days).

Neurological assessment: (24 males and 24 females/group from as many litters as possible; auditory, visual, and righting reflex tests were conducted on PND21; water maze tests were conducted between PND30 and PND 50)

There were no treatment-related effects on auditory, visual, or right reflex responses.

There were no apparent treatment-related effects on learning, memory, relearning, or motor coordination when tested in a water maze.

Reproduction: (24 males and 24 females/group from as many litters as possible; when all F_1 females were at least 70 days old, each F_1 female was placed into cohabitation with an F_1 male from the same dose group, avoiding sibling mating)

There was no apparent treatment-related effect on the cycling of females, male and female mating behavior, fertility, fecundity, number of corpora lutea, or early embryonic development in F_1 animals.

F_2 Generation (F_1 pregnant females assessed on GD14)
Survival:
There were no test article-related effects noted for any intrauterine parameter. The numbers of corpora lutea and implantation sites were similar across groups.

Body weight: Not conducted

External evaluation: Not conducted

9.4 Juvenile Toxicology

Study title: Twice daily subcutaneous dosing. Juvenile toxicity (14-day) dose range-finding study in the rat

Study number:	JUP0012 (sponsor); (b) (4)
Study report location:	Module 4.2.3.5.4
Conducting laboratory:	(b) (4)
Date of study initiation:	01 June 2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	AVE0010, Batch #B004, 88.1% pure

Key Study Findings

- There were no unscheduled deaths. Treated animals were lethargic for approximately 15 minutes after the AM and PM doses, which was most notable on Days 1 to 3, which correlated with markedly reduced food intake from Days 1 to 3.
- Group mean body weight gain was lower from Days 1 to 11 in treated males and from Days 1 to 4 in treated females.
- 1000 µg/kg BID was generally well tolerated and recommended for the pivotal 5-week juvenile toxicology study.

Methods

Species/Strain:	Rat/Sprague-Dawley
Doses:	Control and 2000 µg/kg/d
Frequency of dosing:	Twice daily, approximately 8 hours apart from post-natal day (PND) 25 to 38
Route of administration:	Subcutaneous
Dose volume:	0.5 mL/kg/dose
Formulation/Vehicle:	0.9% NaCl
Number/Sex/Group:	5
Age at first dose:	25 days
Weight at first dose:	70.9 to 78.9 g (males), 65.7 to 77.8 g (females)
Toxicokinetic groups:	33/sex/group
Protocol deviations:	On Day 12, All animals were over-dosed by approximately 10 times the required dose. Because of this, animals were not given the PM dose.

Toxicokinetics:

Sex	Dose (µg/kg/day)	C_{max} (ng/mL)		AUC_{0-24} (ng.h/mL)	
		Day 1	Day 14	Day 1	Day 14
Male	2000	86.1	185	275	419
Female	2000	99.7	128	220	429

Study title: Twice daily subcutaneous dosing 5 week juvenile toxicity study in the rat

Study number:	JUV0026 (sponsor); (b) (4)
Study report location:	Module 4.2.3.5.4
Conducting laboratory:	(b) (4)
Date of study initiation:	12 August 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AVE0010, Batch #B004, 88.1% pure

Key Study Findings

- There were no unscheduled deaths. Clinical signs consisted of post-dose salivation and mouth rubbing in all treated groups.
- Mean body weight gain showed a clear dose-related decrease over the first 4 days at all dose levels, which correlated with decreased food consumption. Body weight gains during the rest of the study were variable but generally lower than controls.
- Mean long bone measurements showed no treatment-related effect. At the end of the dosing period, higher bone mineral density (BMD) was observed for all treatment group males compared with control males. The higher BMD was thought to be due primarily to lower bone area that correlated with lower mean body weights. This effect was not noted after the recovery period.
- Mean balano-preputial separation time was slightly increased at 2000 µg/kg/d compared with controls, although was within the expected range for rats and was not considered to be a significant adverse effect. A slight increase in the day of vaginal opening was noted for the treated groups, but was not considered to be biologically significant. These minor effects on delayed time to sexual maturation were likely related to the pharmacologically-mediated decrease in body weight gain.
- Group mean locomotor activity was slightly decreased for all treated groups, although not in a clear dose-related manner. There were no apparent effects on the auditory startle response, learning, or memory.
- A slight, dose-related increase in mean plasma concentrations of potassium, calcium, and inorganic phosphate were observed for males and females. These findings were no longer elevated after the recovery period. A slight increase in mean plasma urea was also noted.
- There were no toxicologically meaningful effects on mean organ weights.
- There were no treatment-related macroscopic or microscopic findings, including bone.
- Exposures increased in a slightly less than dose-proportional manner on Day 1. Exposures on Day 35 were greater than on Day 1, likely the result of ADAs.
- The NOAEL was determined to be 2000 µg/kg/d for males and females. The sponsor placed the NOAEL for males at 200 µg/kg/d based on very occasional paddling, slightly increased balano-preputial time, and reversible decreased glucose; however, this reviewer feels that these effects are not sufficiently adverse to warrant a lower NOAEL for males.

Methods

Species/Strain: Rat/Sprague-Dawley
Study Design: (sponsor-generated table)

Group Number	Group Description	Total Daily Dose Level (µg/kg/day)	Dose level BID (µg/kg/ occasion)	Number of animals in group	
				Males	Females
Main Study Juvenile Animals					
1	Control	0	0	10	10
2	Low	20	10	10	10
3	Intermediate	200	100	10	10
4	High	2000	1000	10	10
Recovery Juvenile Animals				**Number of animals in group**	
1	Control	0	0	20	20
2	Low	20	10	20	20
3	Intermediate	200	100	20	20
4	High	2000	1000	20	20
Toxicokinetic Juvenile Animals				**Number of animals in group**	
1	Control	0	0	6	6
2	Low	20	10	24	24
3	Intermediate	200	100	24	24
4	High	2000	1000	24	24

Frequency of dosing: Twice daily, approximately 8 hours apart from post-natal day (PND) 25 to 61
Route of administration: Subcutaneous
Dose volume: 0.5 mL/kg/dose
Formulation/Vehicle: 0.9% NaCl
Age at first dose: 25 days
Weight at first dose: 62.2 to 82.8 g (males), 59.0 to 83.5 g (females)
Unique endpoints: Bone density and sexual maturation measurements
Protocol deviations: There were no major deviations from the protocol and any minor deviations were deemed to not affect the integrity or outcome of the study results.

Observations and Results

Mortality: (Twice daily)

There were no unscheduled deaths.

Clinical Signs: (Twice daily; detailed physical exams on days body weight days)

Salivation was observed in up to two females at 20 and 200 µg/kg/d and in six males and five females at 2000 µg/kg/d during the treatment period. After dosing, mouth rubbing was seen in all treated groups with frequency increasing with dose level. Very occasional paddling was seen at 2000 µg/kg/d.

Body Weights: (Twice weekly)

Dose-related effects on body weight gain were observed at all dose levels throughout the study, with the greatest effects occurring during the first 3 to 7 days. By the end of the 5-week dosing period, decrements in body weight gain were similar across all treated groups. During recovery, body weight gains for all treated groups were similar to or slightly higher than the control group, with final body weights still slightly lower than controls by the end of recovery.

Dose (µg/kg/d)	0		20		200		2000	
Sex	M	F	M	F	M	F	M	F
Weight (g) – PND 25	74.3	70.7	74.0	69.7	74.5	69.5	74.1	72.2
Weight (g) – PND 60	356.5	236.8	320.2	225.9	324.4	218.6	318.0	226.9
Weight gain (g) PND 25-28	20.3	16.8	15.0**	14.2**	14.3**	11.1**	10.2**	10.2**
Weight gain (g) PND 25-60	282.2	165.3	246.2	156.2	249.9	149.1	243.9	154.7
Diff from control (g)			-36.0	-9.1	-32.3.	-16.2	-38.3	-106
% diff from control			↓13%	↓6%	↓11	↓10%	↓14%	↓6%

**p<0.001; F = female; M = male.

Feed Consumption: (Twice weekly)

Mean food consumption for all treated groups was generally slightly lower than the control group throughout the treatment period. The degree of decreased food consumption was similar between dose groups except for the first 3 days during which the HD male group showed a slightly greater decrement.

Long bone measurement: (Once weekly starting at PND24)

The ulna was measured using a set of digital calipers, measuring from the elbow of the animal to the bent paw joint.

There was no apparent treatment-related effect on lone bone growth during the treatment period.

Sexual maturation:

Vaginal opening (main study females) and balano-preputial separation (main study males) were assessed daily from PND30 and PND40, respectively, until development was complete.

A slight, dose-related increase in the mean time to both vaginal opening and balano-preputial separation was observed at all dose levels with a statistically significant difference for HD males (see sponsor-generated tables below).

Vaginal opening

Group	Number of animals with complete development Post Natal Day: 30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	Mean post natal day for complete development	Mean weight (g) on day of complete development
1F			1		1	1	1	2	2	1	1							36.6	137.7
2F					1	1	2	2	1	2			1					37.3	132.7
3F					2		1	4	1	1							1	37.5	131.6
4F						1	1		1	2	2	1					2	40.0	149.4
																		DR*	
Statistics																		J	A

J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon
A = ANOVA, dose response and Dunnett's

* P<0.05
** P<0.01
*** P<0.001
DR = significant dose response test

Balano-preputial separation

Group	Number of animals with complete development on Post Natal Day: 40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	Mean post natal day for complete development	Mean body weight (g) on day of complete development
1M							4	2	1		1	2								47.8	264.3
2M							1		3	1	2		1	1	1					49.8	250.7
3M						1	1	1	2		1	1		1		2				49.8	248.5
4M									1	1	3	1		1	1		1		1	51.9**	260.8
Statistics																				J	A

J = Kruskal-Wallis, Terpstra-Jonckheere, Wilcoxon
A = ANOVA, dose response and Dunnett's

* P<0.05
** P<0.01
*** P<0.001

Motor activity:

Ten recovery animals/sex/group were assessed on PND54 (males), PND 59 (females), and PND84 (both genders) in an automated photocell activity recorder for 30 minutes for motor activity. Activity counts were recorded at 2-minute intervals.

Although highly variable, total mobile counts and total activity were decreased for treated males during the 30 minute observation on PND 54. Slight, non-dose-related decreases in total mobile counts and total activity were noted for females. When tested again on PND84 (recovery), total mobile counts and total activity were similar across all male dose groups, whereas a dose-related increase was noted for females.

Summary of Motor Activity Results

Dose Group (µg/kg/d)	Total Mobile Counts		Total Activity	
	PND 54	PND 84	PND 54	PND 84
MALES				
0	253	270	1246	1311
20	140*	292	693***	1085
200	146*	185	793**	1067
2000	112**	294	625***	1096
FEMALES				
0	308	170	1126	804
20	224	270*	826*	986
200	250	321**	1099	1096*
2000	267	380***	880	1154**

Total mean counts = total beam breaks where change in position is greater than mobile threshold.
Total activity = total fast beam breaks (fast and slow, static and mobile)
*p<0.05; **p<0.01; ***p<0.001

Sensory function:

The auditory startle response of 10 recovery animals/sex/group was assessed on PND54/55 and PND82/83.

Auditory startle response was normal for all animals.

Learning ability:

Ten recovery animals were assessed on PND 54/55, 61/62, 82/83, and 89/90 for their learning ability and behavior in the swimming maze.

Ophthalmoscopy: Not conducted

Hematology: (PND 61/62 and PND89)

There were no toxicologically meaningful effects on hematology parameters.

Clinical Chemistry: (PND 61/62 and PND89)

Dose (µg/kg/d)	0		20		200		2000	
Sex	M	F	M	F	M	F	M	F
Phosphorous (mmol/L)	2.6	2.1	2.9***	2.6**	3.0***	2.5**	3.2***	2.6**
-Recovery	2.0	1.8	1.8**	1.9	1.7***	1.9	1.8***	1.9
Potassium (mmol/L)	4.1	4.2	4.4*	4.3	4.4	4.4	4.5**	4.4
-Recovery	4.6	3.9	4.7	4.0	4.9	3.9	4.7	3.8
Calcium (mmol/L)	2.77	2.64	2.83	2.66	2.80	2.71	2.86*	2.73**
-Recovery	2.71	2.72	2.63*	2.68	2.65	2.66	2.65	2.64
Urea (mmol/L)	5.5	5.6	7.8**	6.7	7.3*	7.3**	8.3**	6.2
-Recovery	6.5	5.9	6.8	6.1	7.7**	6.2	7.3*	6.5

*p<0.05; **p<0.01; ***p<0.001; F = female; M = male.

Urinalysis: (overnight samples collected on PND61/62 and PND87/88)

Dose (µg/kg/d)	0		20		200		2000	
Sex	M	F	M	F	M	F	M	F
Specific gravity	1.051	1.047	1.034**	1.044	1.034**	1.040	1.041	1.041
-Recovery	1.048	1.042	1.053	1.041	1.043	1.043	1.047	1.044

Gross Pathology: (All main study animals on PND 61/62 and recovery animals on PND87/88)

There were no treatment-related macroscopic findings.

Organ Weights:

Dose (µg/kg/d)	0		20		200		2000	
Sex	M	F	M	F	M	F	M	F
Prostate (g)	0.628		0.514		0.510		0.490	
(Adjusted to BW)	0.632		0.513		0.508		0.488*	
Seminal vesicles (g)	0.536		0.465		0.452		0.425	
(Adjusted to BW)	0.528		0.468		0.455		0.429	
Testes/Epididymis (g)	4.003		3.837		3.965		3.760	
(Adjusted to BW)	3.915		3.861		3.990		3.798	
Ovaries (g)		0.081		0.076		0.067		0.069
(Adjusted to BW)		0.076		0.076		0.072		0.069
Uterus (g)		0.594		0.508		0.537		0.559
(Adjusted to BW)		0.609		0.507		0.523		0.559

*p<0.05; F = female; M = male.

Histopathology: (All main and recovery animals from control and HD groups)

Adequate Battery: Yes

Peer Review: Yes, conducted by Sanofi pathologist

Histological Findings

No apparent treatment-related microscopic findings were noted. Data for potential target organs are presented for informational purposes.

Main Study Groups

TABLE INCLUDES:
SEX=ALL;GROUP=1,4;WEEKS=ALL
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		SEX: --MALE--		-FEMALE-	
		GROUP: -1-	-4-	-1-	-4-
	NUMBER:	10	10	10	10
PANCREAS	NUMBER EXAMINED:	10	10	10	10
--INFLAMMATORY CELL FOCI		0	0	0	1
THYROID	NUMBER EXAMINED:	10	10	10	10
--INFLAMMATORY CELL FOCI		0	1	0	0
--CYST		1	2	1	3
--ECTOPIC THYMUS		1	1	0	1
SEMINAL VESICLE	NUMBER EXAMINED:	10	10	0	0
--CONTRACTION		0	1	0	0
PROSTATE	NUMBER EXAMINED:	10	10	0	0
--PROSTATITIS		0	1	0	0
UTERUS	NUMBER EXAMINED:	0	0	10	10
--PRO-OESTRUS		0	0	2	4
--OESTRUS		0	0	1	0
--METOESTRUS		0	0	5	3
--DIOESTRUS		0	0	2	3

Recovery Groups

TABLE INCLUDES:
SEX=ALL;GROUP=1,4;WEEKS=ALL
DEATH=U;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		SEX: --MALE--		-FEMALE-	
		GROUP: -1-	-4-	-1-	-4-
	NUMBER:	20	20	20	20
PANCREAS	NUMBER EXAMINED:	20	20	20	20
--INFLAMMATORY CELL FOCI		1	0	2	2
--LOBULAR ATROPHY		0	1	1	1
THYROID	NUMBER EXAMINED:	20	20	20	20
--INFLAMMATORY CELL FOCI		0	2	0	0
--CYST		1	3	0	2
--ECTOPIC THYMUS		1	2	2	1
--VACUOLATED FOCUS		0	1	0	0
UTERUS	NUMBER EXAMINED:	0	0	20	20
--PRO-OESTRUS		0	0	6	4
--OESTRUS		0	0	2	2
--METOESTRUS		0	0	6	9
--DIOESTRUS		0	0	6	5

Special Evaluation:

Dual energy X-ray absorptiometry (DEXA)

DEXA was conducted (2 scans/animal) to measure area, bone mineral content, and bone mineral density on PND61/62 (main study animals) and PND87/88 (recovery animals).

The results are summarized in the sponsor-generated table below.

Table 9 – Tabulated DEXA parameters

Group	BMD g/cm^2	Bone Area cm^2	BMC g	Body Weight g
		Main Study Animals		
1M	0.183	1.87	0.34	356.5
2M	0.189	1.74	0.33	320.2
3M	0.191	1.73	0.33	324.4
4M	0.194	1.71	0.33	318.0
1F	0.194	1.54	0.30	236.8
2F	0.192	1.46	0.28	225.9
3F	0.192	1.45	0.28	218.6
4F	0.193	1.50	0.29	226.9
		Recovery Animals		
1M	0.237	2.29	0.54	479.0
2M	0.230	2.18	0.50	451.6
3M	0.226	2.20	0.50	459.8
4M	0.234	2.21	0.52	471.5
1F	0.231	1.78	0.41	276.5
2F	0.223	1.70	0.38	254.4
3F	0.227	1.73	0.39	269.1
4F	0.230	1.74	0.40	264.0

Toxicokinetics:

Sex	Dose (µg/kg/day)	C_{max} (ng/mL)		AUC_{0-24} (ng.h/mL)	
		Day 1	Day 35	Day 1	Day 35
Male	20	6.18	74.2	6.02	309
	200	29.9	1080	56.1	7510
	2000	76.6	1050	202	6720
Female	20	1.96	49.7	4.92	225
	200	29.9	334	40.4	2370
	2000	73.7	779	177	4920

Values are rounded to 3 significant figures

Dosing Solution Analysis

The analysis of formulation samples confirmed that the prepared dosing solutions were within the acceptance range of 90% to 110% of the nominal concentrations and that AVE0010 was absent from the vehicle control.

Study title: AVE0010 - Exploratory 2-week twice a day (BID) subcutaneous pharmacokinetic study in juvenile male dogs

Key study findings:

- No mortalities occurred.
- Clinical signs included redness of ears, eyes, and/or oral mucosa, paleness of oral mucosa, salivation, and reduced skin elasticity at all dose levels; soft, liquid, and/or discolored feces, vomiting, decreased activity, and absence of food intake at the mid- and high-dose levels; and recumbent posture, weakness, cold to touch, uncoordinated gait, trembling, twitching, and emaciated appearance at the high dose. The more serious adverse signs generally dissipated after the first 2 to 5 days of dosing.
- Dose-related decreases in body weight gain occurred for all treated groups with mean body weights lower than starting weight for the mid- and high-dose groups. Effects on body weight correlated decreased food consumption. Body weights rebounded during the 14-day, treatment-free observation period.
- Clinical signs and effects on body weight observed at the mid- and high-dose levels resulted in these groups being supplemented with wet food and having transient dosing holidays. The report does not state what dogs received a dosing holiday or what days doses were withheld.
- There were no treatment-related effects on hematology, coagulation, or clinical chemistry parameters.
- Exposures were similar on Day 1 and Day 14, with mean $AUC_{0\text{-}24h}$ values being 31500, 136000, and 755000 ng·h/mL on Day 14 for the 10, 40, and 400 µg/kg/d dose groups, respectively.
- A slight anti-drug antibody response may have been observed for one dog each in the mid- and high-dose groups.
- Although the adverse clinical signs and effects on body weight dissipated during the second week, because these effects led to treatment holidays, the high dose of 200 µg/kg BID may have exceeded the maximum tolerated dose.

Study number:	DIV1328
Study report location:	Module 4.2.3.5.4
Conducting laboratory:	Sanofi-Aventis Deutschland GmbH, R&D – SCP Disposition, Safety & Animal Research, 65926 Frankfurt, Germany
Date of first dose:	16 March 2010 (study initiation date not provided)
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	AVE0010; Lot #B004; 88.1% purity

Methods

Doses: 0, 5, 20, 200 µg/kg BID (0, 10, 40, and 400 µg/kg/day)
Species/strain: Dog/Beagle (juvenile)
Number/sex/group: 3 males/group
Route and regimen: Twice daily (8 hours apart) subcutaneous for 14 days with 14 day recovery
Dosing volume: 0.5 mL/kg
Vehicle: 0.9% NaCl
Age: 18-19 weeks at dosing
Weight: 5.1 to 6.4 kg at Day -7
TK sampling times (all dogs at all time points): Day 1 and Day 14
Control and Treated animals - 0.5, 1, 2, 3, 8 hours after 1st daily dosing; and 0.5, 1, 2, 3, 8 and 16 hours after 2nd daily dosing
Ab sampling times: Day -4 and Day 14 (all dogs)
Dose formulation analysis: The achieved concentrations met the acceptance criteria (ie, 90-110% of the theoretical concentrations for solutions). It was confirmed that AVE0010 was absent from the vehicle control samples.

Mortality: Twice daily
Clinical signs: 3 times daily
Physical exam: Once daily
Body weights: Daily during dosing period
Food consumption: Daily
Ophthalmoscopy: Not conducted
EKG: Not conducted
Hematology: Day -4 and Day 14 (sponsor-generated tables)
Clinical chemistry: Days -4 and 14 (sponsor-generated tables)
Urinalysis: Not conducted
Gross pathology: Not conducted
Organ weights: Not conducted
Histopathology: Not conducted

Results:

Mortality: No deaths occurred during the study.

Clinical signs:
A summary of clinical signs are shown in the table below. Most signs were only transiently observed. On at least one occasion between Days 1 and 5, high-dose dog #12 exhibited decreased activity (mild and moderate), cold to touch, uncoordinated gait, recumbent posture, whole body twitching, weakness, marked salivation, vomiting (mild), and absence of food intake. High-dose dog #11 showed signs of decreased activity (mild), trembling, vomiting (mild), mild salivation, and absence of food intake. These signs were not observed after Day 5 for these dogs and the third high-dose dog did not

exhibit any of these signs. Dog #11 also appeared emaciated from Days 14 to 23. An increased incidence of soft or liquid feces was observed at the mid- and high-dose levels. An increased incidence of redness of the ears, eyes, and/or oral mucosa was observed for all AVE0010-treated groups.

Clinical signs: (incidence of at least one observation during the dosing period)

Dose (µg/kg BID)	**0**	**5**	**20**	**200**
Clinical Sign **N**	**3**	**3**	**3**	**3**
Decreased activity -mild -moderate	 0 0	 0 0	 1 0	 2 1
Recumbent posture, lateral	0	0	0	1
Weakness	0	0	0	1
Cold to touch	0	0	0	1
Uncoordinated gait	0	0	0	1
Trembling	0	0	0	1
Twitching, whole body	0	0	0	1
Food intake absence	0	0	2	2
Emaciated	0	0	0	1
Salivation -mild -marked	 0 0	 1 0	 1 0	 1 1
Vomiting -foamy -mild -moderate	 0 0 0	 0 0 0	 0 1 1	 1 1 0
Feces, discolored, red	0	0	2	2
Feces, liquid -mild -moderate	 0 0	 0 0	 3 0	 3 2
Feces, soft	1	1	3	3
Paleness, oral mucosa	0	1	1	2
Redness, oral mucosa	0	1	2	1
Redness, both ears	0	2	2	2
Redness, eye(s)	0	3	2	2
Skin elasticity reduced	0	1	1	2

Body weights: Mean body weight loss occurred at the low dose through Day 4 and throughout the study for the mid- and high-dose groups. Weights rebounded during the recovery period, as shown in the sponsor-generated graph of mean body weight below.

Dose (mg/kg BID)	0	5	20	200
Weight (kg) - Day 1	5.93	6.20	6.03	6.50
Weight (kg) -Day 7	6.27	6.23	5.73	5.57*
Weight (kg) -Day 13	6.77	6.53	5.93	5.90
Weight gain (kg) - Day 1-13	0.84	0.33	-0.10	-0.60
Diff from control (g)		-0.51	-0.94	-1.44
% diff from control		↓61%	↓112%	↓171%

*p<0.05

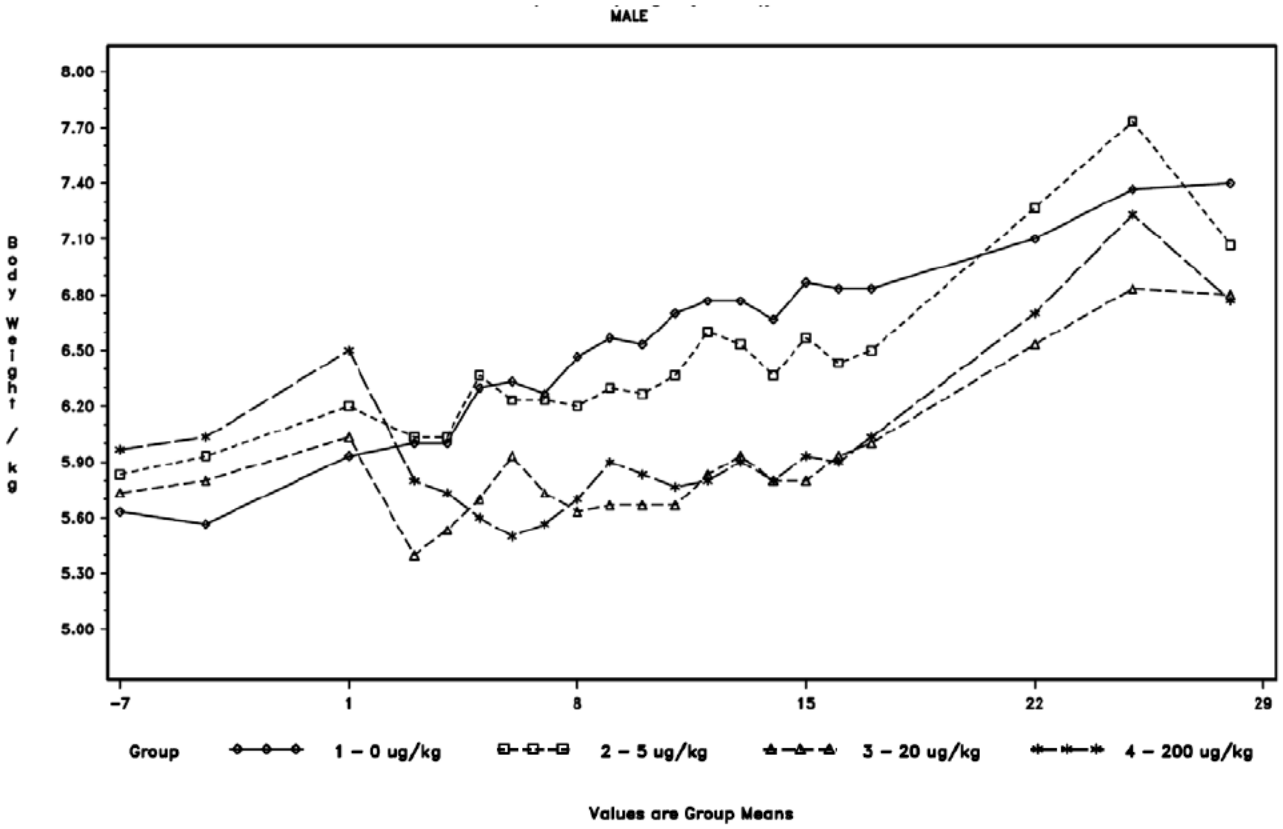


Figure: Graph of Body Weights (sponsor-generated figure)

Food consumption: (sponsor-generated tables)

Pellets: Food Consumption **- Group Summary in g**

Group	Dose in µg/kg BID	Pretest phase	Study phase	Observation period
1	0	5759	13555	14262
2	5	6179	9527	14470
3	20	4891	3679	12034
4	200	6595	2707	12856

Wet Food: Consumption **- Group Summary in g**

Group	Dose in µg/kg BID	Pretest phase	Study phase	Observation period
1	0	no wet food feeding	no wet food feeding	no wet food feeding
2	5	no wet food feeding	no wet food feeding	no wet food feeding
3	20	no wet food feeding	11541	4179
4	200	no wet food feeding	11681	4200

Pellets: Food Consumption **- Group Summary in %**

Group	Dose in µg/kg BID	Pretest phase	Study phase	Observation period
1	0	78.4	92.2	97.0
2	5	84.1	64.8	98.4
3	20	66.5	25.0	81.9
4	200	89.7	18.4	87.5

Wet Food: Consumption **- Group Summary in %**

Group	Dose in µg/kg BID	Pretest phase	Study phase	Observation period
1	0	no wet food feeding	no wet food feeding	no wet food feeding
2	5	no wet food feeding	no wet food feeding	no wet food feeding
3	20	no wet food feeding	87.4	99.5
4	200	no wet food feeding	88.5	100

Hematology: There were no definitive treatment-related effects or toxicologically relevant changes in hematology or coagulation parameters.

Clinical chemistry: There were no definitive treatment-related effects or toxicologically relevant changes in clinical chemistry parameters.

Toxicokinetics:

As shown in the sponsor-generated table below, there was some variability in AUC_{0-24} values between Day 1 and Day 14 for individual animals. For example, some animals had higher values on Day 14 and some had lower values. This variability did not correlate with anti-drug antibody status.

Text table 1 - Summary of toxicokinetic parameters of AVE0010

Species	Sex	Dose (µg/kg/bid)	Dose (µg/kg/day)	C_{max} (ng/mL) [a]		AUC_{0-24} (ng.h/mL) [a]	
				Day 1	Day 14	Day 1	Day 14
juvenile		5	10	3860	3820	30400	31500
dogs	male	20	40	18500	17000	159000	136000
(Beagle)		200	400	73400	84400	723000	755000

[a] Values are rounded to 3 significant figures.

Anti-drug antibody:
Anti-AVE0010 antibodies were analyzed using an assay based on Surface Plasmon Resonance (SPR) employing Biacore technology. The method was validated in human matrix and was applied in this study as an exploratory method for the dog samples.

Two out of 9 treated animals (#9 in the mid-dose group and #10 in the high-dose group) were anti-AVE0010 antibody positive; however the observed immune responses were below the lower limit of quantitation of 3.21 nmol/L. No control animals tested positive. Because of the small number of animals in the study there was no cut-off determination in the screening assay.

Study title: 8-month twice a day (BID) subcutaneous toxicity study in juvenile male dogs with a 2-month recovery period [Interim Report]

Study number:	TXC1462
Study report location:	Module 4.2.3.5.4
Conducting laboratory:	Sanofi-Aventis Deutschland GmbH R&D – SCP Disposition, Safety & Animal Research 65926 Frankfurt, Germany
Date of dose initiation:	17, 18, or 19 August 2010
GLP compliance:	Yes, except for in vivo testicular volume measurements
QA statement:	Yes, but not yet signed; will be included in the final report
Drug, lot #, and % purity:	AVE0010, Batch #B004, 88.1% pure

Key Study Findings

- Initial increased incidences of decreased activity, unstable gait, trembling, reduced skin elasticity, emaciated appearance, and liquid feces were observed at 200 µg/kg QD and/or BID. Increased incidence and severity of vomiting was transiently observed at the MD and HD levels. Incidence and/or duration of injection site induration were observed at all dose levels and injection site swelling was observed at the two 200 µg/kg dose groups.
- An initial, dose-related body weight loss was observed at the MD and HD levels. Mean body weights were statistically significantly lower than control for all treatment groups and the pair-fed control group for the first 4 to 5 months, after which time mean body weights were only slightly lower or similar to controls. The degree of initial body weight loss was slightly greater for the Group 5 (twice daily HD) compared with the pair-fed control group; by the 4th week of treatment and throughout the remainder of the study, mean body weights were similar between these two groups. Effects on body weight correlated with decreased food consumption.
- There were no toxicologically meaningful effects on ophthalmoscopy, ECG parameters, heart rate, or clinical pathology.
- There were no apparent treatment-related effects on the growth and sexual maturation parameters of bone density or in vivo testicular volume measurements.

No differences in mean absolute weights were observed for testis, epididymis, or prostate.

- A slight, dose-related increase in mean absolute and relative thyroid weights was noted.
- An increased incidence of injection sites being red or colored, or having a gelatinous texture at the 200 µg/kg QD or BID. This correlated with an increase in incidence and/or severity of fibrosis, mixed inflammatory cell infiltrate, hemorrhage, and foreign body giant cell granuloma at the injection sites. Injection site findings had diminished or resolved by the end of the treatment period.
- Treatment-related effects in testes included an increased incidence and/or severity of seminiferous tubule dilatation and vacuolation (≥10 µg/kg/d) and hypospermatogenesis or spermatid stasis (≥10 µg/kg/d). In the epididymis dilation, degeneration, and oligospermia/aspermia were observed at ≥200 µg/kg/d. By the end of the treatment period, findings in testis and epididymis were similar to control.
- Based on minimal testicular findings in one male dog at the LD, the NOAEL was considered to <10 µg/kg/d, corresponding to an AUC_{0-24} value of <49.4 ng•h/mL.

Methods

Species/Strain: Dog/Beagle

Design (sponsor-generated table):

Group No.	Final (daily) dose levels (µg/kg/d)	Dose levels (µg/kg BID)	Concentration (µg/mL)	Dose Volume (mL/kg BID)
1	0	0	0	0.125 – 0.25 - 0.5 mL/kg
2	0	0	0	0.125 – 0.25 - 0.5 mL/kg
3	10	1.25 – 2.5 – 5*	10	0.125 – 0.25 - 0.5 mL/kg
4	40	5 – 10 – 20*	40	0.125 – 0.25 - 0.5 mL/kg
5	400	50 - 100 - 200*	400	0.125 – 0.25 - 0.5 mL/kg
6	200	50 - 100 - 200*#	400	0.125 – 0.25 - 0.5 mL/kg

*Three increasing dosing steps with 25%, 50%, and 100% of the final dose every 4th day; final doses were reached on Day 7.
#Group 6 animals were treated once daily treatment.
Group 2 animals were pair-fed matched to Group 5 animals.

Frequency of dosing: Twice daily, split by ~8 hours, except Group 6 (QD)
Route of administration: Subcutaneous
Formulation/Vehicle: 0.9% NaCl
Number/Group: 4 males
Recovery Group: 2 males (Groups 1, 2, 5, 6 only)
Age at start of dosing: 16 to 17 weeks
Weight: 4.7 to 6.1 kg on Day 1
Unique study design: Pair-fed control group
In vivo testicular volume measurements
Bone density measurements

Protocol deviations: There were no deviations that were judged to have had any significant impact on the validity or interpretation of the data.

Observations and Results

Mortality (3 times daily during dosing and once daily during recovery)

There were no unscheduled deaths.

Clinical Signs (3 times daily during dosing and once daily during recovery)

Dose (µg/kg/d)	0	0	10	40	400	200
Group	1	2	3	4	5	6
Decreased Activity						
-mild	0	1/1	0	0	6/3	1/1
-moderate	0	0	0	0	2/1	0
Incoordination gait: unstable	0	0	0	0	2/2	0
Trembling	0	0	0	0	3/1	0
Emaciated	0	0	0	0	0	4/4
Vomiting						
-mild	1/1	1/2	3/3	2/3	5/3	5/2
-moderate	0	0	0	2/1	1/3	2/1
-marked	0	0	0	0	0	1/1
Liquid feces						
-mild	0	0	0	0	6/2	6/2
-moderate	0	0	0	1/1	1/1	1/1
-marked	0	0	0	0	1/1	0
Reduced skin elasticity	0	0	0	0	6/3	1/1
Warm to touch	0	0	0	0	2/4	0
Injection site indurated	1/41	2/5	2/72	3/61	5/101	4/70
Injection site swelling	0	0	0	0	4/37	1/52

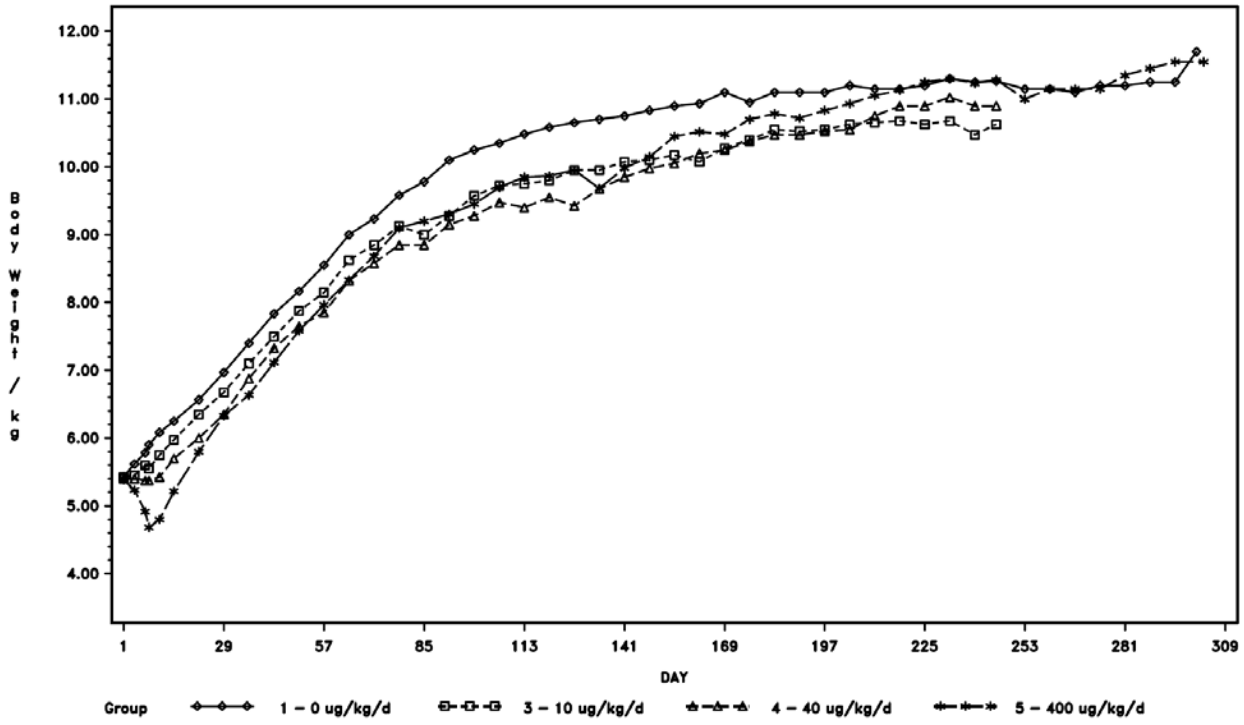
Number of animals / mean number of days with sign

Body Weights (Pretest, Days 1, 4, 7, 8 [except Group 6], 11, 15, then weekly)

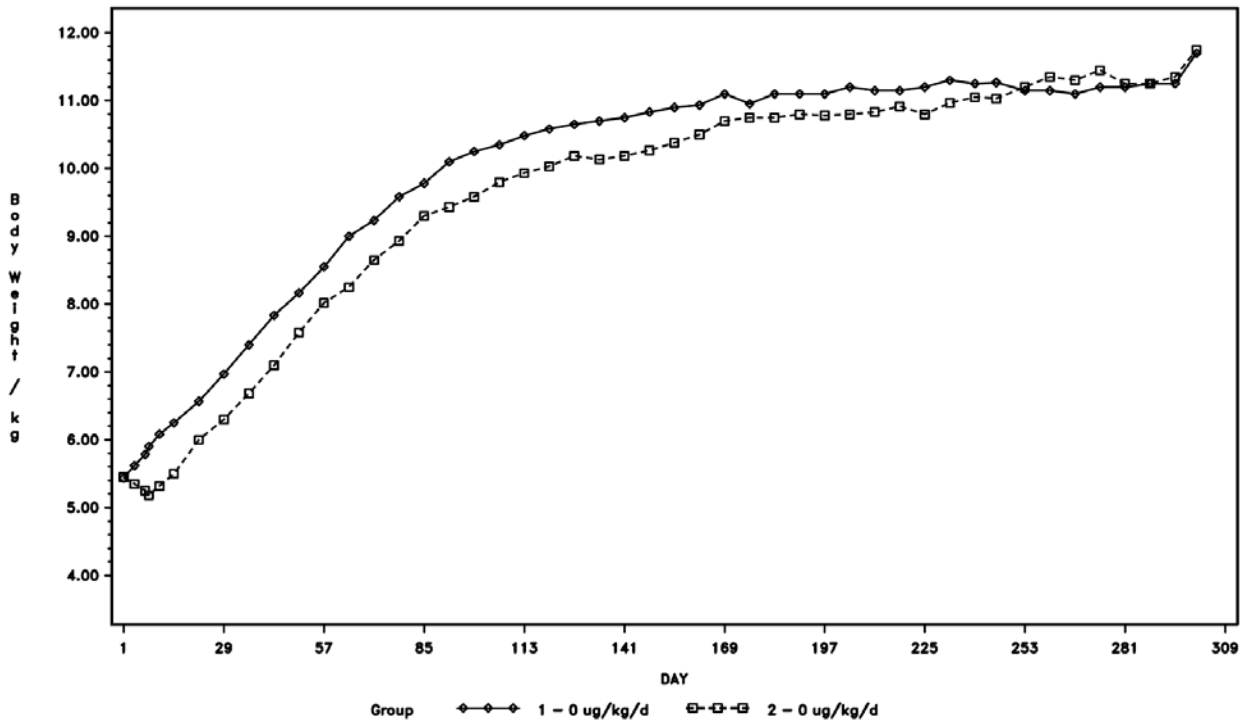
Dose (µg/kg/d)	0	0	10	40	400	200
Group	1	2	3	4	5	6
Weight (kg) - Day 1	5.43	5.45	5.43	5.40	5.38	5.35
Weight (kg) - Day 141	10.75	10.18*	10.08*	9.85*	9.98*	9.97
Weight (kg) - Day 245	11.27	11.03	10.63	10.90	11.28	10.97
Diff from control (kg)		-0.24	-0.64	-0.37	0.01	-0.30
% diff from control		↓2%	↓6%	↓3%	-	↓3%

*p<0.05

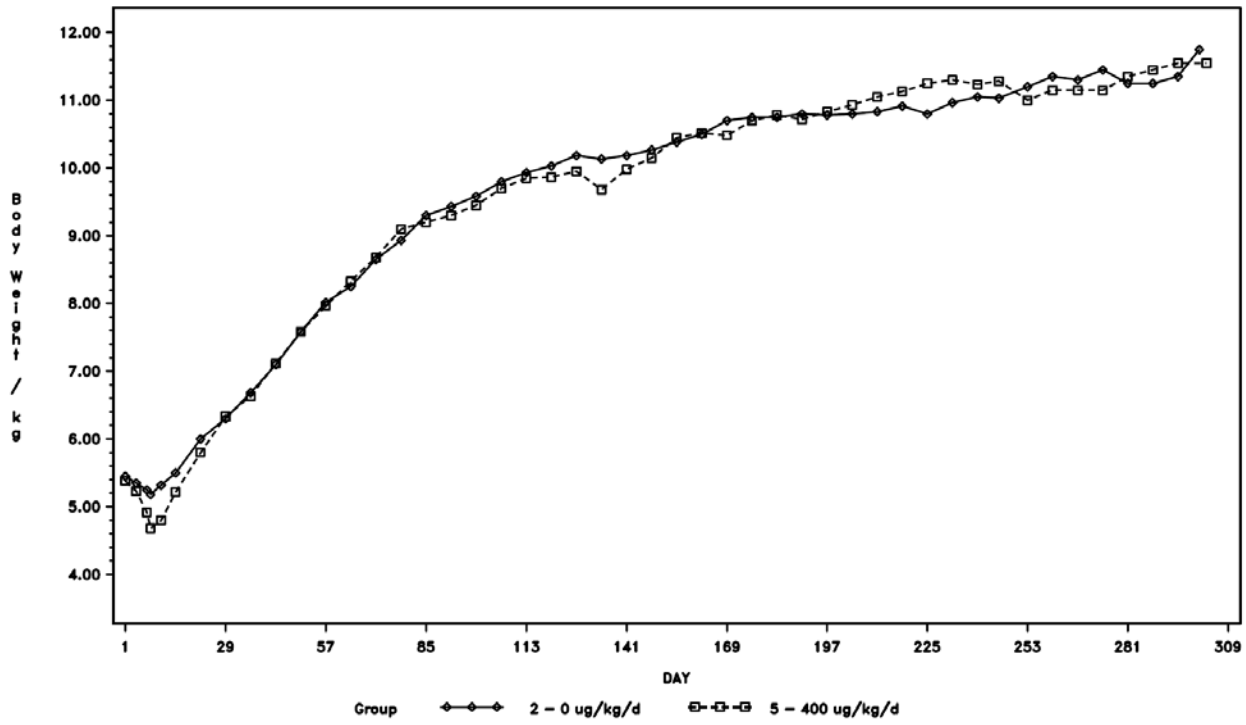
Body weight gain comparison of Groups 1, 3, 4, and 5



Body weight gain of control group 1 compared with pair-fed control group 2



Body weight gain of pair fed control group compared with BID high-dose group



Feed Consumption: (Once daily)

Daily mean food consumption was statistically significantly reduced from Day 1 through 12 at 400 µg/kg/d (~25% to 75%), Day 2 through 11 at 40 µg/kg/d (~45% to 60%), and Day 2 through 12 at 10 µg/kg/d (~25% to 55%). Incidences of reduced food consumption occasionally occurred on other days during the first few months of dosing at all dose levels. In general, mean food consumption was similar across all groups during the second half of the study. Group 2 animals were fed the same amount of food as consumed by Group 5 during the previous day.

Ophthalmoscopy (Pretest, Day 86/87, and Day 237/238/240)

There were no effects on pupillary light reflex or menace reflex. No abnormal ophthamoscopic findings were observed.

ECG (Pretest, Days 84, 239, and 301/303)

There were no treatment-related effects on heart rate or ECG parameters.

Hematology: (Pretest, Days 85, 245, and 300/302)

Dose (µg/kg/d)		0	0	10	40	400	200
Group		1	2	3	4	5	6
Platelets (10^9/L)	Day -10	379.5	325.3	384.8	399.5	358.2	417.7
	Day 85	298.2	302.7	373.8	350.3	456.5*	407.2*
	Day 245	265.5	276.5	302.3	350.0*	**419.2***	341.0*
Eosinophils (10^9/L)	Day -10	0.282	0.213	0.260	0.268	0.338	0.270
	Day 85	0.285	0.263	0.355	0.453	0.347	0.423
	-Day 245	0.380	0.267	**0.600**	**0.530**	**0.755***	**0.530**

*p<0.05.

Clinical Chemistry: (Pretest, Days 85, 245, and 300/302)

There were no toxicologically meaningful effects on clinical chemistry parameters.

Urinalysis: (Pretest, Days 85 to 88, 230 to 234, and 300 to 303)

There were no toxicologically meaningful effects on urinalysis parameters.

In vivo Examination of Testicular Volume (Non-GLP; pretest, Day 86/87, Day 237/238/240, Day 300/302)

There was no apparent effect on testicular volume at the interim time point by caliper measurements or at the end of treatment by caliper measurements or water displacement.

Organ Weights (all main and recovery group animals)

A slight decrease in mean absolute and relative liver weights was observed for treated groups (statistically significant at the MD group; ↓16%) compared with Group 1 controls. However, when compared with the pair fed control group, a difference in mean liver weights was not observed. At the end of recovery, a slight decrease (↓10%) in liver weight was still observed at the HD level.

At the end of treatment, no biologically meaningful differences in mean absolute weights were observed for testis, prostate gland, or epididymis. Testicular weights were slightly lower than Group 1 control values, although in a non-dose related manner. At the end of recovery, mean absolute and relative testicular weights were higher for the 200 µg/kg/dose (BID) and 200 µg/kg/dose (QD) groups when compared with both control group values (~↑40%).

A dose-related, but non-statistically significant increase in absolute and relative thyroid weights was observed (see table below). After recovery, Group 5 weights were still higher than Control 1 (↑19%) but similar to Control 2 (↑4%) and Group 6 thyroid weights were lower than both Control 1 (↓16%) and Control 2 (↓27%).

Summary of Noteworthy Organ Weights

Dose (µg/kg/d)	0	0	10	40	400	200
Group	1	2	3	4	5	6
Thyroid (g)	0.72250	0.62000	0.83500	0.84333	0.84333	0.82500
(%BW)	0.00644	0.00581	0.00813	0.00791	0.00862	0.00758
% difference from control (absolute wt)		↓14%	↑15%	↑17%	↑34%	↑14%

Gross Pathology (all main animals on Day 246 and all recovery animals on Day 302)

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

Sex	Males					
Dose Group	C0	C1	D1	D2	D3	D4
Animals Examined	4	4	4	4	4	4
INJECTION SITE						
– Color, light/pale, red	–	–	–	–	2	1
– Color	–	–	–	–	1	–
– Focus/area, red	1	–	1	2	2	2
– Texture, gelatinous	–	–	–	–	2	4

Histopathology (all main and recovery group animals)

Adequate Battery: Yes

Peer Review: Yes

Histological Findings:
A summary of noteworthy microscopic findings is presented in the abbreviated sponsor-generated tables below. The primary findings were observed in the testis/epididymis and the injection site. No abnormal pancreatic microscopic findings were observed in any group.

Summary of Histological Findings:

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL PHASE EUTHANASIA (K0)

Sex	Males					
Dose Group	C0	C1	D1	D2	D3	D4
No. Animals per Dose Group	4	4	4	4	4	4
INJECTION SITE LEFT No.Examined	4	4	4	4	4	4
– Infiltrate: mixed inflammatory cell	1	2	2	3	4	4
Grade 1	1	2	1	–	–	–
Grade 2	–	–	1	2	–	1
Grade 3	–	–	–	1	4	3
– Fibrosis: subcutaneous	1	2	1	3	4	3
Grade 1	–	1	–	1	–	–
Grade 2	1	1	–	1	–	–
Grade 3	–	–	1	–	2	1
Grade 4	–	–	–	1	2	2
– Hemorrhage	–	–	–	–	2	1
Grade 1	–	–	–	–	1	–
Grade 2	–	–	–	–	1	1
– Granuloma: foreign body giant cell	–	–	–	2	2	2
Grade 1	–	–	–	1	1	1
Grade 2	–	–	–	1	1	1
INJECTION SITE RIGHT No.Examined	4	4	4	4	4	4
– Infiltrate: mixed inflammatory cell	1	–	2	2	4	3
Grade 1	1	–	1	–	–	1
Grade 2	–	–	1	–	2	1
Grade 3	–	–	–	2	2	1
– Fibrosis: subcutaneous	–	–	1	2	4	1
Grade 3	–	–	–	2	2	–
Grade 4	–	–	1	–	2	1
– Hemorrhage	–	–	–	2	2	–
Grade 1	–	–	–	1	1	–
Grade 2	–	–	–	–	1	–
Grade 3	–	–	–	1	–	–
– Granuloma: foreign	1	–	1	2	1	–
Grade 1	1	–	1	1	1	–
Grade 2	–	–	–	1	–	–
PITUITARY GLAND No.Examined	4	4	4	4	4	4
– Cyst	4	4	4	3	4	3
Grade 1	4	3	3	1	2	2
Grade 2	–	–	1	1	1	–
Grade 3	–	1	–	1	1	–
Grade 4	–	–	–	–	–	1

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: TERMINAL PHASE EUTHANASIA (K0)

Sex	Males					
Dose Group	C0	C1	D1	D2	D3	D4
No. Animals per Dose Group	4	4	4	4	4	4
TESTIS No.Examined	4	4	4	4	4	4
– Atrophy: seminiferous tubule	–	1	1	–	–	–
Grade 1	–	–	1	–	–	–
Grade 2	–	1	–	–	–	–
– Dilatation: seminiferous tubule	–	–	1	3	3	3
Grade 1	–	–	1	1	–	1
Grade 2	–	–	–	1	2	2
Grade 3	–	–	–	1	1	–
– Vacuolation: seminiferous tubule	4	4	4	4	4	4
Grade 1	4	4	3	1	1	2
Grade 2	–	–	1	2	2	2
Grade 3	–	–	–	1	1	–
– Hypospermatogenesis	2	3	1	4	2	2
Grade 1	2	3	1	2	1	2
Grade 2	–	–	–	2	1	–
– Stasis:spermatid	1	–	–	1	1	3
Grade 1	1	–	–	1	1	3
EPIDIDYMIS No.Examined	4	4	4	4	4	4
– Dilation: initial segment and efferent ducts	–	–	–	–	2	3
Grade 1	–	–	–	–	1	–
Grade 2	–	–	–	–	1	3
– Degeneration: epithelium of initial segment	–	–	–	–	1	3
Grade 1	–	–	–	–	–	2
Grade 2	–	–	–	–	1	1
– Oligospermia/Aspermia	–	–	–	–	1	1
Grade 2	–	–	–	–	–	1
Grade 3	–	–	–	–	1	–
THYMUS No.Examined	4	4	4	4	4	4
– Atrophy	1	–	2	3	2	–
Grade 2	1	–	2	3	2	–
– Hemorrhage	1	1	1	1	–	1
Grade 1	1	1	1	1	–	1
– Cyst	1	–	–	3	2	1
Grade 1	1	–	–	3	2	1

RECOVERY

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
Necropsy Status: RECOVERY PHASE EUTHANASIA (R1)

Sex	Males					
Dose Group	C0	C1	D1	D2	D3	D4
No. Animals per Dose Group	2	2	–	–	2	2
INJECTION SITE LEFT No.Examined	2	2	–	–	2	2
– Infiltrate: mixed inflammatory cell	1	1	–	–	1	–
Grade 1	1	1	–	–	1	–
– Fibrosis: subcutaneous	–	–	–	–	1	–
Grade 2	–	–	–	–	1	–
INJECTION SITE RIGHT No.Examined	2	2	–	–	2	2
– Infiltrate: mixed inflammatory cell	1	–	–	–	–	1
Grade 1	1	–	–	–	–	1
– Fibrosis: subcutaneous	–	–	–	–	1	1
Grade 2	–	–	–	–	1	1
– Granuloma: foreign	–	–	–	–	2	–
Grade 1	–	–	–	–	2	–
TESTIS No.Examined	2	2	–	–	2	2
– Atrophy: seminiferous tubule	–	–	–	–	1	–
Grade 1	–	–	–	–	1	–
– Vacuolation: seminiferous tubule	2	2	–	–	1	2
Grade 1	2	2	–	–	1	2
– Hypospermatogenesis	1	1	–	–	2	–
Grade 1	–	1	–	–	2	–
Grade 2	1	–	–	–	–	–
– Stasis:spermatid	–	1	–	–	1	1
Grade 1	–	1	–	–	1	1
– Swollen spermatocytes	1	–	–	–	–	–
Grade 1	1	–	–	–	–	–

Special Evaluation

Bone Mineral Density

Ex vivo dual-energy X-ray absorptiometery (DXA) measurements (right femurs of all main study animals; recovery animals were not assessed)

Mean femur mineral density (expressed in g/cm^2)

Group	1	2	3	4	5	6
Dose-level (µg/kg BID)	0	0	5	20	200	200[a)]
Right femur						
- global	0.649	0.643	0.634	0.644	0.642	0.623
- proximal part	0. 564	0.574	0.551	0.572	0.582	0.544
- distal metaphysis	0.571	0.588	0.562	0.569	0.561	0.559
- diaphysis	0.651	0.654	0.645	0.636	0.634	0.628

[a)]: 200 µg/kg/day (once daily).

Ex vivo peripheral quantitative computed tomography (pQCT) measurements (right femurs of all main study animals; recovery animals were not assessed)

Mean femur mineral density (expressed in g/cm^3)

Group	1	2	3	4	5	6
Dose-level (µg/kg bid)	0	0	5	20	200	200[a)]
. Right femur						
. Metaphysis						
- total density	509.3	528.1	500.0	518.0	515.9	515.4
- trabecular density	273.5	277.5	245.7	256.8	260.3	245.9
- (sub) cortical density	843.9	818.5	832.4	840.4	844.0	860.2
. Diaphysis						
- cortical density	1115.7	1119.1	1114.4	1133.0	1128.1	1128.8

[a)]: 200 µg/kg/day (once daily).

Toxicokinetics (Day 7 Day 91, and Day 245)

Groups 1-5
Day 7 [first day of final dose level], 91, and 245: Samples were collected before (Day 7 only) and 1, 2, 3, 8, hours after the 1st daily dose and 1, 2, 3, 8, and 16 hours after the second daily dose.

Group 6
Day 7, 91, and 245: Samples were collected before (Day 7 only) and 1, 2, 3, 8, 10 and 24 hours after the 1st and only daily dose.

Sex	Dose (µg/kg/day)	C_{max} (ng/mL)			AUC_{0-24} (ng.h/mL)		
		Day 7	Day 91	Day 245	Day 7	Day 91	Day 245
Male	10	3.31	4.97	5.48	29.9	40.5	49.4
	40	14.1	13.4	120	125	121	2360
	400	76.8	228	1580	1050	4070	32500
	200	59.3	115	303	522	1420	4660

Values are rounded to 3 significant figures
(sponsor-generated table)

Anti-Drug Antibody Assessment

(pretest, Day 85, Day 245, and Day 300/302 [Recovery period])

Samples were collected but the results are still pending. The results will be included in the final report or in an amended report.

Dosing Solution Analysis

Test article was not detected in any of the vehicle control formulations. Eleven out of 26 formulations were outside of the acceptance range. When out of range, the LD and MD formulations tended to be lower than the nominal concentration (as low as 59%) and the HD formulations tended to be higher than nominal (as high as 240%). These deviations did not impact the validity of the study.

Histopathology Inventory for Juvenile Toxicology Studies

Study	5 Weeks	8 Months
Species	**RAT**	**DOG**
Adrenals	X*	X*
Aorta	X	X
Bone Marrow (in bone section)	X	X
Bone (femur + femorot bial joint)	X	X
Brain	X*	X*
Cecum	X	X
Colon	X	X
Duodenum	X	X
Epididymides	X*	X*
Esophagus	X	X
Eyes	†	X
Gall bladder	NA	X
Gross lesions	X	X
Harderian gland	†	
Heart	X*	X*
Ileum	X	X
Injection sites	X	X
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	†	X
Larynx	†	†
Liver	X*	X*
Lungs	X	X*
Lymph node	X (mandibular)	X (retropharyngeal)
Lymph node, popliteal	X*	
Lymph node, mesenteric	X	X
Mammary gland	X	X
Nasal cavity	†	†
Optic nerves	†	X
Ovaries	X*	NA
Oviducts	X	NA
Pancreas	X	X
Peyer's patches	X	X
Pharynx	†	
Pituitary	X	X*
Prostate	X*	X*
Rectum	X	X
Salivary gland (parotid, mand bular, sublingual)	†	X
Sciatic nerve	X	X
Seminal vesicles	X*	
Skeletal muscle (quadriceps femoris and diaphragm [dog only])	†	X
Skin	†	X
Spinal cord (cervical only for dog)	X	X
Spleen	X*	X
Sternum with bone marrow	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid + parathyroid	X*	X*
Tongue	†	X
Trachea	X	X
Ureters	X	X
Urinary bladder	X	X
Uterus + cervix	X*	NA
Vagina	X	NA
Zymbal gland	†	

X, histopathology performed; *, organ weight obtained;
†tissues collected but not evaluated microscopically

10 Special Toxicology Studies

Mechanistic Studies – Thyroid C-cells

GLP-1 receptor profiling in rat thyroid tissue (Study DIV1353)

Objective: The purpose of this study was to determine the expression of GLP-1 receptor in thyroid C-cells in comparison to follicular cells of untreated rats.

Study Design: Thyroid tissue from untreated 4-month old (2 independent experiments) or 12-month old (3 independent experiments) male rats was isolated, fixed in 10% neutral buffered formalin, and stained by immunohistochemistry using anti-calcitonin antibody. C-cells and follicular cells were excised by laser capture microdissection. Total RNA was isolated and expression levels were assessed by using real-time PCR. Note that cycle threshold (CT) values greater than 35 approach the sensitivity limits of the real-time PCR system. Beta actin expression was used for quality control and normalization.

Results: The mean expression CT value (cycle threshold) of calcitonin RNA for C-cell fractions (n = 18) was 18.1 with a range of 16.4 to 21.8. The mean CT value for the follicular cell fraction was 29.2 with a range of 24.3 to 38.2. No age related differences were noted.

The mean expression CT value of GLP-1 receptor RNA for C-cell fractions (n = 15) was 27.4 with a range of 24.1 to 29.5. In one C-cell fraction, the expression level of GLP-1 receptor was undetermined. The mean CT value for the follicular cell fraction was undetermined for 11 out of 13 fractions. Only in two samples high CT values of 32.2 and 36.3 were observed, which might indicate expression traces (if at all). No age related differences were observed. Results are summarized in the sponsor-generated tables below.

Table 2 - Expression ranges (experiment 1 – 5)

CT ranges experiment 1 - 5	Cell type	CT ACTB	CT Calca	CT Glp1r
Experiment 1 - 5	C-cells	21.7 - 24.9 [n=18]	16.4 - 21.8 [n=18]	24.1 - 29.5 [n=15]
Experiment 1 - 5	follicle cells	20.8 - 27.6 [n=16)	24.3 - 38.2 [n=16]	undetermined [n=11] 32.2 - 36.3 [n=2]

Table 3 - CT mean values not normalized / normalized to beta actin (experiment 1 – 5)

Exp. 1 - 5	Cell type	CT mean Actb	CT mean Calca	CT mean Glp1r	ΔCT mean Calca	ΔCT mean Glp1r
	c-cell mean	23.5	18.1	27.4	-5.4	3.8
	c-cell SD	1.1	1.3	1.5		
	follicle cell mean	24.6	29.2	undetermined*	4.6	**
	follicle cell SD	1.8	4.1	**	**	**

* Glp1r expression was undetermined in 11 of 13 samples

** calculation not possible

Actb = beta actin; Calca = calcitonin.

Conclusions: Under the conditions of this study, GLP-1 receptor RNA expression was observed in C-cells but generally not in follicular cells. No difference in RNA expression was noted for calcitonin or GLP-1 receptor between 4-month old rats and 12-month old rats in either the C-cells or follicular cells.

Functional activity of lixisenatide in thyroid c-cells of rat and human origin in vitro (Study DIVT0007)

Objective: To measure ligand-induced cellular cAMP response in immortal thyroid C-cell lines derived from two different species, 6-23 from rat and human C-cell line TT.

Study Design: Rat C-cell line 6-23 and human C-cell line TT were treated with lixisenatide (AVE0010), liraglutide, or GIP receptor agonist. Cells were dispensed into the wells of 96-well plates. For each ligand, two individual dose dilution series were performed in one microtiter plate, with one case spanning the concentration range from 1 µM to 10 pM in 1:10 serial dilutions and each concentration in triplicate; cells in a second plate were treated with concentrations spanning from 10 nM to 0.1 pM, again with 1:10 serial dilutions and triplicate measurements. Receptor-mediated cAMP production was assessed using an assay based on commercial immunoassay technology with HTRF readout. Data from the two dilution series were pooled and dose response curves were generated.

Results:
Results are summarized in the sponsor-generated tables and figures below.

Table 1 - In vitro functional assay data for cAMP response of the compounds Exendin-4, Liraglutide, GIP, Lixisenatide and GLP-1(7-36) amide in the rat thyroid C-cell line 6-23

Compound	Emin%	Emax%	EminC [pM]	EmaxC [pM]	Slope	EC_{50} rel [pM]	EC_{50} rel (LCB95%) [pM]	EC_{50} rel (UCB95%) [pM]	EC_{50} rel (CV)
Exendin-4	-2.56	114.606	0.1	1000000	1.09	**4.49**	4.01	5.03	5.6%
Liraglutide	0.561	113.927	0.1	1000000	1.15	**715**	621	824	6.9%
GIP	0.741	75.691	0.1	1000000	0.93	**1320**	1110	1560	8.6%
Lixisenatide	1.25	106.756	0.1	1000000	1.10	**8.31**	7.44	9.28	5.4%
GLP-1(7-36)	6.974	105.827	0.1	1000000	1.33	**6.88**	6.29	7.52	4.4%

Table 2 - In vitro functional assay data for cAMP response of the compounds Exendin-4, Liraglutide, GIP, Lixisenatide and GLP-1(7-36) amide in the human thyroid C-cell line TT

Compound	Emin%	Emax%	EminC [pM]	EmaxC [pM]	Slope	EC_{50} rel [pM]	EC_{50} rel (LCB95%) [pM]	EC_{50} rel (UCB95%) [pM]	EC_{50} rel (CV)
Exendin-4	.	.	.	.	.	.	.	.	.
Liraglutide	1.091	25.967	0.1	1000000	0.66	**38600**	1090	1370000	453%
GIP	-2.437	101.75	0.1	1000000	0.98	**181**	136	242	14%
Lixisenatide	.	.	.	.	.	.	.	.	.
GLP-1(7-36)	2.735	15.453	0.1	1000000	1.04	**56.0**	3.77	830	218%

Figure 1 - Dose response curves from in vitro functional assay data for cAMP response of Exendin-4, Liraglutide, GIP, Lixisenatide and GLP-1(7-36) amide in the rat thyroid C-cell line 6-23

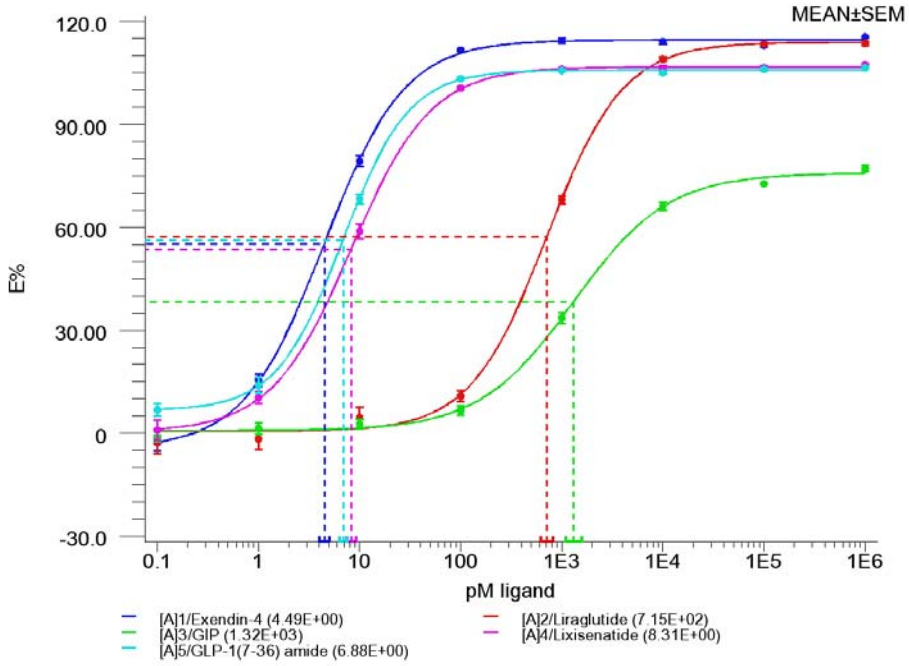
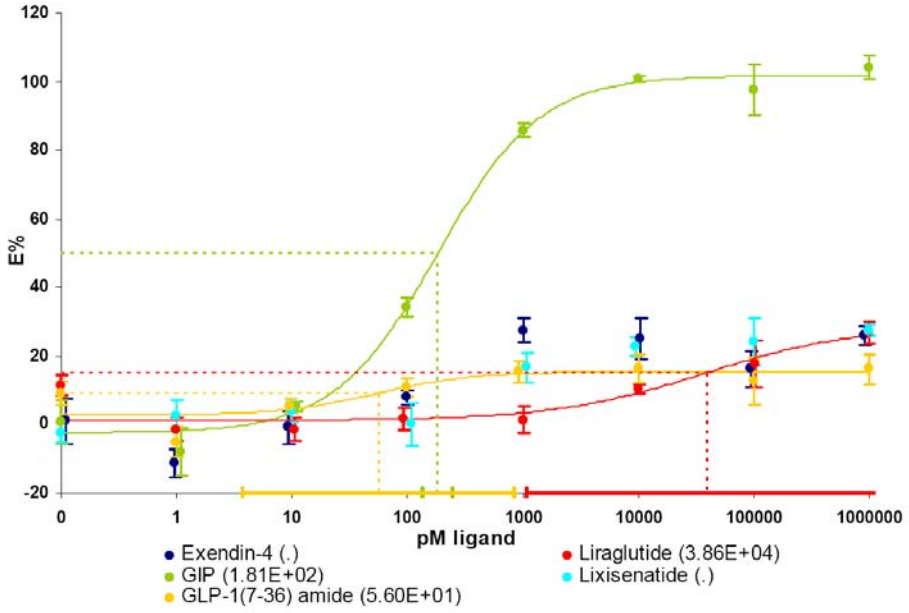


Figure 2 - Dose response curves from in vitro functional assay data for cAMP response of Exendin-4, Liraglutide, GIP, Lixisenatide and GLP-1(7-36) amide in the human thyroid C-cell line TT



Small variability in doses is intentional for better visibility of the response for each ligand

Conclusions: In rat cell line 6-23, E_{max}% values were all similar for all four GLP-1 receptor agonists (106-115%), while E_{max}% of GIP was lower (76%). Relative potency ranking (EC_{50} rel values) of the four GLP-1 receptor agonists and the one GIP receptor agonist in the rat C-cell line 6-23 was:

exendin-4 ~ lixisenatide ~ GLP-1(7-36) amide >> liraglutide > GIP

In the human cell line TT, E_{max}% values for all four GLP-1 receptor agonists were low, but these values were again similar among themselves (15-26%). The somewhat larger deviation for GLP-1 may be due to the larger experimental variability at the higher concentrations for this compound. However, E_{max}% for GIP that does not activate the GLP-1 receptor but the GIP receptor ligand GIP was much higher (102%) in TT cells. A relative potency sequence of the four GLP-1 receptor ligands involving lixisenatide and exendin-4 cannot be demonstrated due to the poor fits.

Overall, there was strong signaling through the GIP receptor by native GIP in both cell lines, while signaling through the GLP-1 receptor by the four GLP-1 receptor agonists was only strong in the rat C-cell line, but weak in the human C-cell line.

GLP-1 receptor profiling in human normal tissue (Study DIV1422)

Objective: The purpose of this study was to determine the expression of GLP-1 receptor in thyroid C-cells in comparison to follicular cells in normal human tissues; **the thyroid gland was not included in this panel**.

Study Design: Twenty-four human peripheral tissues and twenty-one human brain regions were profiled on a panel of ~370 human G protein coupled receptors (GPCRs), including GLP-1 receptor, using the Transcript Low Density Array (TLDA) technique (Applied Biosystems). Three to five samples were collected for each tissue with the exception of adrenal gland, duodenum, jejunum, and skeletal muscle (n=2). Human tissues and tissue RNA were obtained from hospitals, academic tissue banks, and commercial resources.

After total RNA isolation and quality control, cDNA was synthetized and quantitative Real Time RT-PCR was performed. A panel of six housekeeping genes was also measured on the same TLDA. Cyclophilin A (PPIA) was the most stable mRNA of the six, and was used to normalize the data. The cycle threshold (CT) values greater than 35 approach the sensitivity limits of the real-time PCR system.

Results/Conclusions: GLP-1 receptor was detected at low to very low levels in most peripheral tissues. It was undetectable in aorta, skeletal muscle and vas deferens. Highest expression in the periphery was found in heart and pancreas. It was medium in

both. In brain, GLP-1 receptor mRNA was undetectable in thalamus, low in caudate, corpus callosum, nucleus basalis, hypothalamus, nucleus accumbens, globus pallidus and putamen, and very low all other regions. A summary of the results is shown in the sponsor-generated tables below.

Table 1 - GLP1R expression in human normal peripheral tissues

GLP1R (Assay = hs00157705_m1)	Relative abundance versus PPIA	Standard deviation*	Number of measures	Number of measures with detected expression (Ct<=35)	Raw Ct values
Adipose_SubCut	43	66	3	1	35 / 40 / 40
Adipose_Visc	243	246	3	2	32.9 / 36 / 33.9
Adrenal gland	132	39	2	-	36.3 / 36
Aorta	11	6	3	-	40 / 40 / 40
Bladder	87	141	3	-	40 / 40 / 35.2
Colon Intertaenia (no mucosa)	86	23	3	-	35.9 / 36.4 / 35.9
Colon Mucosa	47	69	3	-	35.9 / 40 / 40
Colon Tenia (no mucosa)	105	88	3	-	36 / 35.9 / 40
Duodenum	517	694	2	1	40 / 33.9
Esophagus	73	70	3	1	40 / 34.7 / 35.9
Heart	11507	9703	3	3	32.6 / 29.1 / 28.9
Ileum	604	900	4	3	34.6 / 36.3 / 33.6 / 30.9
Ileum Mucosa	1155	1944	3	1	37 / 40 / 31.1
Ileum (no mucosa)	705	540	3	3	34 / 33.9 / 32.5
Jejunum	132	172	2	-	40 / 35.9
Liver	7	3	3	-	40 / 40 / 40
Lung Alveolar	706	262	4	4	32.9 / 33.4 / 31.9 / 32.9
Ovary	37	54	4	-	40 / 40 / 40 / 35.9
Pancreas	6693	2293	3	3	31.5 / 30.9 / 30.8
Skeletal muscle	14	0	2	-	40 / 40
Spleen	54	39	3	-	36.9 / 35.7 / 40
Testis	113	165	3	1	39.2 / 35 / 40
Urethra	105	171	3	1	40 / 34.3 / 40
Vas Deferens	7	2	3	-	40 / 40 / 40

* Please note that the number of samples is small, thus the standard deviation is not significant and is provided as an indication only

Table 2 - GLP1R expression in human brain regions

GLP1R (Assay = hs00157705_m1)	Relative abundance versus PPIA	Standard deviation*	Number of measures	Number of measures with detected expression (Ct<=35)	Raw Ct values
Amygdala	843	261	3	3	31.9 / 31.7 / 33.8
Caudate	1387	534	3	3	31.9 / 31.5 / 31.9
Cerebellum	444	184	3	3	33.1 / 32.9 / 34.9
Cervical Spinal Cord	470	235	3	1	35.9 / 34.2 / 35.9
Cingulum	457	177	3	3	32.8 / 31.7 / 31.9
Corpus Callosum	1522	707	3	3	33.9 / 32.6 / 33.5
Entorhinal Cortex	656	381	3	3	34.2 / 34.9 / 33.6
Frontal Cortex	204	170	3	2	35.4 / 34.6 / 34.0
Globus Pallidus	2106	1089	3	3	31.1 / 31.6 / 30.9
Hippocampus	765	206	3	3	32.8 / 33.0 / 33.9
Hypothalamus	3622	817	3	3	30.1 / 30.3 / 29.9
Lateral Septum	865	145	3	3	32.7 / 32.9 / 31.9
Nucleus Accumbens	3963	689	3	3	31.1 / 30.7 / 29.9
Nucleus Basalis	1882	1143	3	2	31.3 / 35.9 / 33.7
Parietal Cortex	347	231	3	3	33.0 / 33.1 / 34.1
Prefrontal Cortex	607	291	3	3	31.7 / 32.4 / 30.9
Putamen	2626	743	3	3	30.7 / 30.9 / 30.5
Substantia Nigra	248	90	3	3	35.0 / 33.2 / 33.9
Temporal Cortex	201	106	3	3	33.9 / 32.8 / 33.8
Thalamus	47	38	3	-	35.7 / 35.9 / 40->Not Detected
Ventral Tegmental Area	347	317	3	2	40->Not Detected / 32.6 / 32.9

* Please note that the number of samples is small, thus the standard deviation is not significant and is provided as an indication only

GLP-1 receptor profiling in human thyroid FFPE tissue from (b) (4) (Study DIV1391)

Objective: To determine the expression of GLP-1 receptor in thyroid gland c-cells in comparison to follicular cells in human.

Study Design: Thyroid gland samples from 5 male human donors (age between 44 and 82 years) were purchased from (b) (4) Quantitative Real Time PCR was performed to assess the expression levels of beta actin, calcitonin, and GLP-1 receptor.

Results:
RNA quality was found to be poor. RNA from unstained whole sections was converted to cDNA and quantitative Real Time PCR was performed to investigate if expression levels of beta actin, calcitonin, and GLP-1 receptor could be expressed despite the poor RNA quality. Expression of beta actin could not be determined in 2 of 5 samples due to poor RNA quality. Expression of calcitonin and GLP-1 receptor was undetermined in 3 of 5 samples. When PCR results were obtained, they tended to be highly varied and inconsistent.

Conclusion:
Due to the poor quality of RNA in the thyroid tissues that were purchased for this investigation, a conclusion regarding the expression levels of calcitonin and GLP-1 receptor could not be made.

GLP-1 Receptor profiling in normal human thyroid gland tissue from (b) (4)
(Study DIV1487)

Objective: To determine the expression of GLP-1 receptor in thyroid follicular cells and C-cells in thyroid glands without C-cell pathology.

Study Design:
Four frozen human thyroid gland samples were purchased from (b) (4). Immuncytochemical staining of thyroid glands with polyclonal rabbit calcitonin antibody was conducted to differentiate C-cells from follicular cells. C-cell and follicular cell fractions of the human thyroid gland tissue were excised by laser capture microdissection.

Thyroglobulin expression levels were used as an indicator for thyroid gland tissue. Calcitonin expression levels were used as an indicator for the purity of the different cell fractions. Gene expression was evaluated by using quantitative real-time PCR. The housekeeping gene beta actin was used for quality control. CT-values (cycle threshold) were used to represent the PCR cycle at which an increase in reporter fluorescence was first detected above baseline. A CT value of 35 approaches the sensitivity limits of the assay.

Results:
The RNA quality was found to be acceptable, with the isolated RNA fragments being up to 2000 base pairs or longer. The PCR results are summarized in the sponsor-generated tables below.

8.2.2 CT-mean values for C-cell fractions

Table 4 - CT mean values for C-cell fractions (n=2)

C-cell fractions	ACTB	CALCA	GLP1R	TG
CT mean value	23.21	20.67	33.59	21.47
SD	0.369	0.990	0.615	0.655

SD = standard deviation

8.2.3 CT-mean values for follicular cell fractions

Table 5 - CT mean values for follicular cell fractions (n=4)

Follicular cell fraction	ACTB	CALCA	GLP1R	TG
CT mean value	21.40	undetectable	undetectable	18.73
SD	1.216	*	*	0.805
* Calculation not possible				

SD = standard deviation

ACTB = beta actin; CALCA = calcitonin

Conclusions:
Under the conditions of this study, the results demonstrate that GLP-1 receptor RNA expression in thyroid C-cells is marginal, whereas GLP-1 receptor RNA is generally not expressed in thyroid gland follicular cells.

GLP-1 Receptor profiling in normal human thyroid gland tissue provided by the [redacted] (b) (4) (Study DIV1498)

Objective: To determine the expression of GLP-1 receptor in thyroid follicular cells and C-cells in thyroid glands without C-cell pathology.

Study Design:
Paraffin samples from subjects without C-cell pathology (surgery because of papillary microcarcinoma) were screened by immunocytochemistry [redacted] (b) (4) in order to identify areas with C-cells. From 10 human thyroid gland samples without C-cell pathology (non-neoplastic C-cells) 10 unstained sections from each were checked for C-cell presence were prepared in the [redacted] (b) (4) and shipped to the laboratories of Sanofi-Aventis (Germany) for GLP-1 receptor expression analysis from formalin-fixed paraffin-embedded tissue. Thyroid gland tissue samples were excised by laser capture microdissection. Due to insufficient number of C-cells in combination with RNA quality only 7/10 samples were assessed for expression in C-cells and 8/10 for expression in follicular cells.

Thyroglobulin expression levels were used as an indicator for thyroid gland tissue. Calcitonin expression levels were used as an indicator for the purity of the different cell

fractions. Gene expression was evaluated by using quantitative real-time PCR. The housekeeping gene beta actin was used for quality control. CT-values (cycle threshold) were used to represent the PCR cycle at which an increase in reporter fluorescence was first detected above baseline. A CT value of 35 approaches the sensitivity limits of the assay.

Results:
RNA quality may not have been adequate, as the RNA was partially fragmented, with fragment length less than 200 base pairs in most samples. Variability in gene expression results was noted in this study and the sponsor stated that it may have been related to low starting RNA amount in combination with moderate RNA quality as indicated by fragmentation analysis. The PCR results are summarized in the sponsor-generated tables below.

8.2.2 Main experiment CT-mean values for C-cell fractions

Table 4 - CT mean values for C-cell fractions (n=7)

C-cell fractions	ACTB	CALCA	GLP1R	TG
CT mean value	24.60	26.64	Undetectable *	24.98
SD	3.832	2.841	Not calculated	3.969

SD = standard deviation

* undetectable in 6/7 samples, marginal value (CT 34.86) in 1/7 samples, sensitivity limit of the system is at CT 35.

8.2.3 Main experiment CT-mean values for follicular cell fractions

Table 5 - CT mean values for follicular cell fractions (n=8)

Follicular cell fraction	ACTB	CALCA	GLP1R	TG
CT mean value	26.40	Undetectable *	Undetectable	25.35
SD	3.214	Not calculated	Not calculated	3.678

SD = standard deviation

* undetectable in 7/8 samples, marginal value (CT 34.62) in 1/8 samples, sensitivity limit of the system is at CT 35.

ACTB = beta actin; CALCA = calcitonin.

Conclusions:
Calcitonin RNA was expressed in C-cell fractions but not in follicular cell fractions, demonstrating proper C-cell and follicular cell separation. GLP-1 receptor RNA expression could not be detected in C-cell fractions or follicular cell fractions of the human thyroid gland samples evaluated in this study. This may have been the result of a combination of low expression compounded with partially degraded RNA.

Title: GLP-1 receptor profiling in normal human thyroid gland tissue from (b) (4) (Study DIV1416)

Five frozen human thyroid glands were obtained for GLP1R profiling. After processing and staining for calcitonin, C-cells and follicular cells were collected separately by using laser capture microdissection, total RNA was isolated, and RT-PCR was conducted to measure the expression of GLP1R, calcitonin, beta actin, and thyroglobulin. A threshold cycle value of 35 was considered to be at the sensitivity limit of detection.

A summary of gene expression is shown in the sponsor-generated tables below. These data indicate that calcitonin was expressed in C-cell fractions, but not in follicular cell fractions. GLP1R RNA expression in C-cells and follicular cells was low, with 2/5 C-cell samples showing low expression and 1/5 follicular cell samples showing low expression.

Table 3 - CT mean values for samples from whole section (n=5)

Whole section samples	ACTB	CALCA	GLP1R	TG
CT mean value	15.0	22.2	30.7	12.5
SD	1.03	5.89	3.46	1.35

SD = standard deviation

Table 4 - CT mean values for C-cell fractions (n=5)

C-cell fractions	ACTB	CALCA	GLP1R	TG
CT mean value	24.3	21.5	32.1 *	22.3
SD	1.33	1.13	2.55	1.75
* only two out of five samples provided a CT-value.				

SD = standard deviation

Table 5 - CT mean values for follicular cell fractions (n=5)

Follicular cell fraction	ACTB	CALCA	GLP1R	TG
CT mean value	23.8	undetectable	32.5**	21.0
SD	1.96	*	*	2.40
* Calculation not possible				
** only one sample out of five provided a CT-value.				

SD = standard deviation

Title: GLP-1 receptor profiling in human thyroid gland tissue with C-cell pathology in comparison to normal tissue provided by the (b) (4) (Study DIV1478)

The purpose of this study was to determine the expression of GLP1R in follicular cells and C-cells in human thyroid glands with non-neoplastic and neoplastic C-cell hyperplasia, sporadic C-cell carcinomas, and C-cell carcinomas in the setting of multiple endocrine neoplasia (MEN2) in comparison to expression levels from normal tissue,

which was assessed in Study DIV1498 reviewed above. A summary of the tissues studied is presented in the sponsor-generated table below.

Group	Sample describtion	Number of samples
A	Human thyroid tissue without C-cell pathology	10
B	Human thyroid tissue with sporadic C-cell carcinoma	10
C	Human thyroid tissue with C-cell carcinomas in the setting of multiple endocrine neoplasia (MEN2)	10
D	Human thyroid tissue with C-cell hyperplasia: Group D1: non-neoplastic C-cell hyperplasia Group D2: neoplastic C-cell hyperplasia	 5 5

Samples were screened by immunohistochemistry to identify areas with non-neoplastic and neoplastic cells. C-cells and follicular cells were collected separately by using laser capture microdissection, total RNA was isolated, and RT-PCR was conducted to measure the expression of GLP1R, calcitonin, beta actin, and thyroglobulin. A threshold cycle value of 35 was considered to be at the sensitivity limit of detection. A summary of the RT-PCR results for C-cells and follicular cells is presented in the sponsor-generated tables below.

Table 8 - CT mean values for C-cell fractions

C-cell fractions	ACTB	CALCA	GLP1R	TG
Group A - CT mean value	24.60	26.64	Not detectable (*)	24.98
Group A - SD	3.83	2.84	Not calculated	3.97
Group B - CT mean value	25.41	23.74	35.22(**)	29.27(***)
Group B - SD	4.98	4.91	1.17	3.21
Group C - CT mean value	28.73	24.97*	Not detectable**	31.97***
Group C - SD	2.02	2.81	Not calculated	1.89
Group D1 - CT mean value	28.61	29.17	Not detectable	28.11
Group D1 - SD	1.24	1.04	Not calculated	2.00
Group D2 - CT mean value	28.36	26.96	Not detectable	28.18
Group D2 - SD	2.37	3.34	Not calculated	4.20

SD = standard deviation

(*) not detectable in 6/7 samples, marginal value (CT 34.86) in 1/7 samples, sensitivity limit of the system is at CT 35

(**) not detectable in 8/10 samples, detectable in 2 /10 samples with a CT value of 34.4 and 36.0 and at the sensitivity limit of the system

(***) not detectable in 1/10 samples

*not detectable in 2 / 10 samples,

**not detectable in 9/10 samples, marginal value (CT 35.1) in 1/10 samples, sensitivity limit of the system is at CT 35

***not detectable in 5 / 10 samples

Table 9 - CT mean values for follicular cell fractions

Follicular cell fraction	ACTB	CALCA	GLP1R	TG
Group A - CT mean value	26.40	Not detectable *	Not detectable	25.35
Group A - SD	3.21	Not calculated	Not calculated	3.68
Group B - CT mean value	26.21	26.87**	Not detectable	25.69***
Group B - SD	6.17	4.30	Not calculated	6.87
Group C - CT mean value	25.46***	31.99****	Not detectable	26.40
Group C - SD	1.54	Not calculated	Not calculated	4.10
Group D1 - CT mean value	27.07	Not detectable	Not detectable	25.22
Group D1 - SD	2.27	Not calculated	Not calculated	1.69
Group D2 - CT mean value	25.92	Not detectable	Not detectable	24.41
Group D2 - SD	3.54	Not calculated	Not calculated	4.82

SD = standard deviation
* not detectable in 7/8 samples, marginal value (CT 34.62) in 1/8 samples, sensitivity limit of the system is at CT 35
Group B: n=6, for 4 samples no follicular cell areas available
** n=2, follicular cell areas composed with C-cells
***not detectable in 1/6 samples
Group C: n=7, for 3 samples no follicular cell areas available
***not detectable in 1/6 samples
****n=1, follicular cell area composed with C-cells

Calcitonin RNA was expressed in the C-cell fractions but not in the follicular cell fractions indicating that proper C-cell and follicular cell microdissection. Based on the limited number of samples evaluated in this study, no differences in the expression levels of GLP1R from samples with C-cell pathology (non-neoplastic and neoplastic C-cell hyperplasia, sporadic C-cell carcinomas, and C-cell carcinomas in the setting of multiple endocrine neoplasia were observed in comparison to the expression levels of GLP1R from samples without C-cell pathology.

Title: Expression Profiling of GLP-1, insulin, and IGF receptors in pancreatic, thyroid, lung, and colon tumors compared to normal counterparts using quantitative real-time PCR (Study DIV1404)

The purpose of this study was to determine the potential co-expression of GLP1R with insulin receptor INSR, variant A (INSRA) and variant B (INSRB), and insulin-like growth factor 1 receptor (IGF1R). RT-PCR was performed on available in-house panels of human tissue cDNAs from normal and tumor samples (pancreas, thyroid, lung, colon, kidney, and stomach. The housekeeping gene ribosome protein L37A (RPL37A) was used for quality control and normalization. Threshold cycles (C_T) of 35 or greater are below the sensitivity limits of the RT-PCR system.

Expression in each sample was calculated as: = $2^{-\Delta\Delta CT}$ x 1000, where ΔΔCT = the difference of threshold cycles for a target and reference in a measured sample and calibrator. Expression ranges were defined as follows:

-Expression below 2:

C_T >35: below detection level
C_T 34-35: at detection level
C_T 32-34: very low
C_T 30-32: low

-Expression between 2 and 20: medium

-Expression >20: high

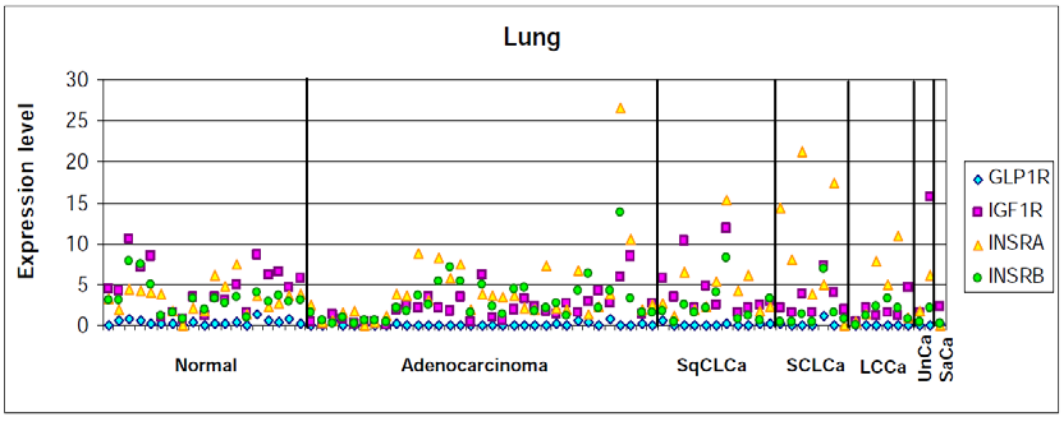
A summary of the types of tumors that were evaluated is shown in the sponsor-generated table below.

	Normal	AdCa	Adeno-ma	Ca	LCCa	SCLC	SqCa	UnCa	Tumor	Pancre-atitis	Total diseased
Kidney	9	/	/	8	/	/	/	/	/	/	8
Pancreas	18	17	/	2	/	/	/	/	1	1	21
Stomach	8	9	/	/	/	/	/	/	/	/	9
Thyroid	10	/	5	8	/	/	/	/	/	/	13
Lung	18	31	/	/	5	6	12	3	/	/	57
Colon	19	24	8	/	/	/	/	/	/	/	32

The RT-PCR results are summarized below in the sponsor-generated tables and figures.

Lung

Five of 57 lung tumors (including adenocarcinoma, large cell carcinoma, sarcomatoid carcinoma, squamous cell carcinoma, and untyped carcinoma) analyzed showed a moderately increased expression of INSRA. In these tumors GLP1R mRNA was very low to undetected (C_T 33-39), as in normal tissue. None of the other receptor examined was significantly higher than normal.



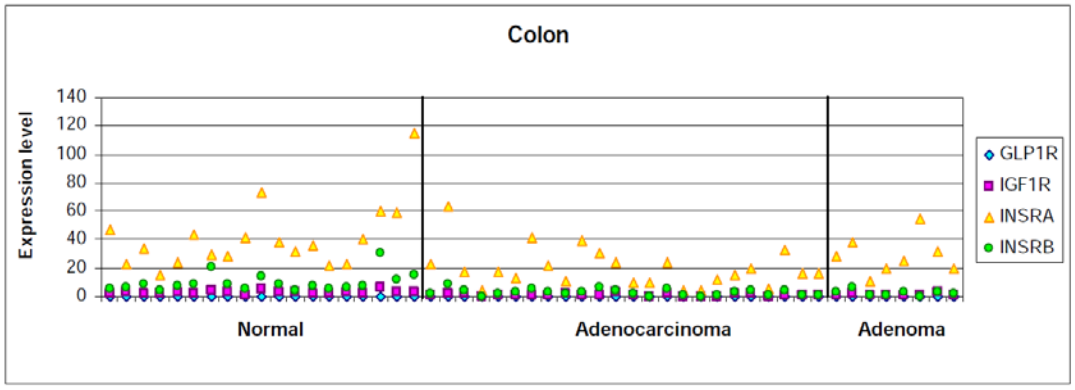
SqCLCa: squamous cell lung carcinoma; SCLCa: small cell lung carcinoma; LCCa: large cell lung carcinom; UnCa: untyped lung carcinoma
SaCa: sarcomatoid lung carcinoma

Sample_Tissue_Pathology	GLP1R	IGF1R	INSRA	INSRB
Min normal	0.0	0.8	1.3	0.7
Median normal	0.2	4.4	3.6	3.1
Max normal	1.3	10.4	7.4	7.8
140877_Lung_AdCa	0.0	5.9	31.2	13.8
140829_Lung_Ca_SCLC	0.0	3.8	25.3	1.3
140869_Lung_Ca_SCLC	0.1	2.1	14.4	0.4
BSV75_Lung_Ca_SCLC	0.0	4.0	17.4	1.5
140861_Lung_SqCa	0.1	11.8	15.3	8.2

significantly higher than normal
significantly lower than normal

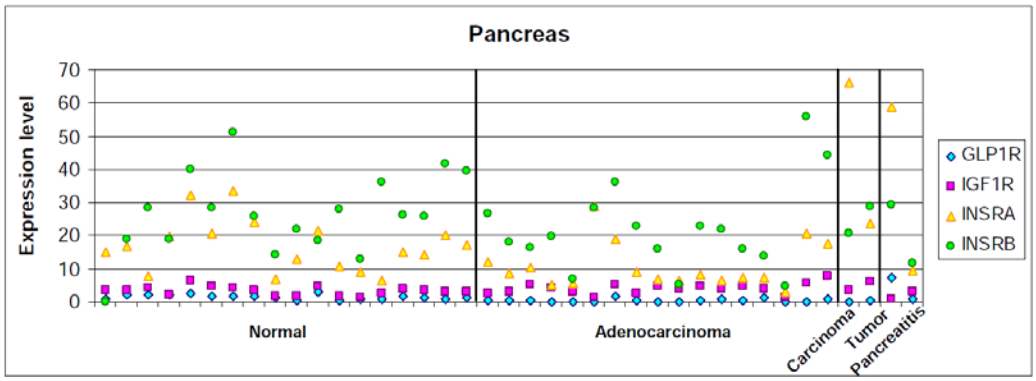
Colon

In colon, GLP1R expression was below detection limit in all samples, either normal or tumor tissue. None of the 32 tumor samples (including adenoma and adenocarcinoma [grades 1, 2, and 3, mucinous, and metastatic in liver] analyzed showed a significant increase in expression in any of the four examined receptors.



Pancreas

In pancreas one metastatic neuroendocrine tumor out of 20 tumors (including adenocarcinoma, ductal adenocarcinoma, mucinous adenocarcinoma, neuroendocrine metastatic to liver, and carcinoma) showed an increased expression of GLP1R (4.5X), at a medium level, together with normal or lower level of the other genes. Another tumor (carcinoma) expressed INSRA higher than normal (4.1X), and altogether significantly underexpressed GLP1R (0.006X normal median).

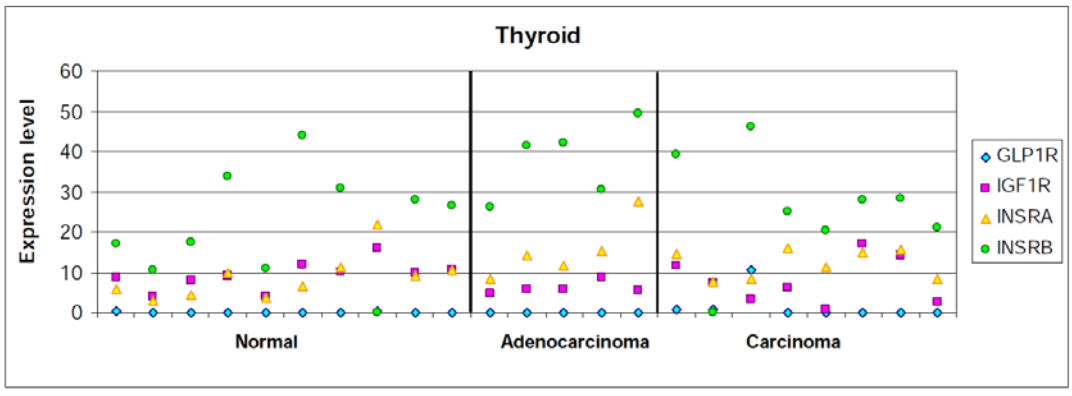


Pathology	GLP1R	IGF1R	INSRA	INSRB
Min normal	0.6	1.5	6.4	12.8
Median normal	1.5	3.4	15.9	26.3
Max normal	2.8	6.6	33.5	51.2
Panc_tum_141049	7.2	0.7	58.7	29.4
Panc_Ca_188849	0.0	3.3	66.0	20.6

significantly higher than normal
significantly lower than normal

Thyroid

In thyroid, 3 papillary carcinomas out of 8 tumors (including papillary and follicular) expressed GLP1R higher than normal, at low, low, and medium levels. INSRA and INSRB expression were not different from normal in these tumors. One of these 3 carcinomas did show a concomitant downregulation of IGF1R. In adenomas (follicular or unspecified), no upregulation of any of the 4 genes was observed.

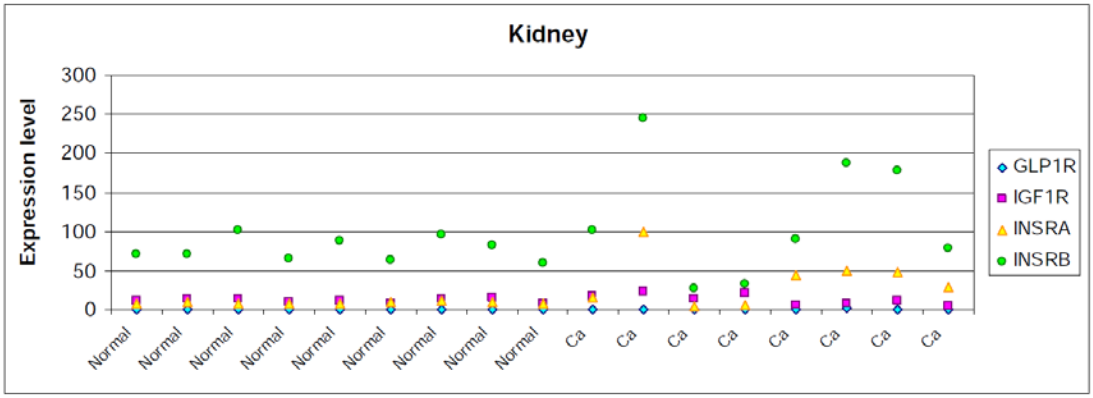


Pathology	GLP1R	IGF1R	INSRA	INSRB
Min normal	0.0	4.1	3.1	10.6
Median normal	0.1	9.5	7.8	26.7
Max normal	0.2	16.1	21.6	43.9
Thyr_Ca_141273	0.8	11.5	14.4	39.3
Thyr_Ca_141275	0.9	7.3	7.6	discarded
Thyr_Ca_141277	10.5	3.3	8.2	46.2

significantly higher than normal
significantly lower than normal

Kidney

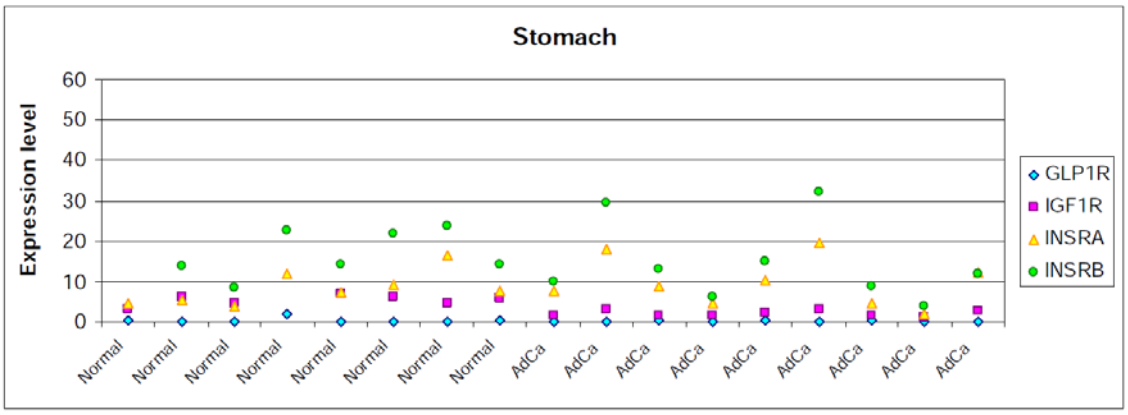
In kidney, a higher expression of INSRA, INSRB, and GLP1R was observed in 6/8, 3/8, and 1/8 kidney carcinomas (not otherwise specified), respectively, while INSRA, INSRB, and IGF1R had lower expression in 2/8, 2/8, and 1/8 tumors, respectively. In the GLP1R overexpressing tumor, GLP1R was very lowly expressed (C_T 32.24; expression 1.14). INSRA was increased by 5.7X in this sample compared with normal tissue, while the increase of INSRB was within the noise (2.6X).



Pathology	GLP1R	IGF1R	INSRA	INSRB
Min normal	0.0	7.3	6.9	58.7
Median normal	0.0	11.3	8.5	71.3
Max normal	0.3	15.0	10.7	100.6
Kidn_Ca_140703	0.1	16.4	16.2	101.4
Kidn_Ca_140705	0.4	23.4	99.0	244.8
Kidn_Ca_140707	0.0	13.4	4.5	27.1
Kidn_Ca_140709	0.1	21.2	5.6	33.0
Kidn_Ca_140711	0.0	5.5	43.6	89.5
Kidn_Ca_140713	1.1	7.6	48.9	188.2
Kidn_Ca_140715	0.0	11.7	46.9	176.8
Kidn_Ca_140717	0.0	3.4	29.4	78.4

significantly higher than normal
significantly lower than normal

Stomach
In stomach, none of the adenocarcinomas was found that expressed any of the 4 genes at higher levels than in normal tissue.



Conclusions
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). A concomitant increase in both GLP1R expression together with INSRA, INSRB, or IGF1R was only detected in one kidney carcinoma (increased expression of INSRA and GLP). In this tumor, GLP1R expression was in the low to very low range. Overall it was concluded that there was no evidence of a general concomitant increase in GLP1R together with IGF1R or one of the two insulin receptors in the tumor tissues examined.

GLP1R 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 GLP1R 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 GLP1R expression levels and neoplasia.

Study title: 1-day QD and BID subcutaneous toxicity study in mice with a 5-day recovery (Study DIV1333)
CD-1 mice (10/sex/group) were administered a single or twice daily subcutaneous dose of vehicle (0.9% NaCl) or 2,000 µg/kg/dose followed by a 5-day recovery period. Animals were assessed for mortality, clinical signs, body weight, food consumption, and gross pathology. A serum calcitonin profile (Day 1) and thyroid gene expression analysis (Day 2) by real-time PCR were conducted. Because of the small size, whole mouse thyroid was used for RNA expression analysis, which included the following genes: cyclin D1, cyclin D2, cyclin D3, C-myc, Bcl-xl, CDK1A (p21), CDK1B (p27), GLP1R, calcitonin, and beta-actin.

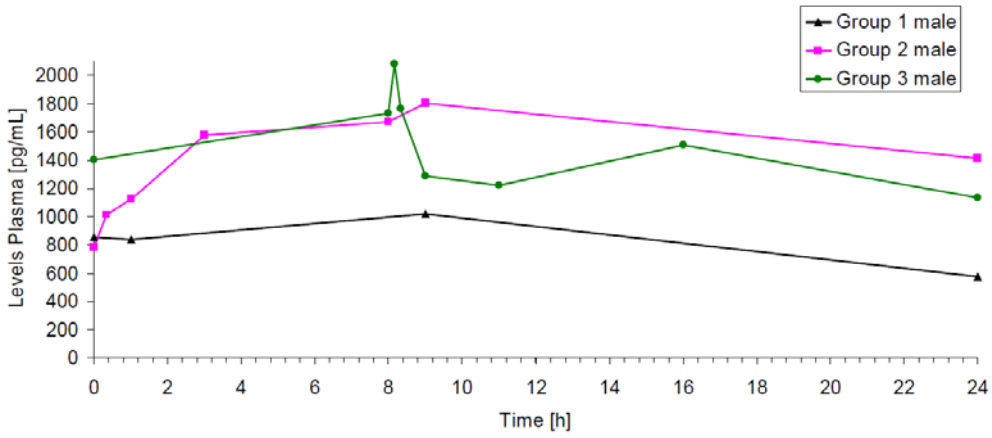
There were no unscheduled deaths or adverse clinical signs. Decreased body weight occurred for treated groups that correlated with decreased food consumption. No macroscopic findings were noted. There was no evidence for biologically relevant changes in gene expression of proliferation markers, calcitonin, or GLP1R in thyroid (all cell types analyzed together; sponsor-generated tables below). AVE0010 induced an increase in serum calcitonin (sponsor-generated figures below).

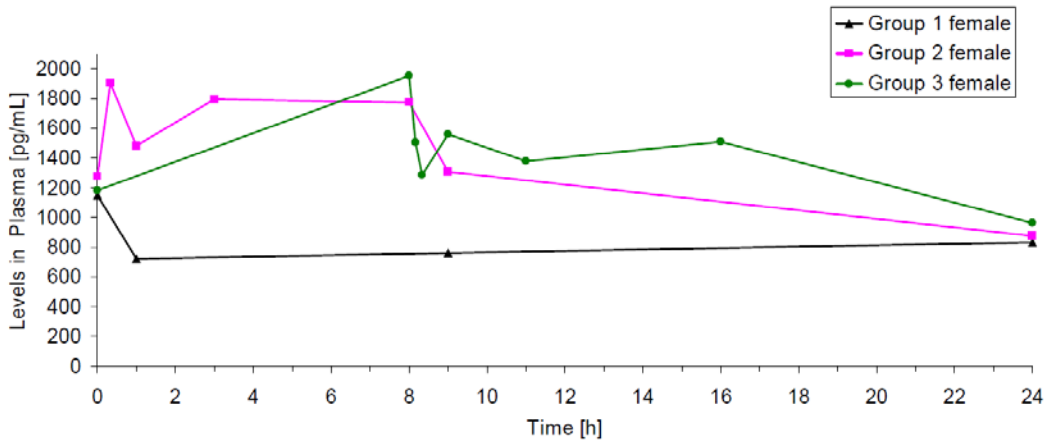
Males (fold change in gene expression compared with control)

	Ccnd1	Ccnd2	Ccnd3	C-myc	Bcl-xl	p21	p27	Glp1r	Calca
group 2	1.27	1.31	1.06	1.15	1.31	1.32	1.41	1.44	1.40
group 3	-1.09	1.28	1.07	1.07	1.49	1.23	1.28	1.21	1.81

Females (fold change in gene expression compared with control)

	Ccnd1	Ccnd2	Ccnd3	C-myc	Bcl-xl	p21	p27	Glp1r	Calca
group 2	-1.03	-1.06	1.01	-1.14	-1.10	-1.08	-1.10	-1.16	1.18
group 3	1.23	1.50	1.18	1.02	1.42	1.25	1.22	1.63	1.84





Study title: 1-day QD and BID subcutaneous toxicity study in rats with a 5-day recovery (Study DIV1332)

Sprague-Dawley rats (10/sex/group) were administered a single or twice daily subcutaneous dose of vehicle (0.9% NaCl) or 2,000 µg/kg/dose followed by a 5-day recovery period. Animals were assessed for mortality, clinical signs, body weight, food consumption, and gross pathology. A serum calcitonin profile (Day 1) and thyroid gene expression analysis (Day 2) by real-time PCR were conducted. C-cells and follicular cells were collected separately by laser capture microdissection based on immunohistochemistry staining for calcitonin. RNA expression analysis included the following genes: cyclin D1, cyclin D2, cyclin D3, C-myc, Bcl-xl, CDK1A (p21), CDK1B (p27), GLP1R, calcitonin, and beta-actin.

There were no unscheduled deaths or adverse clinical signs. Decreased body weight occurred for treated groups that correlated with decreased food consumption. No macroscopic findings were noted. Unlike in mice, AVE0010 did not induce an increase in serum calcitonin in rats. There was no evidence for biologically relevant changes in gene expression of proliferation markers, calcitonin, or GLP1R in thyroid (all cell types analyzed together; sponsor-generated tables below).

9.1.1 Fold changes in male animals – C-cell fractions

Male	Calca	Cyclin D1	Cyclin D2	Cyclin D3	Glp1r	c-myc	Bcl-xl	p21_Cip	p27_Kip
Group 2	- 1.31	-2.34	-2.20	-3.68	1.87	1.06	-1.75	-2.17	2.01
Group 3	-1.30	-1.43	-1.78	-1.35	1.86	-1.06	1.08	-1.37	2.06

9.1.2 Fold changes in female animals – C-cell fractions

	Calca	Cyclin D1	Cyclin D2	Cyclin D3	Glp1r	c-myc	Bcl-xl	p21_Cip	p27_Kip
Group 2	-1.24	-1.40	-1.28	-2.29	-1.58	-1.79	-1.70	-1.32	-1.55
Group 3	1.48	-1.16	1.95	1.73	1.63	2.55	1.73	-3.09	1.80

9.1.3 Fold changes in male animals – follicular cell fractions

	Calca	Cyclin D1	Cyclin D2	Cyclin D3	Glp1r	c-myc	Bcl-xl	p21_Cip	p27_Kip
Group 2	-1.12	-1.26	-2.55	-1.75	n.e.	1.59	-1.02	1.40	1.08
Group 3	1.77	-1.79	-1.62	1.16	n.e.	1.64	1.05	3.44	2.85

n.e. = not expressed

9.1.4 Fold changes in female animals – follicular cell fractions

	Calca	Cyclin D1	Cyclin D2	Cyclin D3	Glp1r	c-myc	Bcl-xl	p21_Cip	p27_Kip
Group 2	-3.74	-1.14	-1.69	-1.17	n.e.	-1.57	-1.84	-1.26	-1.36
Group 3	2.18	1.07	1.15	1.57	n.e.	2.67	1.01	1.85	1.89

n.e. = not expressed

Study title: **AVE0010 - Exploratory 14-day subcutaneous (BID) toxicity study in GLP1 -R (-/-) KO and wild-type mice**

Key study findings:

- No mortality or adverse clinical signs were noted.
- Mean body weights were decreased (-0.7 g) for treated wild-type males during the first week compared to a slight gain in body weight for untreated wild-type males (0.7 g). Mean body weights were decreased during the second week for treated KO males (-2.4 g) and females (-0.9 g) compared with a slight decrease for untreated KO males (-0.6 g) and a slight gain for untreated KO females (0.6 g). These effects on body weight did not consistently correlate with effects on food consumption.
- Treatment with lixisenatide resulted in increased plasma concentrations of calcitonin after 14 days in wild-type mice but not KO mice, indicating that GLP-1 agonist-induced secretion of calcitonin is GLP-1 receptor dependent. However, it is still unclear whether GLP-1 agonist-induced C-cell proliferation is dependent on the GLP-1 receptor because this aspect was not investigated in this study. It is also uncertain whether the extent of calcitonin secretion directly relates to the potential for C-cell proliferation, and therefore, uncertain whether calcitonin is a useful biomarker to indicate early C-cell proliferation.

Study number: TSA1481
Conducting laboratory: Sanofi-Aventis Research and Development
371 rue du Pr. J. Blayac
34184 Montpellier Cedex 04, France
Date of study initiation: 17 June 2011
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: AVE0010, Batch #B004, 88.1% pure
Vehicle/formulation: Stock solution contains 50 mM sodium citrate, pH 5.3, at a concentration of 12.6 mg/mL. Dosing solution was made by diluting stock solution with 0.9% sodium chloride

Methods

Species/strain: Mice/CD-1 (wild type) and GLP-1 receptor (-/-) knock out
Design: see sponsor-generated tables below

AVE0010 dose levels

Group	Dose level (mg/kg BID[c])	Concentration (mg/mL)	Dose volume (mL/kg)
1[a]	0.9% sodium chloride	0	2.5
2[a]	1	0.4	2.5
3[b]	0.9% sodium chloride	0	2.5
4[b]	1	0.4	2.5

a CD1 mice
b GLP1-R (-/-) KO mice
c BID = twice daily

AVE0010 dose levels and number of animals per group

Mouse genotype	Dose level (mg/kg BID[b])	Group	No. of animals		Animal identity number	
			Males	Females	Males	Females
CD1, wild type	0[a]	1	2	6	1-2	3-8
CD1, wild type	1	2	3	5	9-11	12-16
GLP1-R -/-	0[a]	3	2	6	17-18	19-24
GLP1-R -/-	1	4	3	5	25-27	28-32

a BID= twice daily
b Vehicle: 0.9% sodium chloride

Number/sex/group (recovery): No recovery groups
Route and regimen: Subcutaneous, twice daily (2nd dose ~8 hours after the first)
Age: 6-7 weeks at initiation of dosing
Weight: (Males) 34.4 to 36.6 g (CD1) and 26.1 to 30.7 g (KO)
(Females) 24.0 to 28.1 g (CD1) and 18.9 to 24.8 g (KO)
TK sampling: Not conducted
Ab sampling: Not conducted
Analysis of dosing preparations: The sponsor states that "The results of the analysis of samples from study number TSA1481 confirm that the prepared formulations of lixisenatide in 0.9% sodium chloride solution are within the limits specified for solutions (90% - 110% of the nominal concentration). It was confirmed that API was absent from the vehicle control sample."

Observation and Times:
Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Not conducted
EKG: Not conducted
Hematology: Not conducted
Clinical chemistry: Calcitonin only; 2 hours after last dose on Day 14
Urinalysis: Not conducted
Gross pathology: Not conducted

Organ weights: Not conducted
Histopathology: Not conducted
Unique study design: The left lobe of the thyroid glands (with trachea and larynx) from all animals was preserved in 10% neutral buffered formalin, and the right lobe was freshly sampled and preserved in RNA-later buffer.

Results:
Mortality: No unscheduled deaths occurred during the study.

Clinical signs: No treatment-related adverse clinical signs were noted.

Body weights: (sponsor-generated table)

Text Table 1 – Mean body weight changes (g) in CD1 and GLP1-R (-/-) KO mice

Mouse genotype	CD1, wild type				GLP1-R (-/-) KO			
Group	1		2		3		4	
Dose AVE0010 (mg/kg/BID)	0		1		0		1	
Sex	M	F	M	F	M	F	M	F
No. of animals	2	6	3	5	2	6	3	5
Days 1-8	0.7	1.3	**-0.6**	1.0	1.8	1.7	1.4	1.5
Days 8-15	0.1	0.1	1.7	0.2	-0.6	0.6	**-2.4**	**-0.9**

M: males; F: females
In **bold**: treatment-related changes

Food consumption: (sponsor-generated table)

Text Table 2 – Variations in mean food consumption (%) in treated CD1 and GLP1-R (-/-) KO mice versus control groups (Groups 1 and 3, respectively)

Mouse genotype	CD1, wild type		GLP1-R (-/-) KO	
Groups	2		4	
Dose (mg/kg/BID)	1		1	
Sex	M	F	M	F
No. of animals in the cage	3	5	3	5
No. of cage	3	4	7	8
Days 1-8	**-31%**	-18%	-12%	+11%
Days 8-15	-14%	+1%	-18%	+15%

M: males; F: females; **Bold** figures: changes attributed to treatment

Clinical chemistry: (sponsor-generated table)

Text Table 3 – Mean calcitonin values (pg/mL) in CD1 and GLP1-R (-/-) KO mice after the 14-day dosing period

Mouse genotype	CD1, wild type				GLP1-R (-/-) KO			
Group	1		2		3		4	
Dose AVE0010 (mg/kg/BID)	0		1		0		1	
Sex	M	F	M	F	M	F	M	F
No. of animals	2	6	3	5	2	6	3	5
mean	7.5 [a]	13.3 [b]	86.6	99.8	16.3 [c]	10.7 [b]	14.9 [d]	6.13 [e]
SD	NA	NA	29.8	50.9	NA	NA	8.66	NA

M: males; F: females; SD: standard deviation; Values rounded to three significant figures
NA: Not applicable as either all values or more than 50% of values were below the limit of quantification

[a]: 2/2 values below LLOQs (see Appendix III)
[b]: 4/6 values below LLOQ
[c]: 1/2 value below LLOQ
[d]: 1/3 value below LLOQ
[e]: 4/5 values below LLOQ

Title: Exploratory 1-month subcutaneous infusion study in mice (ddo1171)

Introduction

CD1 mice (10/sex/group; 6 to 7 weeks old) received vehicle or 50 µg AVE0010/day by continuous (24 hours) subcutaneous minipump implant infusion for 1 month. Plasma samples for TK analysis were collected on Days 2, 8, 15, and 22. On Day 29, thyroids were collected from 5 mice/sex/group and preserved in RNA-later buffer. Total RNA from thyroid was isolated and converted into cDNA for RNA expression analysis of proliferation markers using Real Time PCR. Threshold cycles greater than 35 approach the sensitivity limits of the PCR system. Thyroids from the remaining 5 mice/sex/group were evaluated by light microscopy using both H&E staining and immunohistochemistry (Ki-67 and calcitonin double staining).

Results

There were no mortalities or adverse clinical signs. An initial decrease in body weight gain was observed for treated animals during the first week, which correlated with decreased body weight gain. After the first week of treatment, body weight gain was similar to controls.

Toxicokinetics

Plasma levels of AVE0010 were stable between Day 2 and 8 but increased by approximately 2-fold in males and 4-fold in females between Day 8 and Day 22 (see sponsor-generated table below).

Sex	Dose (µg/animal/day)	C_{mean} (ng/mL)			
		Day 2	Day 8	Day 15	Day 22
Male	50	35.0	32.5	60.1	72.7
Female	50	19.9	18.9	64.4	89.3

Values are rounded to 3 significant figures

Gene Expression Analysis

There was no evidence for biologically relevant changes in gene expression of the selected proliferation markers after treatment with 50µg/day by continuous subcutaneous infusion. GLP-1R expression was 2.8-fold and 3.8-fold higher in treated males and females, respectively and calcitonin expression was 3.6-fold and 4.6-fold higher for treated males and females, respectively. A summary of relative differences in gene expression when compared with control values is shown in the sponsor-generated table below.

	Ccnd1	Ccnd2	Ccnd3	C-myc	Bcl-xl	p21	p27	Glp1r	Calca
male	1.03	-1.12	1.09	-1.11	1.02	-1.10	-1.14	2.78	3.55
female	1.20	1.06	-1.04	-1.08	1.04	-1.04	1.09	3.84	4.59

Control value = 1, means that relative difference of +/- 1 is not different from control

Histopathology

There were no AVE0010-related microscopic findings in the thyroid gland. Thyroid C-cells were identified by staining with anti-calcitonin and proliferating cells were identified by staining with anti-Ki-67. There was no evidence of double labeling for both calcitonin and Ki-67, indicating that C-cells were not actively proliferating at the time of thyroid removal.

Study title: **AVE0010 - 3-month subcutaneous toxicity study in mice**

Key study findings:

- There were no unscheduled deaths or adverse clinical signs
- Female mice gained more weight than controls but this did not correlate with increased food consumption. Males had an initial body weight loss that did correlate with decreased food consumption.
- AVE0010 treatment resulted in increased plasma calcitonin levels 0.5, 2, and 24 hours after dosing (sex-pooled data).
- There were no treatment-related macroscopic effects. There were no microscopic lesions observed in the thyroid.

- AVE0010 treatment did not alter the expression of 7 growth regulatory genes in thyroid cells. RNA expression of GLP-1 receptor and calcitonin in thyroid was increased 4-fold in males and 2-fold in females.
- Immunohistochemical assessment of thyroid tissue using calcitonin and Ki-67 double staining did not reveal an increase C-cells (calcitonin positive) that were undergoing replication (Ki-67 positive).
- It does not appear that the sponsor adequately evaluated thyroid tissue for subtle C-cell hyperplasia, such as diffuse hyperplasia, because C-cell density (C-cells/mm^2) was not evaluated. C-cell number may be greater in treated animals without a significant amount of active proliferation if the proliferative response is slow (e.g., only affects a few C-cells at any one point in time).

Study number: TXC1491
Conducting laboratory: Sanofi-Aventis Deutschland GmbH, R&D - SCP Disposition, Safety & Animal Research Operational Center Frankfurt, 65926 Frankfurt, Germany
Date of study initiation: 04 Jan 2011 (start of dosing)
GLP compliance: Yes (except gene expression analysis)
QA statement: Yes
Drug, lot #, and % purity: AVE0010, Batch #B004, 88.1% pure
Vehicle/formulation: Stock solution contains 50 mM sodium citrate, pH 5.3, at a concentration of 12.6 mg/mL. Dosing solution was made by diluting stock solution with 0.9% sodium chloride

Methods

Species/strain: Mice/CD-1
Design: see sponsor-generated table below

Dose levels and number of animals per group

Group	Dose levels (µg/kg/BID)	Main group animals		
		Number/ sex	Animal numbers	
			Male	Female
1	0	12	1 - 12	13 - 24
2	1000	12	25 - 36	37 - 48

Number/sex/group (recovery): No recovery groups
Route and regimen: Subcutaneous, twice daily (2^{nd} dose ~8 hours after the first)
Dosing volume: 2.5 mL/kg
Age: 8-10 weeks at initiation of dosing
Weight: 32.9 to 38.9 g (males) and 24.1 to 31.0 g (females)
TK sampling: Not conducted
Ab sampling: Not conducted
Analysis of dosing preparations: The results of the analysis of samples confirmed that the prepared formulations of AVE0010 in 0.9% NaCl solution were within the limits specified for solutions (90% to 110%) of the nominal concentrations except samples 4 A-C. Because of the Out of Limits additional

samples of formulations were collected and measured. Because the concentrations of these samples met the acceptance criteria, this single deviation was not considered to have impaired the quality and integrity of the study. It was confirmed that AVE0010 was absent from the vehicle control samples.

Observation and Times:

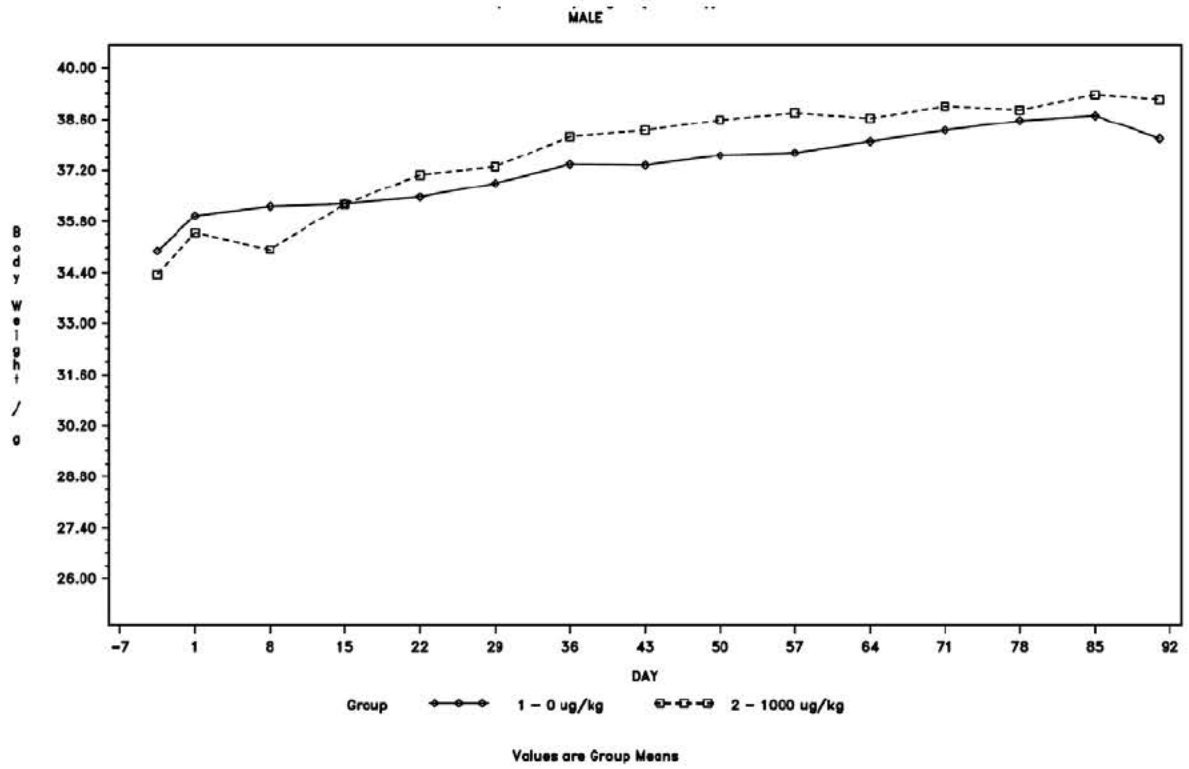
Mortality: Twice daily
Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Not conducted
EKG: Not conducted
Hematology: Not conducted
Clinical chemistry: Calcitonin only; 0.5, 2, 8.5, and 24 hours after dosing (3 animals per time point)
Urinalysis: Not conducted
Gross pathology: All animals
Organ weights: Not conducted
Histopathology: Thyroid glands and tissues with macroscopic observations
Thyroid glands from half the animals (6 animals/sex/group) were preserved in 10% neutral buffered formalin. Two slides per tissue block were prepared, the first stained with H&E and the second prepared for immunohistochemistry, which was conducted with anti-Ki-67 and anti-calcitonin double staining.
The thyroids from the other half of the animals were preserved in RNA-later buffer for gene expression analysis.
Unique study design: Thyroid expression analysis and immunohistochemistry

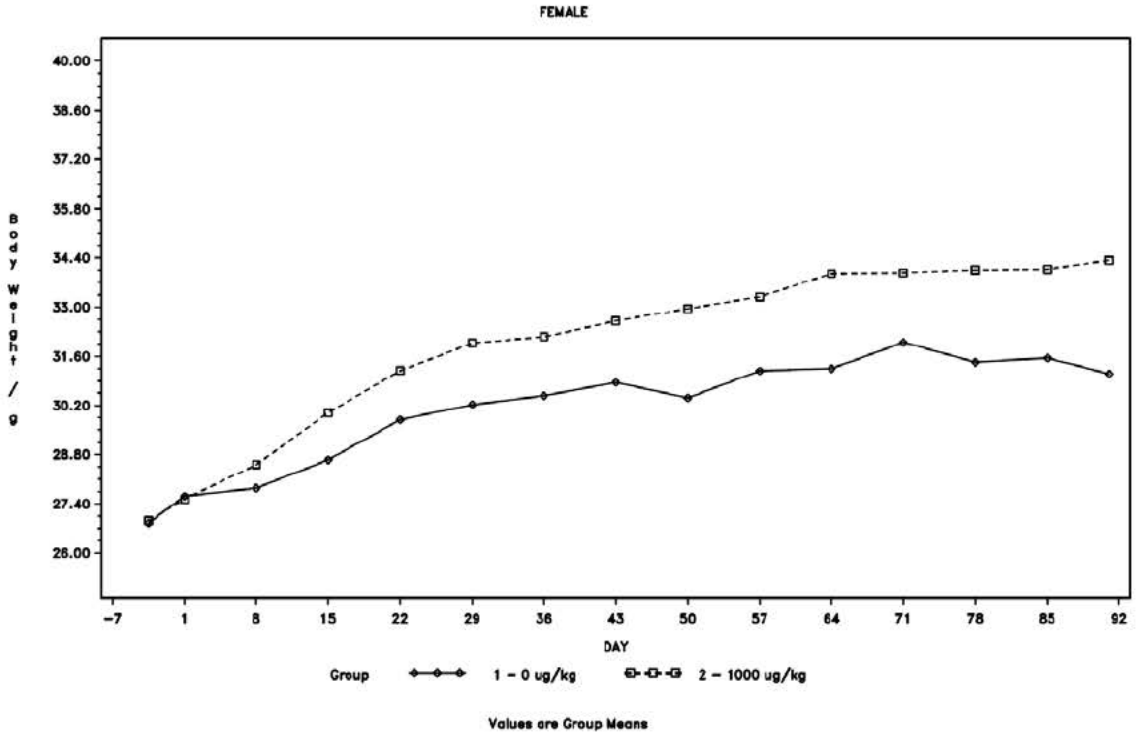
Results:

Mortality: No unscheduled deaths occurred during the study.

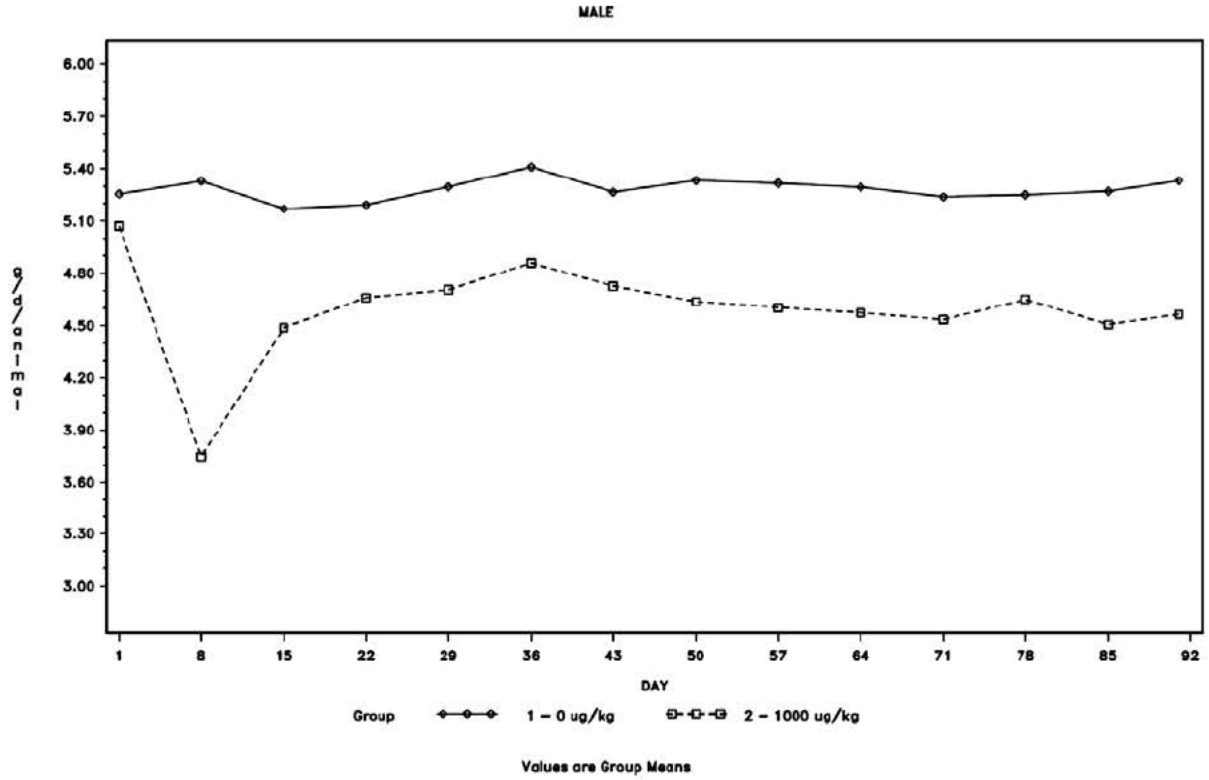
Clinical signs: No treatment-related adverse clinical signs were noted.

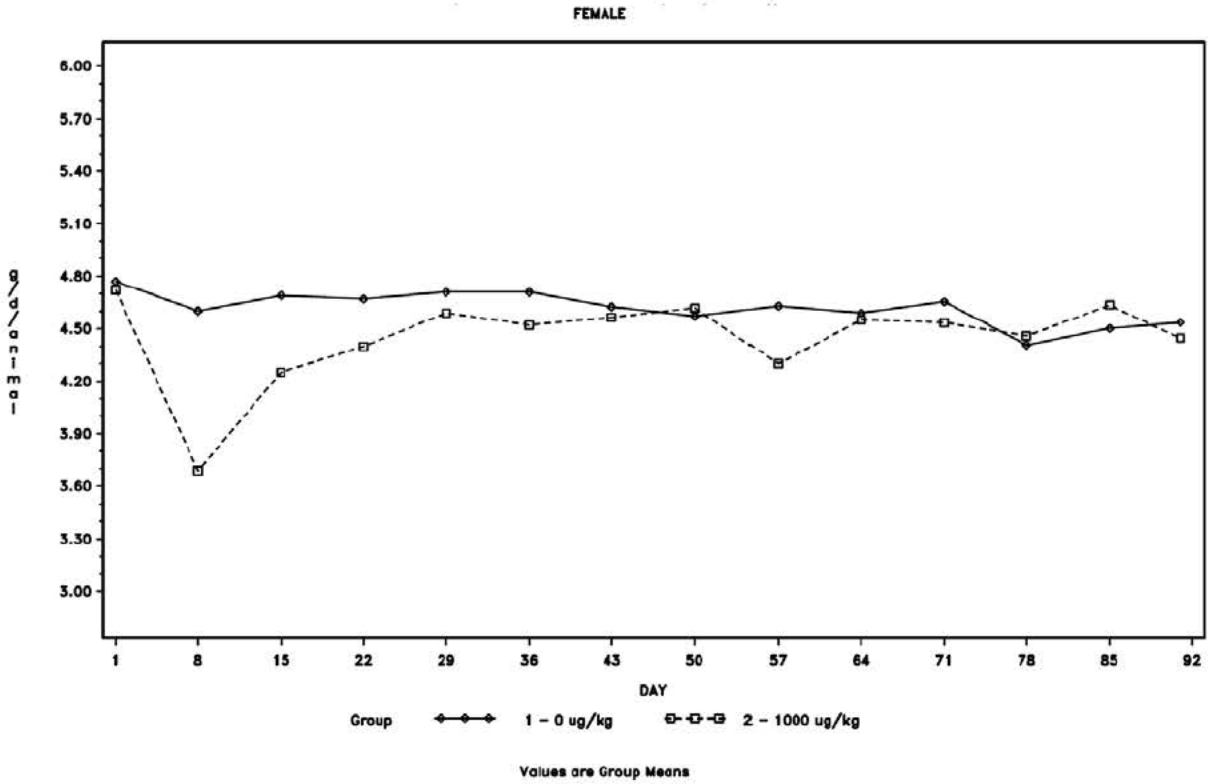
Body weights: There was no significant difference in body weight gain between control and treated males at the end of the study. Males exhibited a slight decrease in body weight between Day 1 and Day 8, which correlated with a decrease in food consumption. Treated females had a statistically significantly higher mean body weight beginning on Day 15, which continued throughout the remainder of the study. Females also showed decreased food consumption at the beginning of the study but it did not translate to lower body weight gain.


MALE
Body Weight / g
40.00
38.60
37.20
35.80
34.40
33.00
31.60
30.20
28.80
27.40
26.00
-7
1
8
15
22
29
36
43
50
57
64
71
78
85
92
DAY
Group
1 – 0 ug/kg
2 – 1000 ug/kg
Values are Group Means


FEMALE
Body Weight / g
40.00
38.60
37.20
35.80
34.40
33.00
31.60
30.20
28.80
27.40
26.00
-7
1
8
15
22
29
36
43
50
57
64
71
78
85
92
DAY
Group
1 – 0 ug/kg
2 – 1000 ug/kg
Values are Group Means

Food consumption: (sponsor-generated figures)



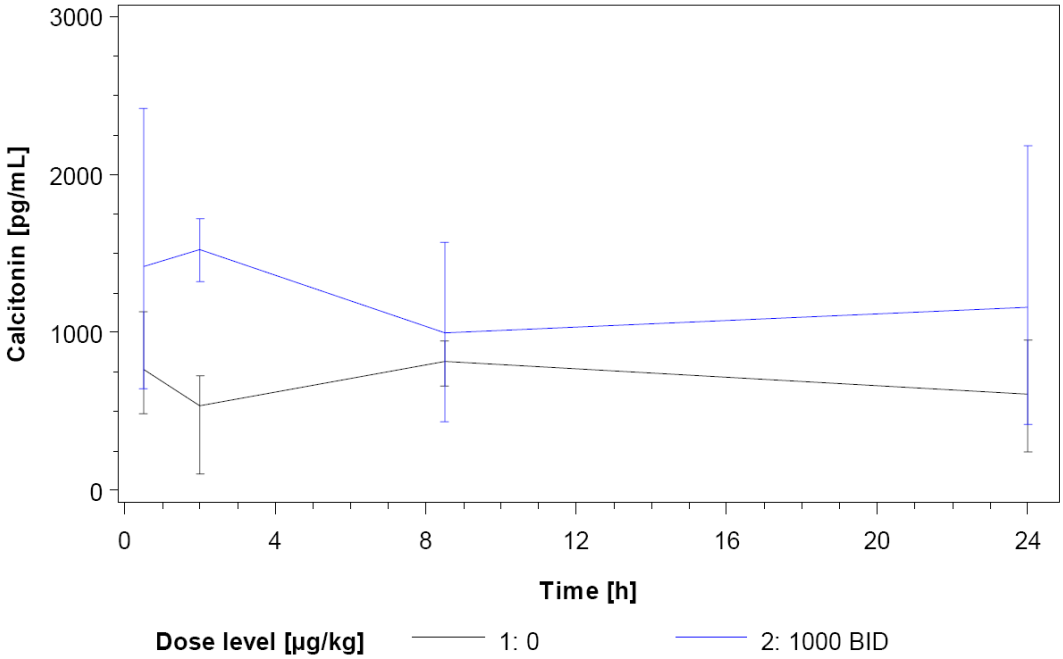


Clinical chemistry: Plasma calcitonin (sponsor-generated table and figure). Similar results were observed for males and females, so only the sex-pooled data are shown here.

Text table 4 - Mean calcitonin values (pg/ml) with standard deviation for sex-pooled data

Time [h]		0.5	2	8,5	24
Group number	1				
Dose level [µg/kg]	0 µg/kg BID				
N		6	6	6	6
Mean		765.32	533.32	816.30	609.50
standard deviation		248.96	250.29	100.31	233.04
Group number	2				
Dose level [µg/kg]	1000 µg/kg BID				
N		6	6	6	6
Mean		1416.17	1524.17	997.12	1160.90
standard deviation		614.63	133.52	490.66	613.73

Text figure 3 - Mean calcitonin levels with min/max values in male and female mice (sex-pooled data) after subcutaneous injection of AVE0010 in a dose of 1000 µg/kg BID and in the untreated control group



Gross Pathology: No macroscopic findings were noted.

Histopathology: No microscopic treatment-related effects were observed.

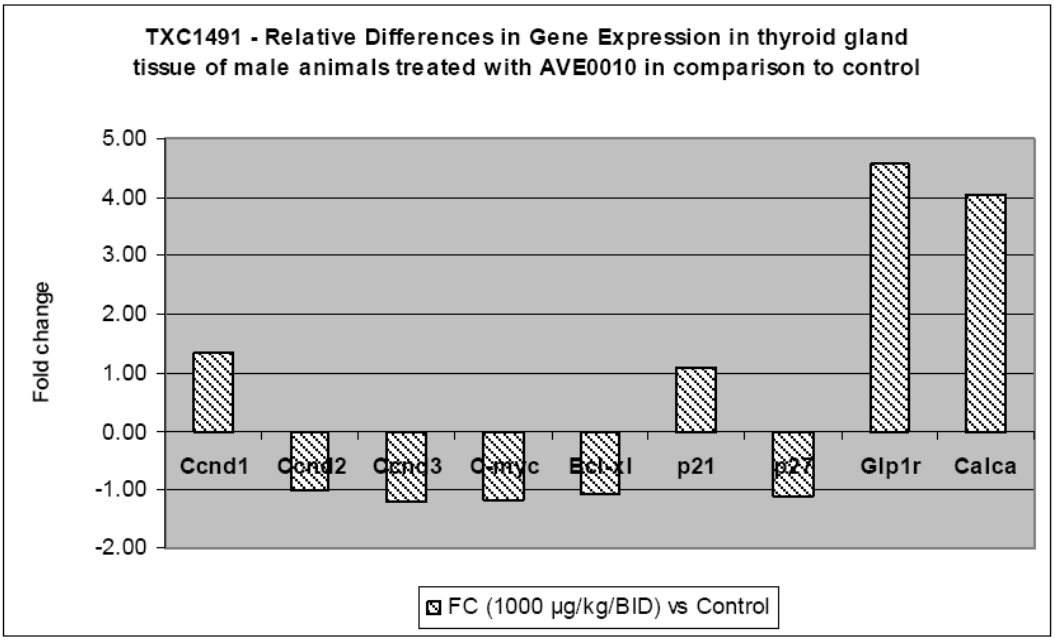
Immunohistochemistry: In the thyroid glands examined C-cells (calcitonin-positive) and/or proliferating cells (Ki-67 positive cells) were detected by immunohistochemistry. However, there was no double staining of cells demonstrating proliferating C-cells.

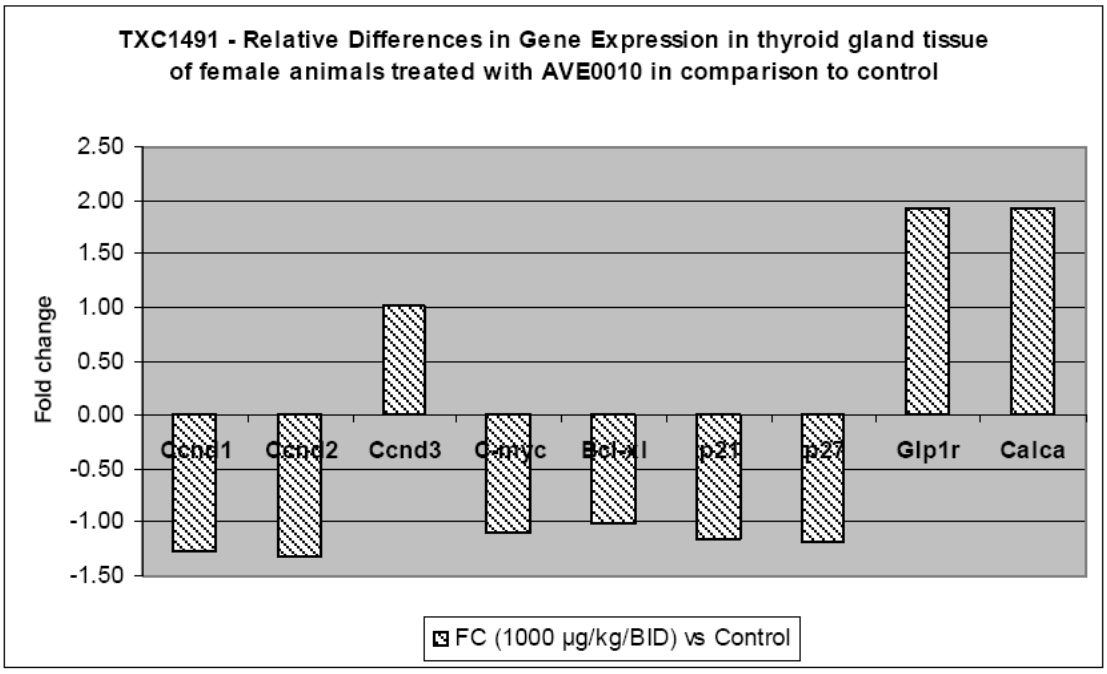
Expression analysis: Results shown in the sponsor-generated table and figures below.

8.1 RELATIVE DIFFERENCES IN GENE EXPRESSION IN COMPARISON TO CONTROL

	Ccnd1	Ccnd2	Ccnd3	C-myc	Bcl-xl	p21	p27	Glp1r	Calca
male	1.32	-1.01	-1.19	-1.18	-1.09	1.08	-1.10	4.56	4.04
female	-1.27	-1.33	1.02	-1.10	-1.01	-1.15	-1.19	1.92	1.92

Control value = 1, means that relative difference of +/- 1 is not different from control


TXC1491 - Relative Differences in Gene Expression in thyroid gland tissue of male animals treated with AVE0010 in comparison to control
Fold change
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
Ccnd1
Ccnd2
Ccnd3
C-myc
Bcl-xl
p21
p27
Glp1r
Calca
FC (1000 µg/kg/BID) vs Control


TXC1491 - Relative Differences in Gene Expression in thyroid gland tissue of female animals treated with AVE0010 in comparison to control
Fold change
2.50
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
Ccnc1
Ccnc2
Ccnd3
C-myc
Bcl-xl
p21
p27
Glp1r
Calca
FC (1000 µg/kg/BID) vs Control

Study title: AVE0010 - 12 week subcutaneous infusion study in mice

Key study findings:

- There were no unscheduled deaths or adverse clinical signs.
- Body weights were significantly less for treated males on Days 8, 57, and 85. Treated females gained slightly more weight than controls, which was statistically significant at some time points.
- Food consumption was lower during the first week for the treated animals.
- AVE0010 treatment resulted in increased plasma calcitonin levels 0.5, 2, and 24 hours after dosing (sex-pooled data).
- There were no treatment-related macroscopic effects. There were no microscopic lesions observed in the thyroid.
- AVE0010 treatment did not alter the expression of 7 growth regulatory genes in thyroid cells. RNA expression of GLP-1 receptor and calcitonin in thyroid was increased 5-fold in males and 2 to 3-fold in females.
- Immunohistochemical assessment of thyroid tissue using calcitonin and Ki-67 double staining did not reveal an increase C-cells (calcitonin positive) that were undergoing replication (Ki-67 positive).
- It does not appear that the sponsor adequately evaluated thyroid tissue for subtle C-cell hyperplasia, such as diffuse hyperplasia, because C-cell density (C-cells/mm^2) was not evaluated. C-cell number may be greater in treated animals without a significant amount of active proliferation if the proliferative response is slow (e.g., only affects a few C-cells at any one point in time).

Study number: TXC1492

Conducting laboratory: Sanofi-Aventis Deutschland GmbH, R&D - SCP Disposition, Safety & Animal Research Operational Center Frankfurt, 65926 Frankfurt, Germany

Date of study initiation: 13 April 2011 (start of dosing)

GLP compliance: Yes (except gene expression analysis)

QA statement: Yes

Drug, lot #, and % purity: AVE0010 prefilled in (b) (4) osmotic pumps, Batch #B004, 88.1% pure

Vehicle/control: (sponsor-generated table)

	Ingredient	Composition per mL
1.	Sodiumacetattrihydrat	(b) (4)
2.	Glycerol 85%	
3.	(b) (4) Methionine	
4.	m-Cresol	
5.	(b) (4) N NaOH	
6.	N HCL	
7.	water for Injection	

Methods

Species/strain: Mice/CD-1

Design: see sponsor-generated table below

Dose levels and number of animals per group

Group	Dose levels (µg/animal/d)	Main group animals			Toxicokinetic animals		
		Number/ sex	Animal numbers		Number/ sex	Animal numbers	
			Male	Female		Male	Female
1	0	20 + 5*	1-20 21-25*	32-51 52-56*	6	26-31	57-62
2	50	20 + 5*	63-82 83-87*	97-116 117-121*	9	88-96	122-130

*Replacement animals (not used)

Dose: 0 and 50 µg/animal/day (~2000 µg/kg/day)

Route and regimen: Subcutaneous, continuous infusion via osmotic pump

Drug concentration in pump and infusion rate: 20 mg/mL; 0.10 µL/h

Age: 6-7 weeks at initiation of dosing

Weight: 29.0 to 39.0 g (males) and 22.4 to 28.6 g (females)

Number/sex/group (recovery): No recovery groups

TK sampling (2-3 animals/time point): One sample on Days 2, 25, and 85; bioanalytical analysis conducted with a non-GLP, partially validated method.

Ab sampling: Not conducted

Analysis of dosing preparations: Not conducted because minipump devices arrived at the lab prefilled with drug.

Observation and Times:

Mortality: Twice daily

Clinical signs: Twice daily

Body weights: Weekly

Food consumption: Weekly

Ophthalmoscopy: Not conducted

EKG: Not conducted

Hematology: Not conducted

Clinical chemistry: Plasma calcitonin only; not fasted (5 animals/sex/time point); 7 am, 10 am, 1 pm, and 4 pm on final day of dosing

Urinalysis: Not conducted

Gross pathology: All animals

Organ weights: Not conducted

Histopathology: Thyroid glands and tissues with macroscopic observations
Thyroid glands from half the animals (10 animals/sex/group) were preserved in 10% neutral buffered formalin. Two slides per tissue block were prepared, the first stained with H&E and the second prepared for immunohistochemistry, which was conducted with anti-Ki-67 and anti-calcitonin double staining.
The thyroids from the other half of the animals were preserved in RNA-later buffer for gene expression analysis.

Unique study design: Thyroid gene expression analysis and immunohistochemistry

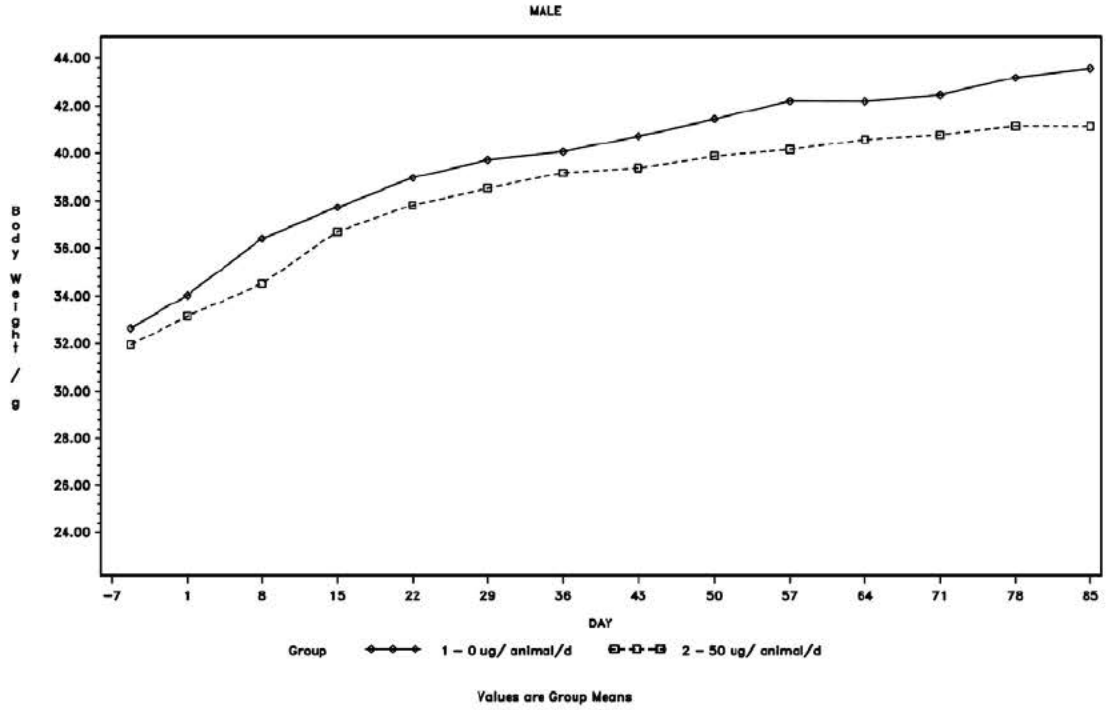
Gene	Intracellular function
Cyclin D1 (Ccnd1)	Functions as a regulatory subunit with cyclin-dependent kinases (CDK) 4 and 6, whose activity is required for cell cycle G1/S transition. Overexpression of this gene has been noted in a variety of tumors.
Cyclin D2 (Ccnd2)	Functions as a regulatory subunit with CDK4 and CDK6, whose activity is required for cell cycle G1/S transition. Overexpression of this gene has been noted in ovarian and testicular tumors.
Cyclin D3 (Ccnd3)	Functions as a regulatory subunit with CDK4 and CDK6, whose activity is required for cell cycle G1/S transition.
C-myc	Transcription factor that promotes cell proliferation. Up-regulates cyclins and down-regulates p21. Up-regulated in many types of cancers.
Bcl-xL	Anti-apoptotic protein in the Bcl-2 family that has been associated with the survival of cancer cells. Involved in the signal transduction pathway of FAS-L.
p21/CIP1/WAF1 (CDKN1A)	Binds to and inhibits the activity of CDK2, CDK4, and CDK6 thereby preventing cell cycle progression/cell proliferation.
p27/Kip1 (CDKN1B)	Binds to Cyclin D either alone or when complexed with CDK4; inhibits CKD4 activity causing cells to arrest in G1 of the cell cycle. Also binds Cyclin E/CDK2 and Cyclin A/CDK2.
Glucagon-like peptide-1 receptor (GLP1r)	G protein-coupled receptor that binds GLP-1. Receptor stimulation activates the adenylyl cyclase pathway resulting in increased insulin synthesis and secretion.
Calcitonin (Calca)	Secreted from thyroid C-cells. Decreases serum calcium by inhibiting absorption and inhibiting renal tubular resorption of calcium; decreases osteoclast activity.

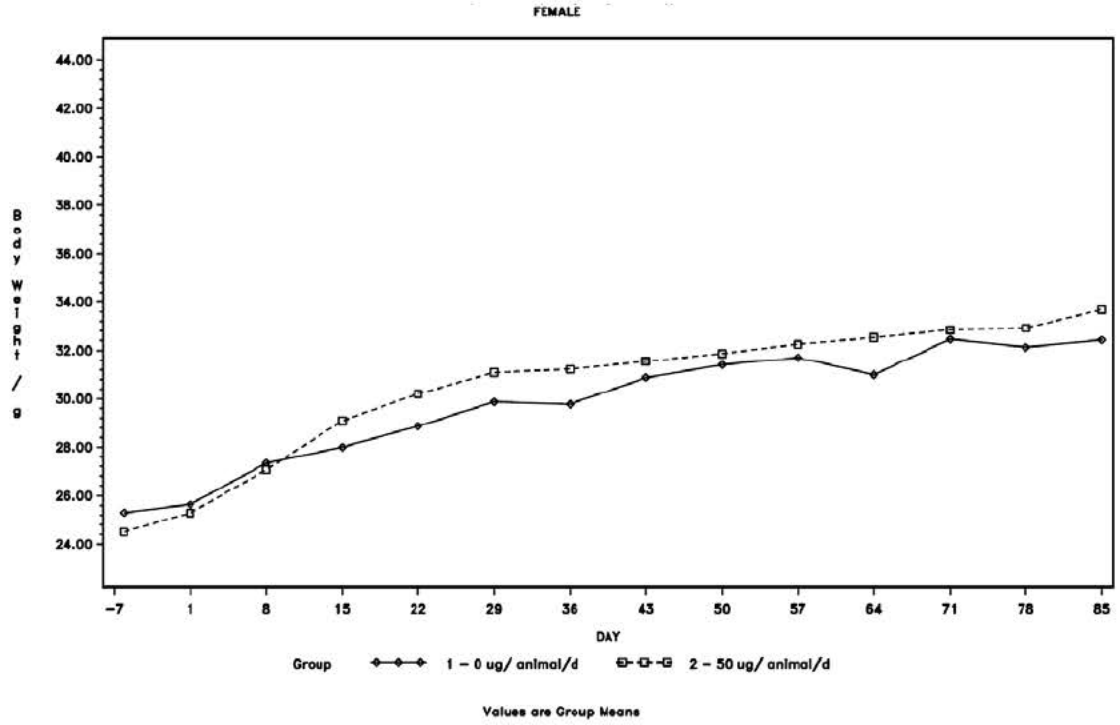
Results:

Mortality: No unscheduled deaths occurred during the study.

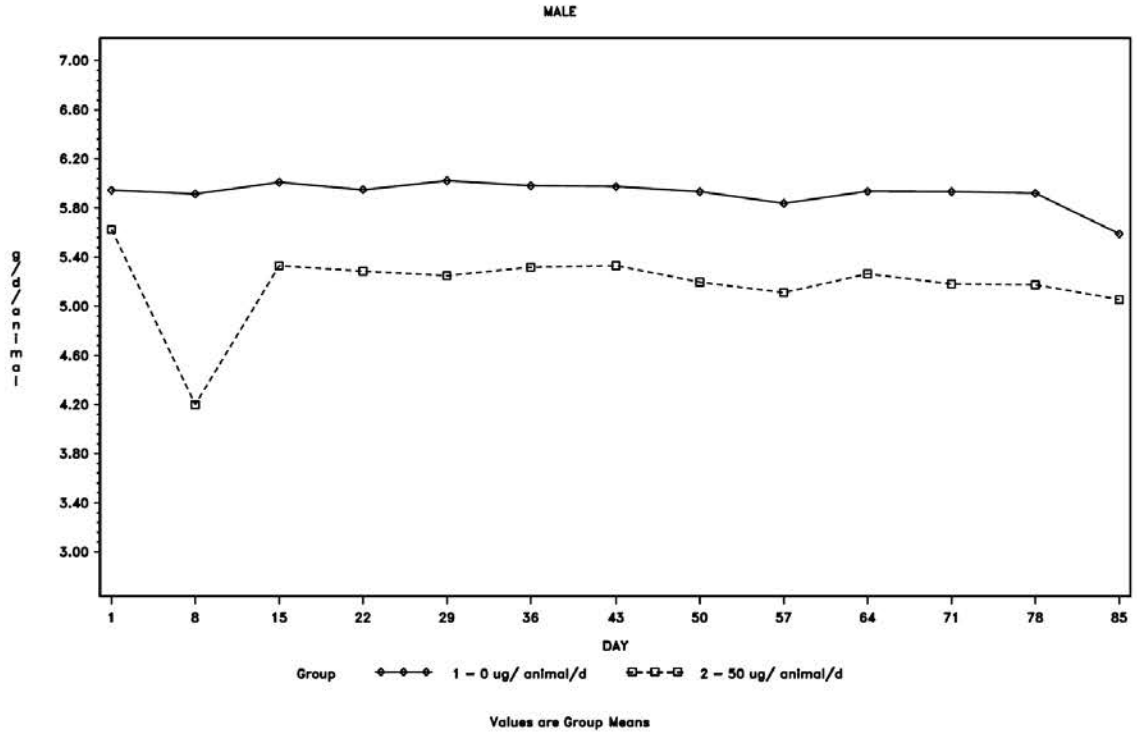
Clinical signs: No treatment-related adverse clinical signs were noted.

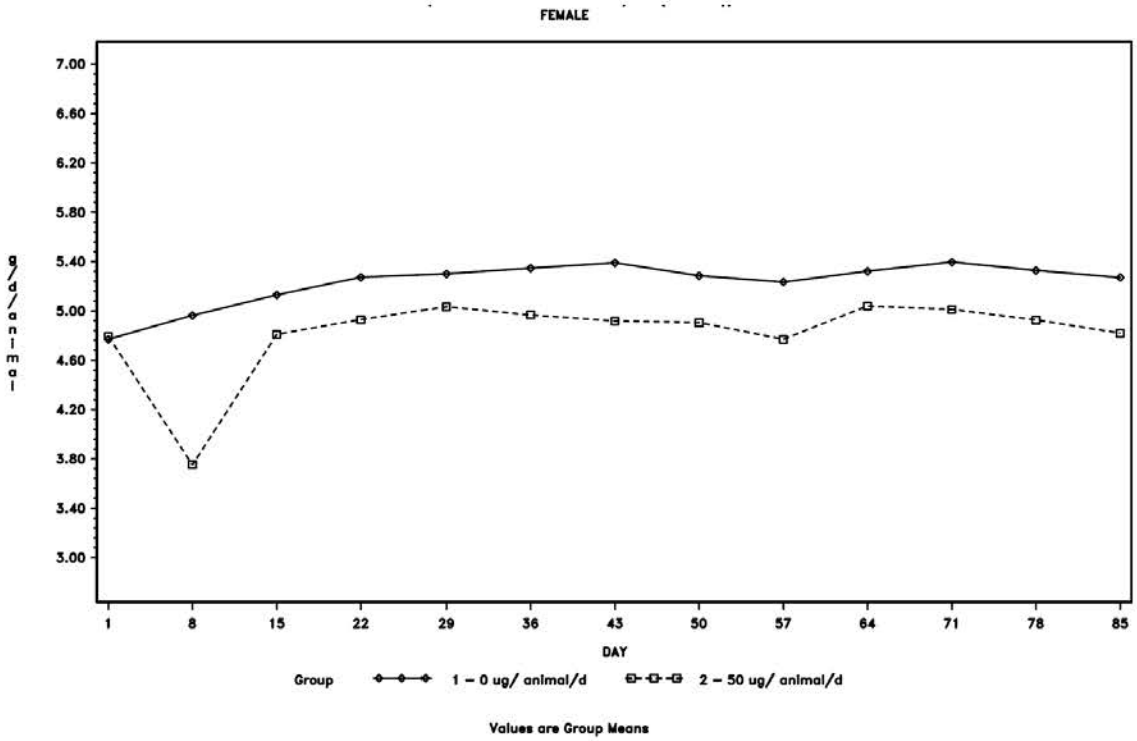
Body weights: (sponsor-generated figures)





Food consumption: (sponsor-generated figures)



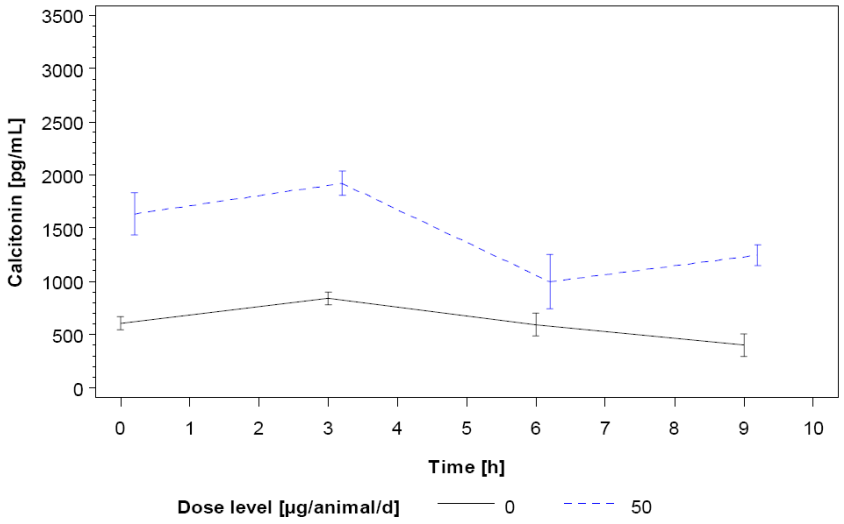


Clinical chemistry: (sponsor-generated table and figures)

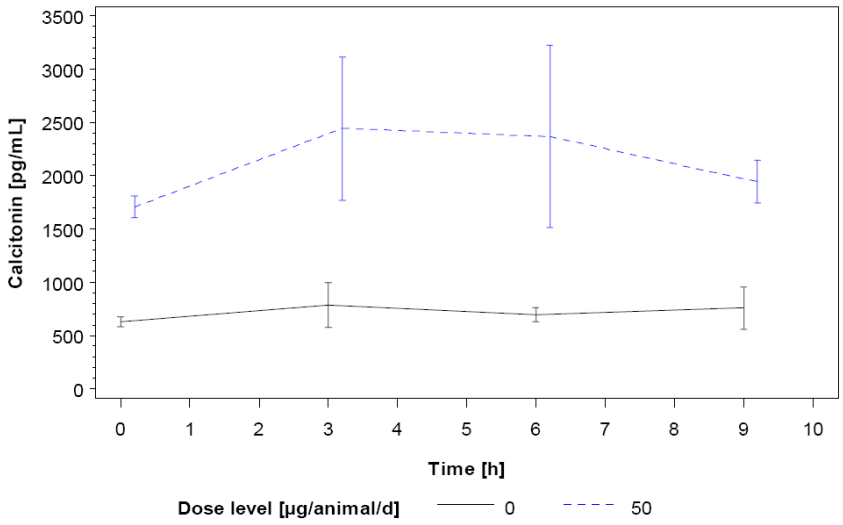
Table 1 – Mean Calcitonin concentrations (pg/mL) in mouse plasma

		Mean Calcitonin Levels [pg/mL]			
		Week 12			
		Time Point [h]			
Group	**Gender**	**0**	**3**	**6**	**9**
1	female	631.5	788.7	696.8	759.3
	male	607.2	841.4	593.2	399.8
2	female	1707	2445	2368	1942
	male	1636	1921	997.1	1248

Text figure 1 - Calcitonin [pg/mL] for male mice over time (mean with standard error)



Text figure 2 - Calcitonin [pg/mL] for female mice over time (mean with standard error)



Gross Pathology: No macroscopic findings were noted.

Histopathology: No treatment-related microscopic effects were observed.

Immunohistochemistry: In almost all thyroid glands examined C-cells (calcitonin-positive cells) and / or proliferating cells (Ki-67 positive cells) were detected by immunohistochemistry. There was no double staining of cells demonstrating proliferating C-cells.

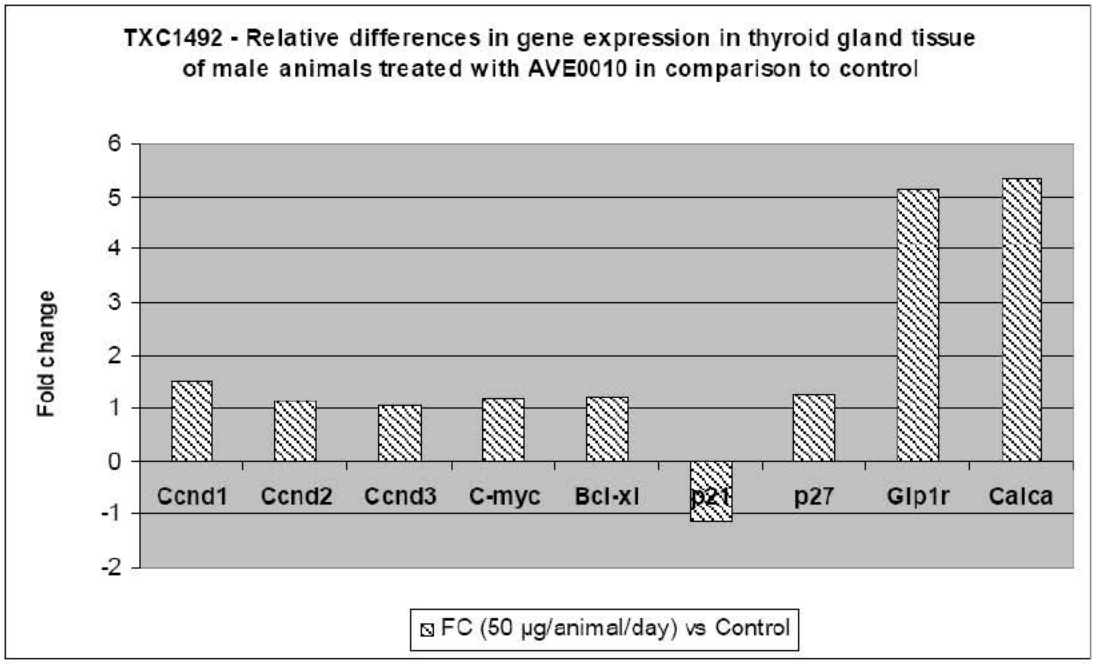
Gene expression analysis: (sponsor-generated table and figures)

8.1 RELATIVE DIFFERENCES IN GENE EXPRESSION IN COMPARISON TO CONTROL

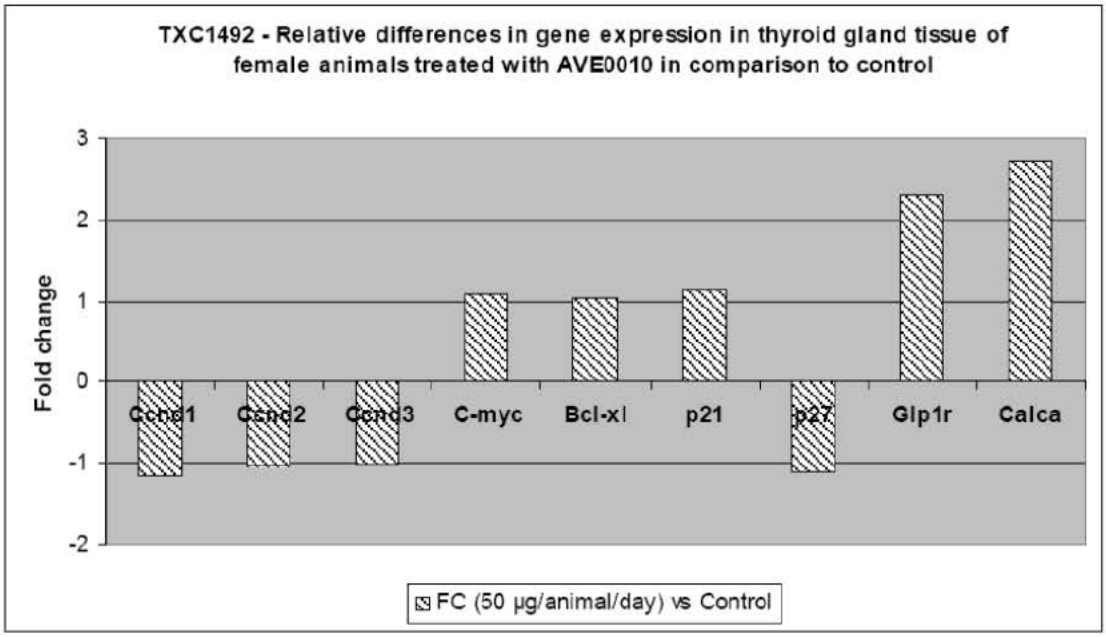
	Ccnd1	Ccnd2	Ccnd3	C-myc	Bcl-xl	p21	p27	Glp1r	Calca
male	1.53	1.14	1.03	1.17	1.23	-1.11	1.26	5.16	5.32
female	-1.15	-1.05	-1.02	1.07	1.04	1.15	-1.10	2.31	2.71

Control value = 1, means that relative difference of +/- 1 is not different from control

Text figure 3 - Gene expression in thyroid gland tissue – males



Text figure 4 - Gene expression in thyroid gland tissue – females



Control value = 1, means that relative difference of +/- 1 is not different from control

Toxicokinetics: As shown in the sponsor-generated table below, AVE0010 exposure was detected at all three time points during the study, although the concentrations were a little inconsistent, especially for females. Based on these data, $AUC_{0\text{-}24h}$ values ranged from ~1000 ng·h/mL for males and females on Day 25 to ~12,000 ng·h/mL for females on Day 85. The highest exposure for males was ~3000 ng·h/mL on Day 2 and Day 85. As a comparison, twice daily dosing of 1000 µg/kg BID in mice resulted in an $AUC_{0\text{-}24h}$ range of 1614 to 2010 ng·h/mL on Day 1 and 5086 to 7114 ng·h/mL after 3 months (Study DSE 2004-0062). Therefore, the exposures observed in this study are mostly consistent with the exposures achieved after twice daily subcutaneous injections of 1000 µg/kg.

Text table 1 - Summary of mean plasma concentrations of AVE0010 in mice (s.c. infusion)

Sex	Dose (µg/animal/day)	C_{mean} (ng/mL)		
		Day 2	Day 25	Day 85
Male	50	126	43.4	122
Female	50	106	42.1	501

Values are rounded to 3 significant figures

Study title: AVE0010 - 3-month subcutaneous infusion study in mice [Note: this study report was submitted to the IND after the NDA submission]

Key study findings:

- There were no definitive treatment-related mortalities. One exenatide-treated male found dead on Day 5 showed decreased motor activity and was cold to touch, which may have been related to the pharmacological effects on food consumption during the first week. Most deaths were procedurally related, either during or after pump implant surgery or after bleeding for calcitonin samples. There were no other noteworthy clinical signs.
- Slight mean body weight decreases occurred for treated groups during the first week, which correlated with a significant decrease in food consumption during this time. Body weights increased thereafter as food consumption increased. Females had similar mean body weights except during the final 2 weeks, whereas the males gained weight but to a lesser extent than controls.
- 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-1 receptor mRNA transcripts in thyroid cells. No meaningful differences in the expression of cell growth regulatory 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.
- Overall, C-cells appeared to respond to treatment by upregulating the expression of calcitonin and GLP-1R and increasing secretion of calcitonin. These data suggest that C-cell proliferation in mice only occurs after a prolonged period of GLP-1 receptor-mediated signaling that results in continuous calcitonin production and secretion.

Study number: TXC1505
Conducting laboratory: Sanofi-Aventis Research & Development Disposition, Montpellier Operational Center, 34184 Montpellier Cedex 04, France
Date of study initiation: 19 April 2012
GLP compliance: Yes (except for calcitonin immunohistochemistry, histomorphometry, and gene expression analysis)
QA statement: Yes
Drug, lot #, and % purity: AVE0010 prefilled in (b) (4) osmotic pumps, Batch #B004, 88.1% pure
Vehicle/control: Sodium acetate trihydrate (b) (4) Glycerol 85% (b) (4), (b) (4) Methionine (b) (4) m-Cresol ((b) (4) NaOH (b) (4) HCl (b) (4) water for injection (1 mL = (b) (4))
Comparator: Exenatide in (b) (4) osmotic pumps

Methods

Species/strain: Mice/CD-1
Design: see sponsor-generated table below

AVE0010 and exenatide dose levels

Group	Test and control article	Dose levels[b] (µg/animal/day)	Concentration (mg/mL)	Flow rate (µL/h)
Main groups				
1[a]	Vehicle	0	0	0.11
2	AVE0010	75	28.41	0.11
3	Exenatide	75	28.41	0.11
Toxicokinetics groups				
4[a]	Vehicle	0	0	0.11
5	AVE0010	75	28.41	0.11
6	Exenatide	75	28.41	0.11

a 3000 µg/kg/day for a mean body weight of 25 g
b Control group: vehicle

Animals/sex/group: 30
Route and regimen: Subcutaneous, continuous infusion via osmotic pump
Age: 6-7 weeks at initiation of dosing
Weight: 30.5 to 44.1 g (males) and 23.5 to 30.3 g (females)

Number/sex/group (TK): 4 (control), 6 (treated)
TK sampling (2-3 animals/time point): One sample on Days 10 and 84.
Ab sampling: Collected but not reported
Analysis of dosing preparations: Formulation samples from Weeks 1, 5, and 9 were analyzed. All formulations were found to be within 96% and 109% of the nominal concentration.

Observation and Times:
Mortality: Once or twice daily
Clinical signs: Once daily; twice daily on days of pump implantation (Days 1, 29, 57)
Pump mobilization: Once weekly
Body weights: Weekly
Food consumption: Weekly
Hematology: Not conducted
Clinical chemistry: Plasma calcitonin only; Day 84
Urinalysis: Not conducted
Gross pathology: All animals
Organ weights: Not conducted
Histopathology: Thyroid glands and tissues with macroscopic observations
Thyroid glands from half the animals (14 or 15 animals/sex/group) were preserved in 10% neutral buffered formalin.
The thyroids from the other half of the animals were preserved in either 10% neutral buffered formalin (left lobe) for calcitonin IHC or RNA-later buffer (right lobe) for gene expression analysis.
Peer review: Yes
Unique study design: Thyroid gene expression analysis and immunohistochemistry

Gene	Intracellular function
Cyclin D1 (Ccnd1)	Functions as a regulatory subunit with cyclin-dependent kinases (CDK) 4 and 6, whose activity is required for cell cycle G1/S transition. Overexpression of this gene has been noted in a variety of tumors.
Cyclin D2 (Ccnd2)	Functions as a regulatory subunit with CDK4 and CDK6, whose activity is required for cell cycle G1/S transition. Overexpression of this gene has been noted in ovarian and testicular tumors.
Cyclin D3 (Ccnd3)	Functions as a regulatory subunit with CDK4 and CDK6, whose activity is required for cell cycle G1/S transition.
C-myc	Transcription factor that promotes cell proliferation. Up-regulates cyclins and down-regulates p21. Up-regulated in many types of cancers.
Bcl-xL	Anti-apoptotic protein in the Bcl-2 family that has been associated with the survival of cancer cells. Involved in the signal transduction pathway of FAS-L.
p21/CIP1/WAF1 (CDKN1A)	Binds to and inhibits the activity of CDK2, CDK4, and CDK6 thereby preventing cell cycle progression/cell proliferation.
p27/Kip1 (CDKN1B)	Binds to Cyclin D either alone or when complexed with CDK4; inhibits CKD4 activity causing cells to arrest in G1 of the cell cycle. Also binds Cyclin E/CDK2 and Cyclin A/CDK2.
Glucagon-like peptide-1 receptor (GLP1r)	G protein-coupled receptor that binds GLP-1. Receptor stimulation activates the adenylyl cyclase pathway resulting in increased insulin synthesis and secretion.
Calcitonin	Secreted from thyroid C-cells. Decreases serum calcium by inhibiting

(Calca)	absorption and inhibiting renal tubular resorption of calcium; decreases osteoclast activity.
Dapdh	House keeping gene

Results:

Mortality: There were 5, 9, and 10 unscheduled deaths in the control, AVE0010, and exenatide groups. One male from Group 2 was found dead on Day 8 and three males and one female from Group 3 were found dead on Days 5, 31, 37, and 59, respectively, without preliminary clinical signs, with the exception of the male that died on Day 5, which showed a decrease in motor activity and was cold to touch before death. The cause of death for these animals was not determined. A summary of all unscheduled deaths is shown in the sponsor-generated table below.

Text Table 2 - Mortality (main study)

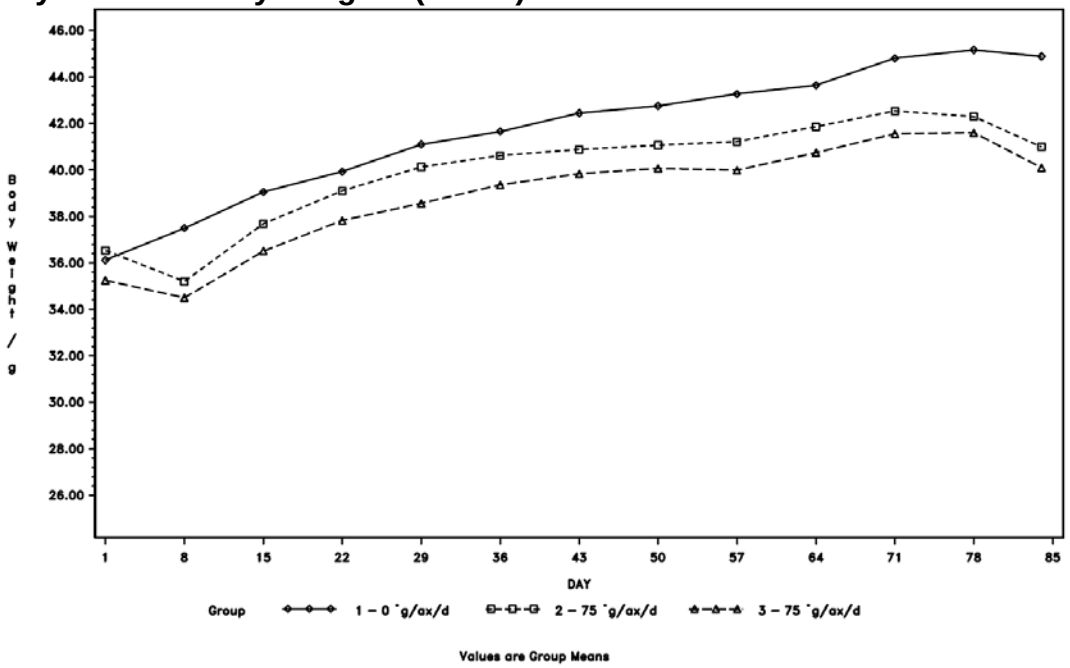
Group	1		2		3	
Dose (mg/animal/day)	0		75		75	
	vehicle		AVE0010		Exenatide	
Sex	M	F	M	F	M	F
No. of animals[a]	34	35	34	35	34	35
Sacrificed for human reason	1	-	-	-	-	-
Found dead	-	-	1	-	3	1
Dead during surgery	4	1	8	5	5	1
Dead after bleeding samples (D84)	-	-	-	1	2	1

a Including replacement animals

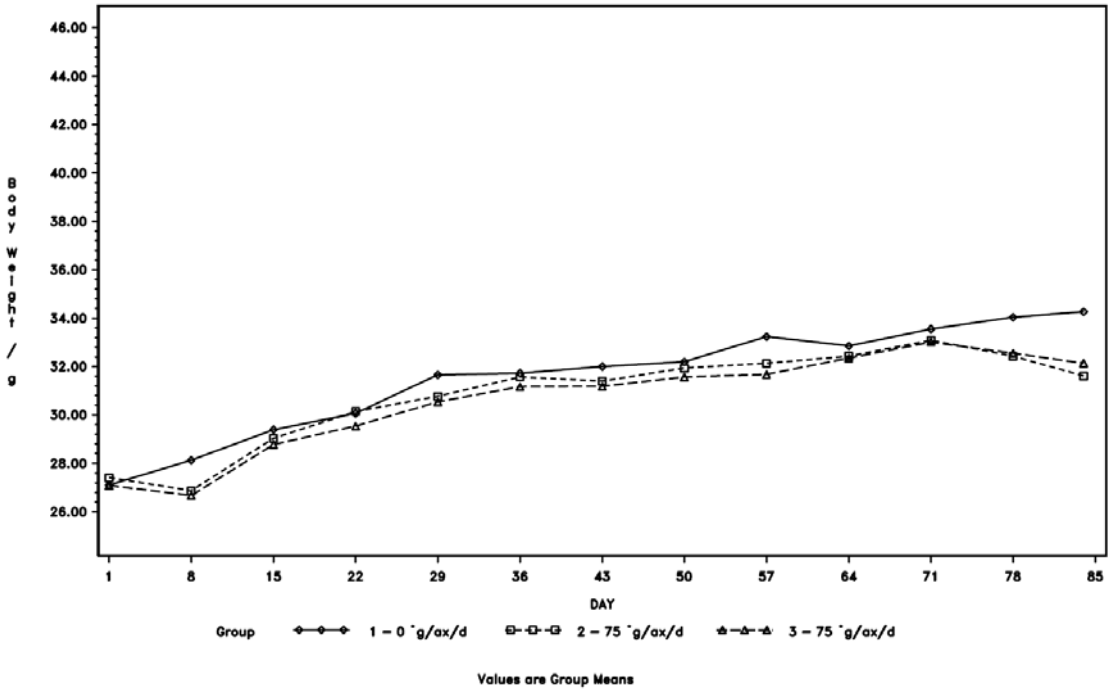
Body weights:

Slight mean body weight loss (≤-4%) occurred for treated groups during the first week of treatment, which correlated with decreased food consumption. After the first week, body weights increased but still remained slightly lower than the control values at the end of the study. A summary of the body weight data is shown in the sponsor-generated figure below.

Summary of Mean Body Weights (males)



Summary of Mean Body Weights (females)



Food consumption:
Treated groups had lower mean food consumption (from -21% to -28%) during Week 1, with a return to normal values during Week 2. From Week 7 to the end of treatment, slightly lower food consumption was observed in males treated with AVE0010 or exenatide.

Clinical signs: With the exception of the animal that died on Day 5, there were no apparent treatment-related adverse clinical signs. The male that died on Day 5 showed a decrease in motor activity and was cold to touch before death, which may have been related to pharmacodynamic effects on food and water consumption.

Calcitonin:
On Day 84, mean calcitonin levels in treated groups (pooled values) were increased by approximately 3 fold compared with the control value. A summary of the data are shown in the sponsor-generated table below.

Table 2 - Summary results for calcitonin concentration

		Calcitonin concentration (pg/mL)		
		Male	Female	pooled
Group 1 Vehicle	Median	12.50	34.70	12.50
	Mean	17.24	66.81	42.03
	STD	14.46	71.10	56.68
	N	30	30	60
	Min	12.5	12.5	12.5
	Max	82.0	231.6	231.6
Group 2 AVE0010 75 µg/day	Median	81.75 *	158.45 *	126.30 *
	Mean	97.76	173.45	136.91
	STD	57.18	70.19	74.24
	N	28	30	58
	Min	12.5	71.4	12.5
	Max	230.5	348.3	348.3
Group 3 Exenatide 75 µg/day	Median	125.50 *, #	121.90 *, #	123.35 *, #NS
	Mean	133.92	136.72	135.37
	STD	59.36	64.61	61.60
	N	28	30	58
	Min	48.2	51.3	48.2
	Max	286.4	302.4	302.4

*: Significantly different ($p \leq 0.05$) from group 1, *NS: not significantly different from group 1, #: Significantly different ($p \leq 0.05$) from group 2, #NS: not significantly different from group 2

Gross Pathology:
There were no apparent treatment-related macroscopic findings noted.

Histopathology:
No major differences in the grade of C-cell staining were observed between treated and untreated groups. Moderately (grade 3) labeled C-cells were noted in four Group 2 (AVE0010) males at a slightly higher incidence than for controls (one animal). A very slight increase in grade of C-cell clusters was also observed. The subtlety of these

effects was not felt to represent a treatment-related effect on C-cells. A summary of the microscopic data is shown in the sponsor-generated table below.

Text Table 1 - Summary of observations on calcitonin-immunolabelled sections

Group		1		2		3	
Treatment		Vehicle		AVE0010		Exenatide	
Dose (µg/animal/day)		0		75		75	
Sex		M	F	M	F	M	F
N =		35	31	35	35	36	32
N observed =		30	30	28	30	27	31
C-cells	Grade 1	16	21	17	13	18	11
	Grade 2	7	6	6	12	7	9
	Grade 3	1	-	4	1	-	1
Clusters of C-cells	Grade 1	6	7	2	5	5	5
	Grade 2	-	-	2	1	-	2
Colloid labeling	Grade 1	9	9	7	8	10	13
	Grade 2	5	3	4	5	-	2

Immunohistochemistry / Histomorphometry: (5 slides per thyroid at approximately 20%, 45%, 50%, 55%, and 80% of the length of the thyroid)

There was no meaningful difference in the number of calcitonin-stained (C-cells) or non-calcitonin stained cells.

Figure 4 -Maximum of Mean Area Positive Cells (µm²) (Analyse Globale)

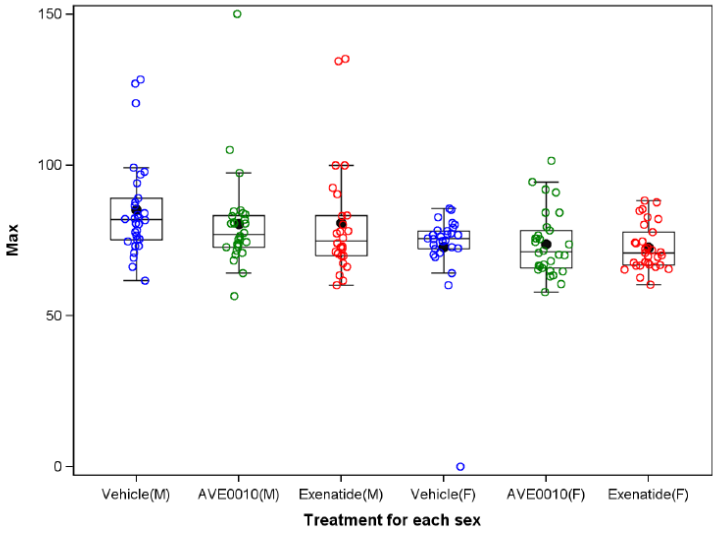


Table 10 -Descriptive Statistics of Mean Area Positive Cells (µm²) (Global analysis)

Sex	Treatment	N	Mean	Median	Max	SD
FEMALE	**Vehicle**	120	49.37	64.42	**85.5**	31.20
	AVE0010	143	46.75	61.17	**101.5**	30.44
	Exenatide	148	48.65	61.28	**88.3**	28.60
MALE	**Vehicle**	141	56.81	71.77	**128.3**	34.16
	AVE0010	127	56.13	68.98	**150.1**	31.61
	Exenatide	130	51.98	64.55	**135.3**	32.46

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Figure 6 -Maximum of Mean Area Negative Cells (µm²) (Global analysis)

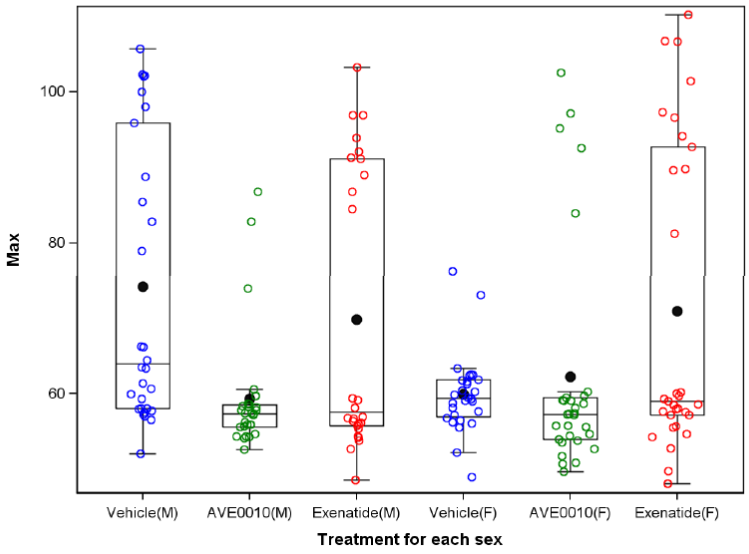

Table 14 -Descriptive Statistics of Mean Area Negative Cells (µm²) (Global analysis)

Sex	Treatment	N	Mean	Median	Max	SD
FEMALE	**Vehicle**	120	57.02	56.84	**76.3**	4.16
	AVE0010	142	59.41	54.78	**102.5**	13.70
	Exenatide	148	67.58	57.40	**110.2**	18.94
MALE	**Vehicle**	141	68.56	60.68	**105.7**	16.47
	AVE0010	127	55.33	54.87	**86.8**	4.95
	Exenatide	130	65.71	56.03	**103.3**	16.95

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Gene expression analysis:

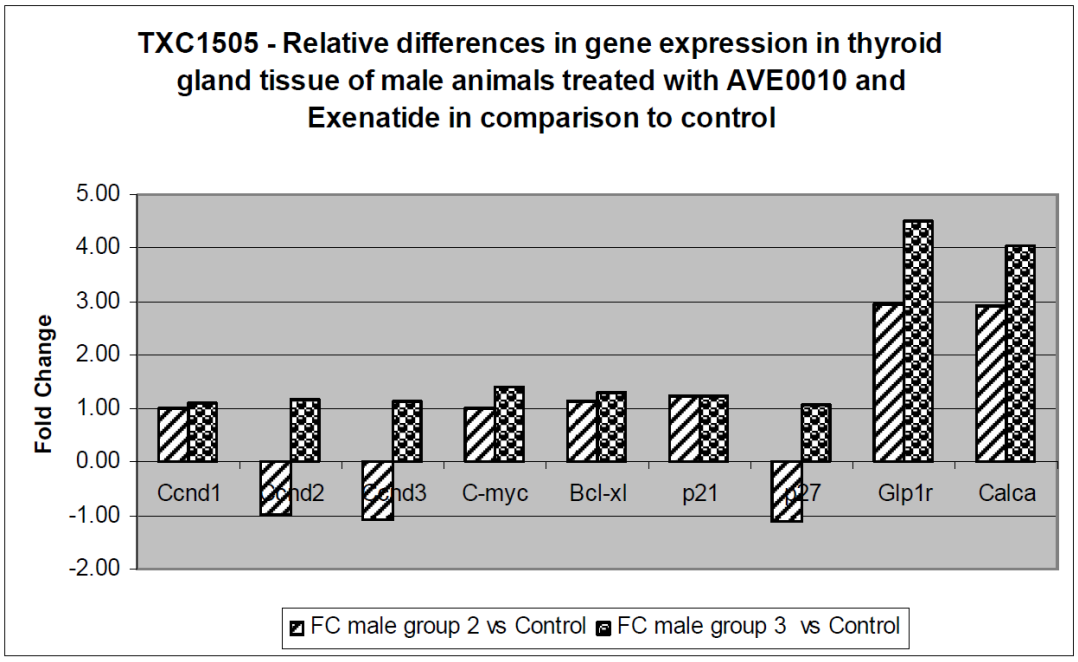
Gene expression analysis was conducted by real-time, quantitative PCR. Threshold cycle values greater than 35 exceeded the sensitivity limits of the real-time PCR system. The data are summarized in the sponsor-generated tables and figures below. Expression levels of GLP-1R and calcitonin were higher in treated animals. There were no apparent biologically relevant changes in expression levels of growth regulatory genes, which may not be surprising considering an increase in C-cell number was not detected through histomorphometry.

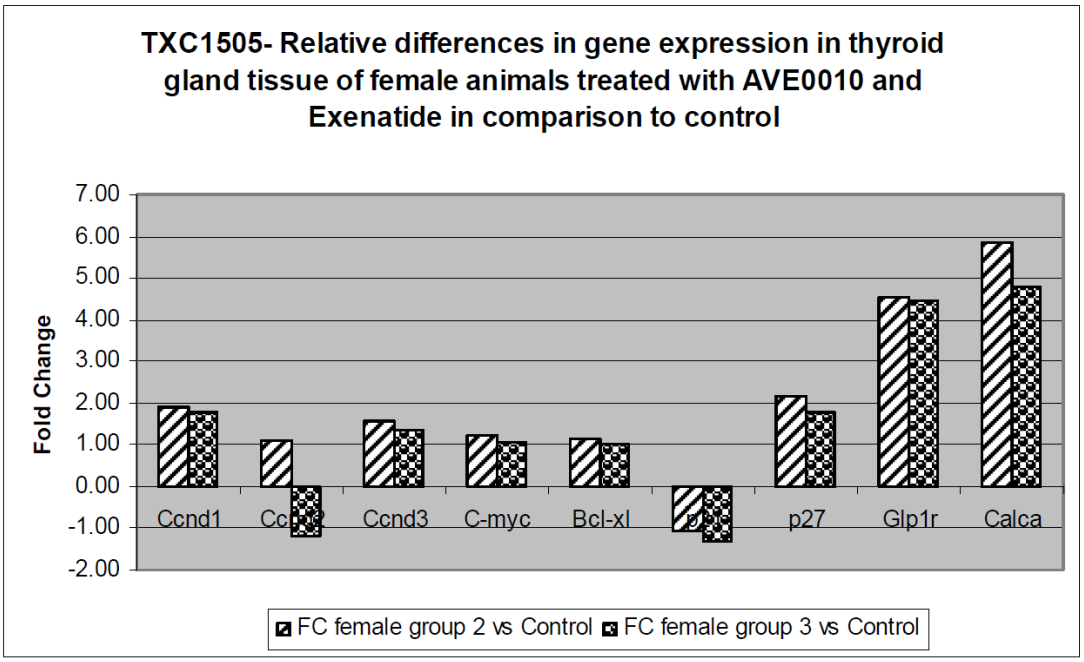
Male animals	Ccnd1	Ccnd2	Ccnd3	C-myc	Bcl-xl	p21	p27	GLP-1R	Calca
Group 2 - AVE0010* (75 µg/animal/day)	1.00	1.02	-1.07	1.02	1.13	1.23	-1.10	2.95	2.91
Group 3 – Exenatide (75 µg/animal/day)	1.11	1.16	1.15	1.41	1.30	1.24	1.08	4.49	4.04

Control value = 1, means that relative difference of +/- 1 is not different from control
* group 2: animal 102 excluded

Female animals	Ccnd1	Ccnd2	Ccnd3	C-myc	Bcl-xl	p21	p27	GLP-1R	Calca
Group 2 – AVE0010 (75 µg/animal/day)	1.92	1.09	1.55	1.23	1.13	-1.07	2.15	4.54	5.87
Group 3 - Exenatide* (75 µg/kg/day)	1.76	-1.21	1.36	1.04	1.02	-1.30	1.76	4.45	4.81

Control value = 1, means that relative difference of +/- 1 is not different from control
*Group 3: animal no. 196 excluded





Toxicokinetics:
AVE0010 exposure increased by approximately 23 fold and 18 fold for males and females, respectively, and exenatide exposure increased by approximately 7 fold and 2.5 fold for males and females, respectively. TK data are summarized in the sponsor-generated table below.

Text Table 1 - AVE0010 and exenatide mean toxicokinetic parameters on Days 10 and 84 (n=3 by sex, dose, sampling day)

Analyte	Dose (µg/animal/day	Day	Sex	C_{mean}* (pg/mL)	R_{acc} (C_{mean})	R_{Cmean} female/males
AVE0010	75	10	male	15400		
			female	17700		1.15
		84	male	357000	23.2	
			female	325000	18.4	0.910
Exenatide	75	10	male	14800		
			female	24900		1.68
		84	male	102000	6.89	
			female	61100	2.45	0.599

* values are rounded to 3 significant figures

Local Tolerance (Occupational Safety Assessment Studies)

Local tolerance studies with lixisenatide were performed in rabbits by SC (clinical route), and intramuscular (IM), paravenous (PV), intraveneous (IV), and intra-arterial (IA) administration routes to cover potential routes of accidental misinjection. Two rabbits per administration route were sacrificed 24 hours and two were sacrificed 120 hours after administration to assess reversibility of tissue alterations (assessed by histological

examination). The test formulation was administered on the right side and the control solution (saline) was administered contra-laterally as an intra-individual control.

The lead formulation was tested with (batch #BR477/35) and without (batch #BR477/39) 100 µg/mL lixisenatide (Study DSE 2005-0519 and Study DSE 2005-0327). These studies revealed a moderate tolerance of the placebo and the lixisenatide formulation after SC administration, which is the intended clinical route. After IV or IA administration of the placebo and the lixisenatide formulation, a good or limited tolerability, respectively, was seen (tolerance assessment is internally scaled in several grades: good, moderate, limited or poor tolerability). Poor tolerability occurred after PV and IM administration of both the placebo and the lixisenatide formulation.

Because of the poor intramuscular tolerability which is a likely accidental false injection route, a new modified formulation, BR477/38, was investigated in an additional local tolerance study (Study DSE 2005-0771 and DSE 2005-0771 Amendment 1). The only difference between formulation BR477/35 and BR477/38 was the absence of EDTA and histidine in this new formulation. Formulation BR477/38 was tested in the intended clinical route (SC) and the two injection routes, which showed poor tolerability in earlier studies (IM and PV). Results are summarized in the sponsor-generated table below.

Formulation code and description [ref]	Route	Results
BR477/39 (placebo formulation) [TS 2.6.7.16C] BR477/35 (lixisenatide formulation) [TS 2.6.7.16A]	SC (0.5 mL)	moderate tolerability
	IM (0.5 mL)	poor tolerability
	PV (0.1 mL)	poor tolerability
	IV (0.5 mL)	good tolerability
	IA (0.5 mL)	limited tolerability
BR477/38 (lixisenatide formulation without EDTA and histidine as current clinical formulation) [TS 2.6.7.16B]	SC (0.5 mL)	good tolerability
	IM (0.5 mL)	good tolerability
	PV (0.1 mL)	poor tolerability

EDTA= Edeate sodium

The poor paravenous tolerability was confirmed with the modified formulation. The immediate release formulation BR477/38 showed a significantly improved local tolerability profile in the rabbit concerning SC and IM injection. It was therefore evident that the moderate/poor SC and IM tolerability of former formulations with lixisenatide was caused by distinct excipients (EDTA and histidine). As a consequence, clinical formulations with lixisenatide did not contain these excipients.

A skin sensitization (local lymph node assay) test was conducted in Balb/c N female mice and an irritation test (skin and ocular) was conducted in male New Zealand white rabbits with [redacted] (b) (4) (Study IST0273). The results indicated that [redacted] (b) (4) is not a sensitizer and is not an irritant to the skin. It was, however, found that the test article was a severe irritant to the eye, not otherwise described.

A skin sensitization (local lymph node assay) test was conducted in Balb/c N female mice and an irritation test (skin and ocular) was conducted in male New Zealand white rabbits with (b) (4)
(Study IST0274). The results indicated that (b) (4) is not a sensitizer and is not an irritant to the skin. It was, however, found that the test article was a severe irritant to the eye, not otherwise described.

Effects on Immune System

Study title: B-Cell Response to SC and PO Administration of ZS42-0010 (AVE0010) in Mice (non-GLP study, Study DIV1155)

Methods:
Twenty female BALB/cJ mice were dosed with AVE0010 either orally (19.95 mg/kg = 4 µmol/kg, n=l0) or SC (1.995 µg/kg= 0.4 nmol/kg, n=l0) five times, once every 2 weeks, over a period of 8 weeks. The presence of antibodies recognizing the peptide AVE0010 was examined by ELISA before dosing of AVE0010 as well as 5 and 9 weeks after initiation of dosing.

Results:
Both SC and PO treatment with AVE0010 gave rise to formation of antibodies against the peptide in the plasma of the treated mice. However, the plasma antibody titers were low or very low, especially in mice treated orally, compared with the anti-AVE0010 titer observed in a reference rabbit antiserum.

Study title: Murine T-Cell Responses to parenteral Administration of ZS42-0010 [AVE0010], Amendment 1 (non-GLP study, #DIV1158)

Methods:
Female BALB/cJ mice were treated once with 65 nmol of AVE0010 dissolved in either a 1:1 mixture of Freunds Complete Adjuvant and Phosphate Buffered Saline (FCA/PBS) or in PBS. Control groups consisted of animals treated with FCA/PBS only (negative control) or with tuberculin purified peptide derivative (PPD, positive control). Ten days after treatment lymph nodes were withdrawn and cell suspensions from the lymph nodes were re-stimulated with dilutions of AVE0010, GLP-1, PPD, or PBS.

Results:
Both GLP-1 and AVE0010 stimulated T-cell proliferation in cell cultures derived from mice primed with AVE0010 dissolved in FCA/PBS. However, GLP-1 or AVE0010 were unable to stimulate T-cell proliferation in cultures derived from mice primed with AVE0010 dissolved in PBS or in mice primed with FCA/PBS only. At very high concentrations of AVE0010 (>5 µM) and GLP-1 (>45 µM) both peptides induced a decrease in the proliferative T-cell response.

11 Integrated Summary and Safety Evaluation

Lixisenatide is a GLP-1 receptor agonist that is an analog of exendin-4, a 39 amino acid peptide. Lixisenatide is a synthesized 44 amino acid peptide; (b) (4) Like exendin-4, lixisenatide is resistant to degradation by DPP4 and therefore has an extended half-life compared with endogenous GLP-1.

In vitro binding studies show that lixisenatide has a strong binding affinity to the human GLP-1 receptor (IC_{50} = 1.43 nM), which is approximately 4 times greater than the receptor binding affinity of endogenous human GLP-1. The antidiabetic properties of lixisenatide were demonstrated in animal models of diabetes, including db/db mice and Zucker fatty diabetic rats. In these models, lixisenatide improved oral glucose tolerance, basal blood glucose, and HbA1c with a rapid onset and sustained duration of action. Enhancement of glucose-stimulated insulin secretion was shown to occur in a glucose-dependent manner. Lixisenatide also prevented the deterioration of pancreatic responsiveness and glucose homeostasis over time and protected β-cells derived from a rat pancreatic dell line from lipid and cytokine-induced apoptosis. Lixisenatide also delays gastric emptying and causes reduced food intake.

In an in vitro secondary pharmacology receptor binding assay, lixisenatide was shown to have low to very low affinity to a wide range of receptors. Of the 91 receptors tested, only the Ca^{2+}-channel (N) showed inhibition of greater than 50%. In a follow-up study using rat cultured dorsal root ganglion neurons, it was confirmed that lixisenatide is a weak and selective blocker of N-type calcium channels. Overall, lixisenatide appears to be a selective and potent agonist for the GLP-1 receptor.

Absorption of lixisenatide is rapid following a single SC or IP administration for all species tested, with mean T_{max} values between 0.25 and 3.75 hours after dosing. Absolute bioavailability for SC dosing was approximately 90% in dogs, 70% in pigs, 36% to 50% in db/db mice, ≥30% in rabbits, and only 3% in rats. In mice, exposures generally increased in a nearly dose-proportional manner between 20 and 2,000 µg/kg BID on Day 1. In rats, exposures increased in a slightly less than dose-proportional manner between 5 and 2,000 µg/kg BID. In dogs, exposures increased in a slightly less than dose-proportional manner between 20 and 1,000 µg/kg BID. After repeated dosing, the development of anti-drug antibodies resulted in large increases in drug exposure in mice, rats, and dogs, likely due to decreased peptide elimination. For example, in rats, exposures increased by as much as 200X within 3 months compared with Day 4 values. Using a cell-based assay with an endpoint of GLP-1 receptor-mediated cAMP production, the sponsor demonstrated that antibody-bound lixisenatide retained biological activity in mice, rats, and dogs.

Plasma protein binding was estimated to be 55%, 49%, and 62% in human, dog, and rat plasma, respectively. After subcutaneous administration of radiolabeled lixisenatide to rats, the highest concentrations of radioactivity at 0.25 hours after injection were found

at the injection site, pancreas, renal cortex, lung, and glandular tissue. Blood, myocardium, and adrenal glands showed moderate levels of radioactivity. Significant amounts of lixisenatide could not be measured in the brain. Distribution studies conducted in pregnant animals showed that a very low amount of lixisenatide transfers across the placenta and could be detected in rat and rabbit fetuses. In in vitro metabolism studies with S9 from liver and kidney, 28 lixisenatide metabolites were identified. These metabolites were determined to be degraded peptide products and are not expected to be pharmacologically active. Lixisenatide did not significantly inhibit or induce CYP450 isozymes. Specific elimination studies were not conducted, but it is anticipated that lixisenatide is primarily cleared in the kidney through peptide degradation. An elimination study in lactating rats showed that a very low amount of lixisenatide is secreted in milk.

Treatment with a single, high dose of lixisenatide (5,000 µg/kg) by SC injection resulted in transient piloerection in rats. In a safety pharmacology Irwin test, no abnormal behavior or coordination was observed after a single subcutaneous dose of 2,000 µg/kg. Rats did show transient hypoactivity after receiving repeated daily subcutaneous doses of ≥20 µg/kg BID. Paddling, salivation, and mouth rubbing were also occasionally observed. A neurobehavioral safety pharmacology study in rats using the intravenous route showed effects at ≥1 µg/kg, including apathy, decreased locomotor activity, decreased body tone, abnormal dispersion within the home cage, and impairment of the righting reflex. At ≥50 µg/kg, a slight decrease in pain response and hypothermia were observed in some animals. In dogs, doses ≥300 µg/kg resulted in vomiting, diarrhea, and sharply reduced food consumption and body weight.

Cardiovascular safety pharmacology studies showed that lixisenatide can block hERG channel currents by approximately 37% at 30 µg/mL. At concentrations up to 0.57 µg/mL, lixisenatide did not induce any changes in resting membrane potential or action potential parameters of rabbit Purkinje fibers. In conscious rats, an intravenous injection of ≥50 µg/kg resulted in increased mean arterial pressure as well as blood glucose. In anesthetized dogs, a single intravenous dose of up to 10 µg/kg did not significantly affect cardiovascular or respiratory function. Additionally, no toxicologically meaningful effects on heart rate or ECG parameters were noted in dogs in toxicology studies using twice daily doses of up to 1,000 µg/kg/dose. Lixisenatide was shown to decrease the rate of gastric emptying in mice when administered by intraperitoneal injection with an ED_{50} of 6.4 nmol/kg, which is similar to the ED_{50} of exendin-4.

There were no definitive, treatment-related adverse microscopic findings in the pancreas of mice or rats after treatment for up to 2 years or in dogs after treatment for up to 1 year. The primary target organs after repeated dosing were the testes and injection sites. In a 6-month repeat-dose study in rats, an increased incidence and severity of microscopic findings in 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 (7,250X of clinical exposure). These effects were found to be mostly reversible after a 1-month recovery period. Additionally, treatment-related microscopic findings were not

observed after 3 months of treatment. In dogs, microscopic findings were also observed in epididymis and testis, including moderate to severe hypospermatogenesis in seminiferous tubules and epididymal dilation, degeneration, oligospermia, or aspermia at ≥200 µg/mg BID after 12 months of treatment (≥4,062X of clinical exposure). Effects on spermatogenesis in dogs could be observed after 13 weeks of treatment, with the incidence and severity increasing with duration of dosing. In an 8-month juvenile toxicology study in dogs at 5, 20, and 200 ug/kg BID (≥7X of clinical exposure), similar findings were observed in the testis and epididymis in a dose-related manner. However, these effects were shown to be reversible after a 2-month treatment-free period.

Using RT-PCR, the sponsor measured the expression level of GLP1R in testis and epididymis from rat, dog, and human. The results of this study showed that GLP1R expression in dog epididymis was 21- to 4,147-fold higher than in rat, depending on the epididymal segment. GLP1R expression in human epididymis was 10-fold lower (total tissue) or 4-fold lower (caput segment) than dog, whereas the expression in the corpus and cauda segments were approximately 11- and 8-fold higher than dog, respectively. In testis, GLP1R expression was at least 100-fold higher than in rats and 3- to 10-fold higher than in humans. These data suggest that the increased GLP1R expression in testis and epididymis make dogs more sensitive to lixisenatide-induced inhibition of spermatogenesis. In support of this hypothesis, once daily doses of 20 µg (the maximum recommended clinical dose) for 6 months in obese men did not result in clinically significant effects on human spermatogenesis (total sperm count, motility, or morphology) or on reproductive hormones (clinical Study TDR11215). Therefore, based on the current available data, it does not appear that human males are at risk for effects on spermatogenesis at the recommended clinical dose.

A slight increase in incidence of hemorrhage, fibroblastic reaction, and abscess at the injection sites was observed for male rats receiving ≥100 µg/kg BID for 6 months (≥2.5X of the clinical dose at the injection site). An increase in inflammation and fibrosis was observed at the injection sites of dogs after treatment with ≥200 µg/kg BID (≥100X of the clinical dose at the injection site) for 1 year.

Treatment of male and female Sprague-Dawley rats with lixisenatide at doses up to 414 µg/kg BID (~2,600X of clinical exposure) for 4 weeks (males) or 2 weeks (females) prior to pairing, throughout the mating period, and during early gestation did not impair fertility, alter mating behavior, or affect early embryonic development. As noted above, reversible effects on spermatogenesis were noted in dogs. It is uncertain whether the observed effects would result in decreased fertility.

Treatment of pregnant rats from GD 6 through GD 17 resulted in delayed fetal growth, and reduced ossification at ≥2.5 µg/kg BID, with more significant effects on growth and ossification occurring at 500 µg/kg BID. Single cases of fetal malformations were observed for each of the treatment groups: one microphthalmia at (2.5 µg/kg BID), one anophthalmia and one diaphragmatic hernia (one fetus each at 35 µg/kg BID) and similar multiple skeletal malformations in one growth-delayed fetus of each dose group.

All malformation data exceeded the historical ranges and mean values. Maternal effects including decreased motor activity, sleepiness, decreased reactivity, piloerection, reduced food consumption, and an initial dose-dependent decrease in body weight were observed at all dose levels. The NOAEL for maternal and developmental toxicity of AVE0010 is <2.5 µg/kg BID, the lowest dose tested.

Treatment of pregnant rabbits from GD 6 through GD 18 resulted in malformations at all dose levels. There were two fetuses each from the 2.5 and 25 µg/kg BID groups and one fetus from the 250 µg/kg BID group with multiple visceral and skeletal malformations, including thorocogastroschisis, amelia of forelimbs, absent bones, malformed bones, fused sternebrae, spina bifida, misshapen heart, malpositioned main arterial vessels, absence of organs, exencephaly, microphthalmia, and omphalocele. Aplasia of the gallbladder was seen in one and two fetuses from the 25 and 250 µg/kg BID dose groups, respectively, and one fetus from the 250 µg/kg BID dose group had a cardiac ventricular septum defect. A statistically significant increase in post-implantation loss occurred at 250 µg/kg BID. Maternal toxicity was noted at all dose levels and consisted of a dose-dependent decrease in mean body weight associated with reduced food consumption, decreased feces, reduced motor activity, and piloerection. The NOAEL for both maternal and developmental toxicity was considered to be <2.5 µg/kg BID (<22X clinical exposure).

In an effort to attain a NOAEL, a second embryo-fetal developmental toxicity study was conducted in rabbits using lower doses. Treatment-related malformations were not observed at doses up to 2.5 µg/kg BID. A slight increase in some developmental variations was observed at ≥1.0 µg/kg BID, including skull (small hole, splitting of bone, and additional suture of parietal bone), 13th rib (supernumerary – short or full), central caudal vertebrae (ossification of less than 15), and forepaw (hyperflexion). Signs of maternal toxicity were observed at ≥1 µg/kg BID and included decreased motor activity, piloerection, and decreased body weight that correlated with decreased food and water consumption. Because the skeletal variations observed at ≥1 µg/kg BID cannot be ruled out as being related to lixisenatide, the NOAEL for embryo-fetal development is considered to be 0.15 µg/kg BID (1X clinical exposure), which is also the NOAEL for maternal toxicity.

Treatment of pregnant rats from GD 6 through Postnatal Day (PND) 20 resulted a slight decrease in suckling in male pups at ≥20 µg/kg BID resulting in decreased body weight gain and a slight increase in pup mortality from PND 0 to 21 and abnormal findings on tails at 200 µg/kg BID. Two cases of multiple skeletal malformations of long bones and ribs were observed in two growth-delayed dead pups at 200 µg/kg BID. A slight, statistically significant decrease in the time to vaginal opening was observed at ≥20 µg/kg BID. There were no adverse effects on reproductive parameters of the F_1 generation. Maternal effects included decreased motor activity and initial body weight loss that correlated with decreased food consumption at all dose levels. The NOAEL for F_0 maternal rats was considered to be <2 µg/kg BID based on the adverse effects on body weight. The NOAEL for F1 pups was considered to be 20 µg/kg BID based on treatment-related mortalities and skeletal and tail defects observed at the high dose.

Lixisenatide was negative for genotoxicity in the standard battery of genetic toxicology assays. A summary of safety margins for the most noteworthy toxicities is presented in the table below. Safety margins for carcinogenicity data are presented in a separate table.

Estimated Safety Margins for Specific Toxicities

Target Organ	Species	NOAEL	$AUC_{0\text{-}24h}$ (ng•h/mL)	Safety Margin Based on AUC
Testis and epididymis	Rat	100 µg/kg BID	2,735	377X*
	Dog	2 µg/kg BID	138	19X*
	Juvenile Dog	<5 µg/kg BID	49	<7X*
Embryo-fetal malformations	Rat	<2.5 µg/kg BID	3.9	<3X†
	Rabbit	<2.5 µg/kg BID	27.2	<22X†
	Rabbit	0.15 µg/kg BID	0.929	1X†
Embryonic loss	Rabbit	25 µg/kg BID	293	240X†

*AUC for Ab+ humans on Day 28: 7.25 ng•hr/ml at 20 µg/day (Study ACT6011).
†AUC for Ab- humans on Day 28: 1.22 ng•hr/ml at 20 µg/day (Study ACT6011).

In a 2-year carcinogenicity study in mice, thyroid c-cell adenomas were noted in 1, 1, and 4 male animals from the 40, 200, and 1000 µg/kg BID groups compared with 0 and 0 from 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 at the mid-dose level cannot be concluded. A statistically significant increase in C-cell adenomas was not observed in females, with only a single incidence at the high-dose level. Accordingly, the NOEL for thyroid C-cell adenomas was determined to be 200 µg/kg BID for males and 1,000 µg/kg BID for females. Exposures at the male and female NOAELs are approximately 272X and 5,000X higher than the anticipated clinical exposure at 20 µg/day.

In a 2-year carcinogenicity study in rats, a statistically significant increase in thyroid C-cell adenomas was noted for all lixisenatide-treated groups. C-cell carcinomas were observed for mid- and high-dose males and females at a low incidence (1 to 3 per group) compared with no animals from either control group having a carcinoma. However, the increase in C-cell carcinomas was not statistically significant. A slight increase in focal C-cell hyperplasia was observed for all treated groups. The NOEL for thyroid c-cell adenomas was not identified (<40 µg/kg BID). The clinical exposure margin based on the low dose used in this study is <1,028X. No other tumor types were determined to be related to lixisenatide treatment.

Carcinogenicity exposure margins
Because of the development of ADA-mediated drug accumulation after repeated dosing, it is somewhat difficult to calculate an accurate, biologically relevant clinical safety margin. The sponsor has proposed to compare the $AUC_{0\text{-}24h}$ values achieved in

the carcinogenicity studies, in which nearly all animals developed ADAs, to the AUC_{0-24h} values achieved in antibody-positive diabetic subjects. When using this comparison, exposure margins become very large due to ADA production. Although the sponsor has demonstrated that Ab-bound lixisenatide has biological activity in a cell-based assay, it is uncertain how relevant these higher exposures are with respect to GLP-1 receptor saturation in target tissues. Although humans also show drug accumulation due to ADA production at the highest clinical dose of 20 µg, the degree of accumulation is lower than in rats and mice (at the higher dose levels; see table below). Therefore, the very large exposure margins based on the highest dose levels tested in the carcinogenicity studies may represent an overestimation of exposure margins.

Lixisenatide Accumulation due to Anti-Drug Antibodies

Species	Dose Level	AUC_{0-24h} (ng·h/mL)		Accumulation
		Initial Exposure	Exposure after Repeated Dosing	
Mouse	40 µg/kg BID	~28 †	59††	2X
	200 µg/kg BID	124†	1338††	11X
	1000 µg/kg BID	787†	28,350††	36X
Rat	40 µg/kg BID	68.1††	7455††	110X
	200 µg/kg BID	255††	35,550††	139X
	1000 µg/kg BID	843††	39,250††	47X
Human	5 µg QD	0.241*	0.210**	1X
	10 µg QD	0.331*	0.388**	1X
	20 µg QD	0.753*	4.78**	6X

†TK data from Day 1 of a 14-day range-finding study (2003-1952).
††Animal TK data from the mouse and rat carcinogenicity studies.
*Geometric mean of antibody negative subjects from Studies ACT6011 and PDY6797; not used for exposure margin calculations (Table 10, Module 2.7.2).
**Geometric mean of antibody positive subjects from Studies ACT6011 and PDY6797; not used for exposure margin calculations (Table 11, Module 2.7.2).

Carcinogenicity Safety Margins

Species	Dose Level	Clinical Safety Margins Based On:		
		Exposure in Ab+ Humans after Repeated Dosing*	Initial Exposure (Day 1 or Day 4) Vs. Ab- humans†	Dose comparison based on Body Surface Area
Mouse	40 µg/kg BID	8X	11X	19X
	200 µg/kg BID	185X	138X	97X
	1000 µg/kg BID	3,910X	702X	486X
Rat	40 µg/kg BID	1,143X	55X	39X
	200 µg/kg BID	5,434X	198X	195X
	1000 µg/kg BID	6,303X	707X	973X

*Animal TK data taken from Day 176 (mouse) or Day 359 (rats) from each respective carcinogenicity study (CAR0085 and CAR0084, respectively).
†Mouse data for Day 1 TK values are taken from Study DSE 2005-0443 and Study DSE 2003-1952.
Rat data for Day 4 TK values are taken from Study CAR0084.
Arithmetic mean AUC_{0-24} for Ab positive subjects from Study ACT6011 is 7.25 ng·h/mL on Day 28 and arithmetic mean AUC_{0-24} for Ab negative subjects from Study ACT6011 is 1.22 ng·h/mL on Day 28.

Carcinogenicity data comparison to other approved GLP-1 receptor agonists

The GLP-1 receptor agonist class has been divided into two subclasses, short-acting and long-acting, based on the apparent half-life-dependent ability to induce C-cell tumors in rodents. Because short-acting agonists (i.e., Byetta) do not result in a steady-state drug concentration after repeated dosing, it is believed that GLP-1 receptor activation occurs in a pulsatile fashion. In contrast, long-acting peptides (i.e., Victoza) or short-acting peptides administered in a subcutaneous depot (i.e., Bydureon) reach a steady-state concentration allowing continuous activation of the GLP-1 receptor. The difference between continuous versus pulsatile GLP-1 receptor activation is thought to directly influence the risk for a particular drug to induce C-cell tumors in rodents. This difference in apparent risk between these two different subclasses has resulted in different labels for short-acting and long-acting receptor agonists, with only the long-acting agonists having a boxed warning for the risk of thyroid C-cell tumors. For labeling purposes, one needs to consider whether the carcinogenicity and PK profiles of lixisenatide is more consistent with a short-acting or long-acting agonist. The carcinogenicity data and corresponding clinical exposure margins for each of the approved GLP-1 receptor agonists are shown in the table below. Additional comparisons are made for half-life and receptor activation potency.

Safety Exposure Margins Across the GLP-1 Receptor Agonist Class

GLP-1 Receptor Agonist	Carc Dosing Regimen	Clinical Dosing Regimen	Gender	Multiple of Human Exposure at LOEL			Exposure Margins of Carc Study Doses[†]
				C-Cell Carcinoma	C-Cell Adenoma	C-Cell Hyperplasia	
RATS							
Exenatide (Byetta)	QD	BID	M	-	-	22X	5, 22, 130X
			F	-	<5X[#]	22X	
Liraglutide (Victoza)	QD	QD	M	<1X	2X	<1X	0.5, 2, 8X
			F	2X	<1X	2X	
Exenatide QW (Bydureon)	Q2W	QW	M	10X	<2X	<2X	1-2, 10, 25X
			F	25X[#]	<1X	<1X	
Lixisenatide	BID	QD	M	340X[#]	<90X	<90X*	90, 340, 1120X
			F	340X[#]	<90X	<90X*	
MICE							
Exenatide (Byetta)	QD	BID	M	-	-	-	7, 26, 95X
			F	-	-	-	
Liraglutide (Victoza)	QD	QD	M	-	10X	2X	0.2, 2, 10, 45X
			F	45X[#]	10X	2X	
Exenatide QW (Bydureon)	NA	QW	M	NOT TESTED			NA
			F				
Lixisenatide	BID	QD	M	-	1045X	165X	37, 165, 1045X
			F	-	-	1045X	

BID = twice daily dosing; carc = carcinogenicity; F = female; LOEL = lowest observed effect level; M = male; NA = not applicable; QD = once daily dosing; QW = once weekly dosing; Q2W = dosing every other week.

[#]nonstatistically significant numerical increase vs. control

*Slight numerical increase vs. control

[†]Values represent the mean of both genders combined

When comparing the rat carcinogenicity data for exenatide immediate release (IR) versus liraglutide or exenatide once weekly (QW), it is clear that the longer acting agonists induce C-cell carcinomas whereas exenatide IR did not. The longer-acting agonists also induced adenomas in rats at clinically relevant exposures whereas exenatide IR induced a slight numerical, non-statistically significant increase in adenomas in females only. No treatment-related C-cell tumors were observed in exenatide IR treated mice; however, mice treated with liraglutide had increased C-cell adenomas and a slight numerical, non-statistically significant increase in C-cell carcinomas in females.

Lixisenatide treatment induced an increase in C-cell adenomas in rats, but only at exposures that are estimated to be at least 90 times higher than clinical exposures. A very slight, non-statistically significant increase in C-cell carcinomas was observed at an exposure that is approximately 340 times higher than clinical exposures. In mice, a slight, but statistically significant increase in C-cell adenomas was observed in males at an exposure that is approximately 1000 times higher than clinical exposures.

Half-Life Comparison

As noted above the duration of activity after dosing influences the risk for the development of C-cell tumors in rodents. As shown in the table below, the half-life of lixisenatide is more similar to exenatide IR than liraglutide and exenatide QW.

Half-Life Comparison Across the GLP-1 Receptor Agonist Class

Drug	Half-Life		
	Mouse	Rat	Human (Ab-)
Lixisenatide	0.5 - 1 hour	0.5 hour	~3 hours
Exenatide (immediate release)	Not reported	1.5 - 3.6 hours	2.4 hours
Exenatide (once weekly)	Not reported (>1 day)	Not reported (>1 day)	~10 days*
Liraglutide	7 hours	3.6 hours	14 -15 hours

*Approximately 10 weeks after discontinuation of Bydureon, plasma exenatide concentrations generally fall below the minimal detectable concentration of 10 pg/mL (Bydureon package insert).

Potency Comparison

In addition to half-life, the potency at which each compound activates the GLP-1 receptor could also influence the minimum dose level required to induce C-cell tumors. Each of the sponsors for lixisenatide, exenatide, and liraglutide conducted in vitro receptor activation studies using cAMP as a measure. Although each sponsor used a different test system, collectively the results show that both exenatide and lixisenatide have similar potency to GLP-1(7-36) to induce cAMP production, whereas liraglutide showed slightly less potency. It is uncertain why the Sanofi results show that liraglutide

has 100-fold less potency than GLP-1(7-36), but the results seem inconsistent with the data from Novo Nordisk. Based on carcinogenicity data, liraglutide appears to have a greater potency to induce C-cell tumors in rodents compared with both exenatide and lixisenatide even though liraglutide has less potency to induce GLP-1R mediated cAMP production in vitro. Therefore, receptor activation potency alone does not appear to be a major factor for the induction of C-cell tumors in rodents.

Sanofi (lixisenatide) - cAMP production in rat thyroid c-cell line 6-23

Drug	Relative EC_{50} (pM)
GLP-1(7-36)	6.88
Lixisenatide	8.31
Exendin-4	4.49
Liraglutide	715

Amylin - (exenatide, NDA 21,773) - cAMP production in RINm5f rat insulinoma pancreatic cell line (data obtained from Dr. Colerangle's review of NDA 21,773)

Drug	EC_{50} (nM)
GLP-1(7-36)	0.23
Exenatide	0.31

Novo Nordisk (liraglutide, NDA 22,341) - cAMP production in baby hamster kidney cells expressing cloned GLP-1R from mice, rats, rabbits, pigs, monkeys, or humans (table taken from Dr. Parola's review of NDA 22,341)

GLP-1 Receptor Species	cAMP Accumulation EC_{50} (pM)	
	NNC 90-1170	GLP-1
Human	5 - 60	1 - 2
Mouse	20	2
Rat	24	6
Rabbit	15	3
Pig	9	3
Monkey	5	1

Carcinogenicity Conclusions

One issue that impacts the ability to evaluate the carcinogenicity safety margins for lixisenatide is the fact that a NOEL for C-cell adenomas was not established for rats because tumors were induced at all doses tested. However, if lower doses were tested in rats, it is highly likely that slight increases in C-cell adenomas would still be observed, which would be similar to what was seen with exenatide IR. Additionally, exenatide IR was administered once daily even though the clinical dosing regimen is twice daily. In

contrast, lixisenatide was dosed twice daily even though the clinical regimen will be once daily. Because exenatide IR has a short half-life and was only tested once daily and it is known that the duration of receptor activation is a critical component of inducing C-cell tumors, it is likely that the exenatide IR carcinogenicity data underestimate the exposure margins for carcinogenicity. It is hypothesized that if twice daily dosing was used for exenatide IR and lower dose levels of lixisenatide were tested, it seems likely that both drugs would show some degree of C-cell adenoma induction near or slightly above clinically relevant exposures. Therefore, even in the absence of data for lower dose levels in rats, it could be argued that the carcinogenicity profiles of lixisenatide and exenatide IR are quite similar in rats.

The NOEL for adenomas in mice was at approximately 165 times the clinical exposure. Considering that exenatide IR was only tested up to approximately 95 times clinical exposure, the carcinogenicity profiles for lixisenatide and exenatide IR appear very similar in mice.

In addition to similar carcinogenicity profiles, like exenatide IR, lixisenatide also has a relatively short half life when compared with liraglutide and exenatide QW. The receptor activation potency of lixisenatide, exenatide, and GLP-1 (7-36) were similar suggesting that a difference in binding potency would not be responsible for any potential differences in tumor induction.

In conclusion, the half-life and the exposure margins at which C-cell tumors were observed in rats and mice were much more similar between lixisenatide and exenatide IR rather than when lixisenatide is compared to liraglutide or exenatide QW. Accordingly, these data support the placement of lixisenatide into the short-acting agonist subclass, and therefore should have a label that is consistent with other short-acting GLP1R agonists with regard to carcinogenic potential.

Mechanistic Studies Regarding the Human Relevance of C-cell Tumors Observed in Rodents

The sponsor conducted several pharmacology studies to investigate the GLP-1 receptor expression and activity in normal rat and human tissues, human tumors, and two immortal cell lines. Rat thyroid C-cells and follicular cells were isolated by laser capture microdissection and an evaluation of GLP-1 receptor RNA expression was conducted for each cell type using RT-PCR (DIV1353). The results showed that GLP-1 receptor RNA was expressed in rat C-cells (mean CT value of 27.4) and was generally not expressed in follicular cells (mean CT value greater than the threshold limit of 35 cycles for 11/13 samples). No differences in GLP-1 receptor expression were noted between 4-month old rats and 12-month old rats.

GLP-1 receptor expression profiling was conducted on several human tissues using the Transcript Low Density Array technique, although the thyroid gland was not included in this analysis (DIV1422). The results showed that GLP-1 receptor RNA expression was detected at low or very low levels in most peripheral tissues and was undetectable in

aorta, skeletal muscle, and vas deferens. Peripheral expression was highest in the heart and pancreas. In the brain, low expression was observed in the caudate, corpus callosum, nucleus basalis, hypothalamus, nucleus accumbens, globus pallidus, and putamen. Expression was not detected in the thalamus and was very low in all other brain regions that were evaluated.

Four studies were conducted to evaluate GLP-1 receptor RNA expression in human thyroid tissue (DIV1391, DIV1487, DIV1498, and DIV1416). C-cell and follicular cell fractions were identified using calcitonin staining and excised by laser capture microdissection. Due to poor RNA quality, results were only interpretable for two studies, in which thyroid tissue from only nine donors was analyzed (DIV1487 and DIV1416). The results of those studies indicated that GLP-1 receptor expression was marginal in C-cells (mean CT values of 33.6 and 32.1 [2 of 5 samples only], with 35 being the threshold limit) and not detectable in follicular cells. GLP-1 receptor expression was not detected in all but one follicular cell sample.

GLP-1 receptor expression profiling was also conducted on human thyroid samples having C-cell pathology, including tissue with non-neoplastic C-cell hyperplasia, neoplastic C-cell hyperplasia, sporadic C-cell carcinoma, and C-cell carcinoma in the setting of MEN2 (DIV1478). Based on the limited number of samples evaluated in this study, no differences in the expression levels of GLP-1 receptor from samples with C-cell pathology were observed in comparison to the expression levels of GLP-1 receptor from samples without C-cell pathology.

In addition to profiling thyroid C-cell tumors, GLP-1 receptor expression was also measured in 140 diseased tissue samples, including 1 pancreatitis sample and 139 cancer samples derived from kidney (8), pancreas (21), stomach (9), lung (57), thyroid (13; papillary and follicular), and colon (32) (DIV1404). GLP-1 receptor 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 GLP-1 receptor 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). The results of this study indicate that an upregulation of the GLP-1 receptor is not a common occurrence in the human tumorigenic process for the tumor types that were evaluated.

Two medullary thyroid carcinoma cell lines, the rat 6-23 line and the human TT line, were used to measure GLP-1 receptor activity using a ligand-induced cellular cAMP response assay (DIVT007). The results showed that exendin-4, GLP-1(7-36 amide), lixisenatide, and liraglutide induced the production of cAMP in the rat cell line with EC_{50}rel values of 4.49, 6.88, 8.31, and 715 pM, respectively, with all compounds inducing an Emax% greater than 100%. In contrast, in the human cell line, Emax% was not able to be calculated for exendin-4 or lixisenatide and GLP-1(7-36 amide) and liraglutide had Emax% values of 15.4% and 26.0%, respectively. For those two later compounds, the EC_{50}rel values of these reduced responses were 56.0 and 38,600 pM,

respectively. Therefore, the sponsor concluded that the rat cell line was more responsive to GLP-1 receptor agonists than the human cell line. The sponsor did not provide GLP-1 receptor expression data for these two cell lines; however, it was previously reported that the GLP-1 receptor is expressed about 14-fold higher in the rat 6-23 cell line compared with the human TT cell line (Knudsen et al., 2010). Therefore, the assumption is that the human cell line produced less cAMP because there are fewer GLP-1 receptors.

An in vivo study was conducted in GLP-1 receptor knock-out (KO) mice to determine the requirement of the GLP-1 receptor for C-cell effects. Using this model, it was demonstrated that the GLP-1 receptor was required for an initial treatment-related increase in plasma concentrations of calcitonin.

Subcutaneous administration of 1000 µg/kg BID or continuous subcutaneous infusion of 2000 µg/kg/day lixisenatide to CD-1 mice for 3 months did not alter the expression of 7 growth regulatory genes in thyroid cells. RNA expression of GLP-1 receptor and calcitonin in thyroid was increased 4 to 5-fold in males and 2 to 3-fold in females. Immunohistochemical assessment of thyroid tissue using calcitonin and Ki-67 dual staining did not reveal an increase in C-cells that were undergoing replication, although C-cell density was not evaluated.

In a second 3-month subcutaneous infusion study, CD-1 mice received 75 µg/animal/day (~2000 µg/kg/day) lixisenatide or exenatide. 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-1 receptor mRNA transcripts in thyroid cells. No meaningful differences in the expression of cell growth regulatory genes were observed between treated and control groups, which is consistent with an apparent lack of C-cell proliferation based on histopathology.

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/s/

BRIAN T HUMMER
08/22/2013

KAREN L DAVIS BRUNO
08/22/2013
concur with recommendaiton

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/s/

TODD M BOURCIER
04/08/2016
Primary nonclinical review

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

Signed off in DARRTs on 9/17/2015

NDA Number: NDA 208471 **Applicant: Sanofi-Aventis US.** **Stamp Date: 7/27/15**

Drug Name: Lixisenatide **NDA/BLA Type: 505(b)(1)**

Drug product: Lixisenatide is supplied as subcutaneous (SC) solution for injection. The initiation dose of lixisenatide is 10 µg, and maintenance dose is 20 µg to be administered via a pen injector once daily.

Brief history: Lixisenatide is a potent and selective DPP-4 resistant GLP-1 receptor agonist. Initially on 12/20/2012, Sanofi-Avenits submitted an NDA application for lixisenatide (AVE0010) as an adjunct to diet and exercise to improve glycemic control in the treatment of adults with Type 2 diabetes mellitus (T2DM). The sponsor withdrew this NDA application on September 11, 2013 in order to await the complete results of the ELIXA cardiovascular outcomes study rather than have the FDA review the interim data. The ELIXA study has been completed, and the sponsor has now submitted this data in the current application (NDA 208471).

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	Yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	Yes		
4	Are all required and requested IND studies in accord with 505 (b)(1) and (b)(2) including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	Yes		All the required pharmacology/toxicology studies were submitted previously on 12/20/2012, and these have already been reviewed by Dr. Tim Hummer under NDA 204961 and signed off in DARRTs. Applicant has submitted some additional 13-week repeat dose PK studies in rats, mice, dogs and some cell based bioassays to determine the neutralizing effects of anti-drug antibodies for mouse and rat plasma.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			In the pre-NDA meeting (IND 62,724, on 6/2015), we communicated to the sponsor that if there are any changes in formulation from the previous submission dated 12/20/2012, additional nonclinical studies may be required. Note that the acceptability of above submitted PK studies and qualification of excipients /ingredients (if new formulation has been used) will be a review issue.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?			The route of administration is via subcutaneous (SC) injection in toxicology studies, which is the intended route in humans.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	Yes		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?		No	Note that no new Pharmacology/toxicology special studies were requested by us in this revised submission.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate including human dose multiples expressed in either mg/m^2 or comparative serum/plasma levels) and in accordance with 201.57?			Yes, the draft labeling submitted in general is in accordance with 21 CFR 201.57 labeling. Sanofi has submitted the label according to the Pregnancy and Lactation Labeling Rule (PLLR), and it is provided in accordance with content of the January 2006 Physicians Labeling Rule. This new PPLR will be reviewed and if necessary, changes will be recommended.
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)		No	We communicated to the applicant in a pre-NDA meeting (IND 62,724) that if there are any changes in formulation (from the previous submission dated 12/20/2012), additional nonclinical studies may be required.
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			Not applicable

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

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

This NDA application is fileable. Note that all the pharmacology/toxicology studies have already been conducted and reviewed when it was first submitted in 2012. Some new PK studies have been submitted in this revised application, which will be reviewed.

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

From the Pharmacology/toxicology point of view, no additional information is needed at this time.

Reviewing Pharmacologist: Indra Antonipillai

Supervisory Pharmacologist: David Carlson.

File name: 208471-filing

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/s/

INDRA ANTONIPILLAI
09/17/2015
From the pharmacology/toxicology point of view, this application is fileable.

DAVID B CARLSON
09/18/2015