

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

208471Orig1s000

CHEMISTRY REVIEW(S)

Recommendation:

NDA: Approval

NDA 208471 Final OPQ Review

Drug Name/Dosage Form	Lixisenatide/solution for injection
Strength	0.05 mg/ml and 0.1 mg/ml ; 10 mcg, 20 mcg in a 0.2ml dose
Route of Administration	Subcutaneous injection
Rx/OTC Dispensed	Rx
Applicant	Sanofi-Aventis U.S. LLC
US agent, if applicable	N/A

SUBMISSION(S) REVIEWED	DOCUMENT DATE	DISCIPLINE(S) AFFECTED
Original	July 27, 2015	All OPQ Disciplines
Amendment	Dec. 17, 2015	Microbiology

Quality Review Team

DISCIPLINE	REVIEWER	BRANCH/DIVISION
Drug Substance	Joseph Leginus	Branch II /New Drug Product API
Drug Product	Ravindra Kasliwal	Branch VI/New Drug Products II
Process	Yuesheng Ye	Branch VI/Process Assessment
Microbiology	Maria Cruz-Fisher	Branch I/Microbiology Assessment
Facility	Vipulchandra Dholakia	Branch III/Inspectional Assessment
Biopharmaceutics	N/A	Branch II/Biopharmaceutics
Regulatory Business Process Manager	Anika Lalmansingh	Branch I/Regulatory Business Process Management I
Application Technical Lead	Danae Christodoulou	Branch VI /New Drug Products II
Laboratory (OTR)	N/A	N/A
ORA Lead	N/A	N/A
Environmental Assessment (EA)	Joseph Leginus	Reviewed in NDA 204961

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Quality Review Data Sheet

1. RELATED/SUPPORTING DOCUMENTS:

A. DMFs:

DMF #	TYPE	HOLDER	ITEM REFERENCED	STATUS	DATE REVIEW COMPLETED	COMMENTS
(b) (4)	III		(b) (4)	Adequate	Feb. 6, 2003	LoA: 2-11-15
	III		Adequate	Jan. 28, 2011	LoA: 2-20-15	
	III		Adequate	Jan. 25, 2013	LoA: 2-10-15	
	III		Adequate	Sep. 6, 2011	LoA: 2-10-15	
	V		Adequate	Dec. 9, 2015	LoA: 2-15-15	
	V		Adequate	Dec. 28, 2015	LoA: 2-15-15	

B. Other Documents:

DOCUMENT	APPLICATION NUMBER	DESCRIPTION
NDA	204961	Lixisenatide injection

2. CONSULTS:

DISCIPLINE	STATUS	RECOMMENDATION	DATE	REVIEWER
Biostatistics	N/A			
Pharmacology/Toxicology	Adequate	Approval	4-8-16	Todd Bourcier B.T. Hummer (NDA 204961)



QUALITY ASSESSMENT



CDRH-OC	Completed	Inspection of Device Assembly Facility	9-23-15 DARRTS	Christopher Brown
Other	N/A			

Executive Summary

I. Recommendations

A. Recommendation and Conclusion on Approvability

1. The application is recommended for approval for CMC. There are no OPQ pending deficiencies and overall cGMP recommendation for manufacturing facilities is "Approve". The review of the device (pen injector) by CDRH is pending at this time.

B. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable: None

II. Summary of Quality Assessments

The application is submitted as a 505(b)(1). Lixisenatide is a New Molecular Entity (NME). The application was originally submitted as NDA 204961 in 12-20-2012 and CMC and Microbiology reviews by Dr. Leginus and Dr. Jessica Cole recommended approval for CMC and Microbiology. NDA 204961 was withdrawn on 9-10-13 for clinical considerations. Dr. Leginus' s reviews of NDA 204961 in DARRTS, dated 5-31-13, 7-25-13 and 9-13-13 and Dr. Cole's review of microbiology dated 9-4-13 will serve as the primary reviews for NDA 208471. The current OPQ review team of NDA 208471 reviewed updates to NDA 204961, mainly the manufacturing process (b) (4)

is comparable to drug product manufactured by the earlier processes with respect to identity, potency, chemical and microbiological purity. Updated drug substance and drug product specifications are provided in the drug substance and drug product sections in this review of NDA 208471.

A. Drug Substance [lixisenatide] Quality Summary

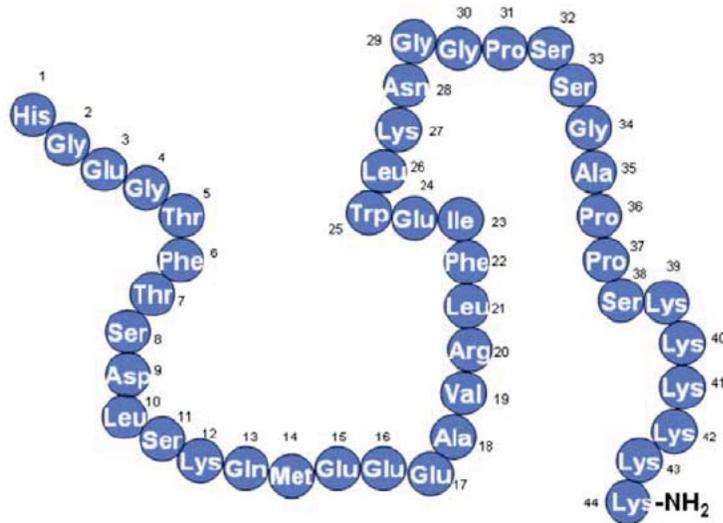
(Structural data and comparisons with exendin-4 and GLP-1 are reproduced from Dr. Joe Leginus' review of NDA 204961 dated 5-31-13.)

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-NH₂
USAN Name: lixisenatide

Molecular Formula: C₂₁₅H₃₄₇N₆₁O₆₅S

Molecular Weight: (b) (4) 4858.5 g/mol (average)

Structural formula:



Lixisenatide, a human glucagon-like peptide-1 (GLP-1) receptor agonist, is a synthetic peptide containing 44 amino acids, which is amidated at the C-terminus amino acid (position 44). The amino acid sequence of lixisenatide is shown above. The structure of lixisenatide is based on that of exendin-4, a hormone found in the saliva of the Gila monster that displays biological properties similar to GLP-1. Lixisenatide has a modified C-terminus with six lysine residues which makes it more able to withstand physiological degradation by dipeptidyl peptidase IV. Amino acid sequences of both exendin-4 and lixisenatide partially overlap that of human GLP-1. Amino acids highlighted in black below show elements of lixisenatide and exendin-4 that differ from human GLP-1. Amino acids highlighted in gray show elements unique to lixisenatide.

GLP-1 (7–37)	H_2N-H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R G $-COOH$
GLP-1 (7–36) amide	H_2N-H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R $-CONH_2$
Exendin-4	H_2N-H G E G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P S $-CONH_2$
Lixisenatide	H_2N-H G E G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P S K K K K K K $-CONH_2$

The manufacturing process for lixisenatide is (b) (4)



The structure of lixisenatide was elucidated by a variety of analytical and spectrophotometric techniques, including N-terminus amino acid analysis and sequencing, (b) (4) and bioactivity.

Specifications for lixisenatide drug substance include appearance, identification by 1) amino acid sequencing, and 2) mass spectrometry, assay (HPLC), chiral purity (amino acid analysis – GC), impurity profile (HPLC), (b) (4) content (HPLC) residual solvents (GC), (b) (4) bioburden and endotoxin. The (b) (4) specification was tightened from (b) (4) % to (b) (4) % in NDA 208741 which improves upon the (b) (4) nature of lixisenatide. Description of analytical methods and validation of these methods is adequate. Batch analyses results, reference standards and container/closure system information is adequate. Based on the Pharmacology/Toxicology review of NDA 204961 by Dr. B.T. Hummer the impurities/degradation products at the proposed limits in the drug substance specifications have been adequately qualified and none of the impurities pose a significant genotoxicity risk.

Based on stability results from 3 commercial production drug substance batches manufactured at the commercial production plant (Sanofi-Aventis Deutschland GmbH, Frankfurt Germany) a retest date of (b) (4) months is granted for the New Molecular Entity lixisenatide when stored (b) (4)

B. Drug Product [lixisenatide injection] Quality Summary

Lixisenatide injection is a sterile, clear, colorless aqueous solution for subcutaneous injection. It is available at dosage strengths of 0.05 mg/mL and 0.1 mg/mL. The compositions of the two strengths (b) (4). The solution contains the excipients 85% glycerol (b) (4), sodium acetate trihydrate (b) (4), methionine (b) (4), metacresol (b) (4) and water for injection. Hydrochloric acid and/or sodium hydroxide may be added to adjust pH to pH 4.5. All excipients are listed in the USP and comply with respective compendial requirements.

The drug product solution is filled into a 3 mL cartridge that is closed with a plunger stopper on one end and a (b) (4) cap on the other end. This cartridge is irreversibly integrated in a disposable pen-injector.

Two disposable pen-injectors are available. The pen-injectors have the same external shape and identical mechanical components. To differentiate the two dosage strengths, the pens have different color, tactile features and label design. The 10 mcg dose (0.2 mL of a 0.05 mg/mL solution) will be supplied in a green pen and the 20 mcg dose (0.2 mL of a 0.1 mg/mL solution) in a (b) (4) pen. Each pen-injector delivers 14 fixed doses.

The manufacturing process of the drug product is typical for this type of dosage form: (b) (4)

Three alternative manufacturing process variants exist, which differ in the (b) (4) initial lixisenatide assay (see diagram in the Process review by Dr. Y. Ye and microbiology assessment in Microbiology review by Dr. M. Cruz-Fisher).

- (b) (4)
- (b) (4)
- (b) (4)

The manufacturing process (b) (4) was implemented for the primary stability batches and clinical trials. For commercial distribution, only drug product manufactured by manufacturing process (b) (4) will be shipped to the United States. Batch analysis and stability data for drug product manufactured by the three process variants show comparability for the drug product.

The proposed release specifications include appearance (visual, clarity and color), lixisenatide identity (HPLC and SEC), metacresol identity and content, lixisenatide and methionine assay (HPLC), individual and total impurities (HPLC), high molecular weight proteins (SEC), volume of injection in container, pH, particulate matter, endotoxin and sterility. The analytical procedures have been adequately described and validated. Batch analysis data from drug products manufactured by the three processes meet specifications. Specification for the impurity “(b) (4)” was updated. In NDA 204961 the limit was < (b) (4) while in this NDA the proposed limit is (b) (4)%. Since the limit is tighter, it is acceptable.

The container closure for both strengths of lixisenatide injection is a clear, colorless 3 ml cartridge (b) (4) closed with a (b) (4) plunger stopper on one side and a (b) (4) cap (b) (4). The cartridge is (b) (4) integrated into a disposable pen-injector which is used to dispense multiple fixed doses of lixisenatide. The pen-injector housing also serves to protect the lixisenatide

injection cartridges from light since lixisenatide was determined to be photosensitive when exposed to intense light.

Review of leachables from the pen injector is performed by the CDRH team.

Although long term stability data from earlier batches are available through 36 months, the applicant re-analyzed data available from all batches and proposed a 24-month expiry for the drug product which is granted for lixisenatide injection 0.1 mg/mL and 0.05 mg/mL strengths when stored at 5°C ± 3°C.

In-use stability: 14 days granted when stored at +30°C.

Due to the findings in the photostability study, labeling will include a statement that the product should be kept in its secondary package with the cap attached during storage and when not in use in order to minimize its exposure to light.

C. Summary of Drug Product Intended Use

Lixisenatide is intended as an adjunct to diet and exercise to achieve glycemic control in patients with type 2 diabetes mellitus. It acts as a glucagon-like peptide-1 (GLP-1) receptor agonist to stimulate insulin release from the pancreatic islets, suppress glucagon secretion, delay gastric emptying and reduce body weight. Lixisenatide has been designed to be resistant to physiological degradation by dipeptidyl peptidase-4 (DPP4), an enzyme responsible for degradation of GLP-1. This is accomplished by synthetic modification of the C-terminus of the peptide with six lysine residues which slows its degradation. The half-life of lixisenatide is 2 – 4 hours, and it is classified as a short-acting GLP-1-receptor agonist. Despite its relatively short half-life, lixisenatide is supplied as a solution to be taken once daily within one hour prior to either the first meal of the day or the evening meal. It is supplied in a reusable disposable pen injector in two strengths: A “Starting Dose” of 10 mcg once daily for 14 days and a “Maintenance Dose” of 20 mcg daily starting on Day 14. Lixisenatide should be injected into the abdomen, thigh region, or outer area of the upper arm.

Proprietary Name of the Drug Product	Proposed (b) (4),,
Non Proprietary Name of the Drug Product	Lixisenatide injection
Non Proprietary Name of the Drug Substance	Lixisenatide (USAN)
Proposed Indication(s) including Intended Patient Population	Adjunct to diet and exercise for glycemic control of patients with Type 2 diabetes mellitus
Duration of Treatment	Chronic as indicated
Maximum Daily Dose	20 mcg
Alternative Methods of Administration	N/A: Subcutaneous injection ONLY

D. Biopharmaceutics Considerations

1. BCS Classification: Not determined
 - Drug Substance:
 - Drug Product:

2. Biowaivers/Biostudies : N/A

- Biowaiver Requests
- PK studies
- IVIVC

E. Novel Approaches: N/A

Any Special Product Quality Labeling Recommendations: Replace the accepted proprietary name for “Lixisenatide”. See Drug Product section labeling review by Dr. R. Kasliwal. Include a statement that the product should be kept in its secondary package (pen injector) with the cap attached during storage and when not in use in order to minimize its exposure to light.

F. Life Cycle Knowledge Information: See Attachment A.

OVERALL ASSESSMENT AND SIGNATURES: EXECUTIVE SUMMARY

Application Technical Lead Signature:

Danae D. Christodoulou -S

Digitally signed by Danae D. Christodoulou -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People,
0.9.2342.19200300.100.1.1=1300132624, cn=Danae D. Christodoulou -S
Date: 2016.04.11 18:00:54 -04'00'

MEMORANDUM

Date: 04-Sep-2013

From: Joseph Leginus, Review Chemist, Branch VII/ONDQA

To: NDA 204961, Lixisenatide Injection

Subject: Response to IR

Background:

The sponsor has submitted an Amendment providing additional responses to the FDA request for information dated 6/5/2013 ("Provide in-use stability results for lixisenatide injection samples (Batch 1F001 for 0.1 mg/mL and batch 1F002 for 0.05 mg/mL) assembled in pen-injectors for samples stored at 12 months under real-time storage conditions that were manufactured using Manufacturing Process (b) (4)). In a 6/27/2013 response for item 9, Sanofi committed to provide additional in-use stability data for the assembled pen injectors once it was available. This data is now available and is provided in this Amendment.

Results:

In-use testing has been performed with the two manufacturing process (b) (4) batches 1F002 (0.05 mg/mL) and 1F001 (0.1 mg/mL) after 24 months storage. When stored at +30°C for 14 days the drug product remains within the acceptance limits. (Total impurities/degradation products increased by (b) (4)% for the 0.1 mg/mL dosage strength and (b) (4)% for the 0.05 mg/mL dosage strength. Assay of lixisenatide decreased by 2.2% of the initial value for the 0.1 mg/mL dosage strength and 2.7% of the initial value for the 0.05 mg/mL dosage strength. The other parameters (HMWP, appearance, clarity and color, pH, content m-cresol, and particulate matter – visible particles) remain practically unchanged).

Conclusion:

The results demonstrate that the change in the process with respect to (b) (4) does not impact the quality of the drug product. In addition, in-use stability has been demonstrated with the drug product in pen injectors using samples at 24 months post manufacture.

Joseph Leginus, PhD
Review Chemist

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

/s/

JOSEPH LEGINUS
09/04/2013



**Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research**

Office of Biotechnology Products
Division of Therapeutic Proteins
Rockville, MD 20852
Tel. 301-827-1790

Memorandum

Date: 05/05/2013

From: Faruk Sheikh, Ph.D., Laboratory of Immunology
Kirshner, Susan, Ph.D., Associate Chief of Lab. of Immunology

Consult: Immunogenicity Consult
For Division of Metabolism and Endocrinology Products

NDA: NDA 204961

Product: Lixisenatide, a Synthetic 44-Amino Acid Peptide including six C-terminal Lys residues.

Indication: Type II Diabetes Mellitus (T2DM, Noninsulin dependent diabetes)

Dose: 10 and 20µg: SC Injection to be taken once daily within one hour prior to (b) (4) the first meal of the day (b) (4) from a prefilled pen.

Sponsor: Sanofi Aventis

Dates for Review Process:

DTP received – 5/5/13

Desired completion date: August 3rd, 2013

Review Team:

Clinical: Suchitra Balakrishnan

Clin/Pharm: Suryanarayana Sista

Quality: Joseph Leginus

RPM: Pooja Dharia

RECOMMENDATION:

The Sponsor submitted the validation package of an analytical procedure for the detection of anti-lixisenatide antibodies in human serum. The considerations made in the validation process are appropriate and consistent with current recommendations. The screening cut-point (SCP) and the confirmatory cut-points (CCP) derived from one hundred human plasma samples obtained from obese individuals were 11.9 and 19.1 respectively, which are good and acceptable for application in clinical sample analysis. Therefore, the validation of anti-lixisenatide antibody screening assay is complete.

Clinical Immunogenicity Findings:

The immunogenicity of lixisenatide in patients with T2DM was assessed in 3 Phase 2 studies (ACT6011, DRI6012, and PDY6797), and 9 Phase 3 studies. In three Phase 2 studies in patients with T2DM, 114 out of 247 subjects (46.2%) were recorded as having anti-lixisenatide antibodies. The duration of Phase III studies was longer (see table 148 at the end of the review). The immunogenicity assessment based on anti-lixisenatide antibody data collected in nine Phase 3 placebo-controlled study pool (EFC6014, EFC6015, EFC6016, EFC6017, EFC6018, EFC10743, EFC10781, EFC10887, and EFC11321) indicated that the percentage of antibody-positive patients in the lixisenatide group increased with time. There were 57.7% (101 out of 175) T2DM patients who tested positive for the presence of anti-lixisenatide antibodies at week 12, reaching 69.6% (1370 out of 1968 patients) after 24 weeks of treatment and 71.5% (913 out of 1277 patients) after 76 weeks of treatment (5.3.5.3 iss [Section 4.3.6.1]). The percent of antibody positive patients slightly decreased or remained the same, at week 100 (70.2%). The Sponsor stated that no clinically relevant impact of antibody status on allergic reactions, incidence of pancreatitis, or thyroid C-cell related AEs was observed (Summary of clinical safety-06/Nov/2012; version number1, page: 237).

The cross-reactivity of the antibodies with endogenous GLP-1 and glucagon was determined in 3 Phase 3 studies (EFC6015, EFC10781, and EFC11321) using the Biacore method and in the Phase 2 Study DRI6012 using radioimmuno-precipitation assay. No cross-reactivity was seen in any patients. Therefore, although the incidence of ADA is high there does not seem to be an impact on clinical safety and efficacy at this time.

PRODUCT BACKGROUND:

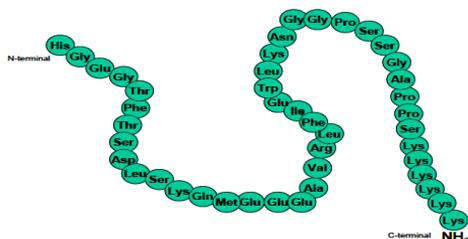
Laboratory code / Product code: AVE0010

International Nonproprietary Name (INN): lixisenatide

Lixisenatide, under development by Sanofi-Aventis, is a novel human glucagon-like peptide-1 receptor (GLP-1R) agonist for the treatment of type 2 diabetes mellitus. The structure of lixisenatide was based on exendin-4 (1-39), which was modified by (b) (4) adding six Lysine residues C-terminally. These modifications enable the product to withstand physiological degradation by dipeptidyl peptidase IV.

Exendin-4, originally isolated from the saliva of a lizard (*Heloderma suspectum*) shares 50% amino acid sequence identity with human GLP-1 (1). Lixisenatide demonstrated affinity and selectivity for the human GLP-1 receptor in preclinical studies, with approximately fourfold higher binding than that of native GLP-1 (2,3,4).

Figure 1 - Structural formula of lixisenatide



Lixisenatide: HGGEGTFTSDL SKQMEEEEAVR LFIEWLKNNG PSSGAPPS (K)₆-NH₂

Exenatide: HGGEGTFTSDL SKQMEEEEAVR LFIEWLKNNG PSSGAPPPS-NH₂

Human GLP-1: HAEGTFTSDV SSYLEGQAAK EFIAWLVKGR-NH₂

Fig: Amino acid sequence of Lixisenatide, Exenatide (exendin-4) and mammalian GLP-1. Font in green color of GLP-1 indicates a sequence overlap with **Exenatide** (5).

From the proposed label, the time to T max is 1 – 3.5 h and the mean terminal half life is ~ 3 h in patients with type 2 diabetes.

Immunogenicity:

The incidence of anti-lixisenatide antibodies ranged from 43% in the 10 µg once daily group to 71% in the 20 µg twice daily group following 13-weeks of treatment with lixisenatide. The antibodies were not characterized further. Because the product is highly immunogenic and has 50% homology to human GLP-1, the anti-drug antibodies may cross-react to the endogenous GLP-1. Therefore, safety and efficacy may be impacted.

STUDY: VALIDATION OF A METHOD FOR THE DETECTION OF ANTI-AVE0010 ANTIBODIES IN HUMAN EDTA PLASMA USING SURFACE PLASMON RESONANCE (SPR) TECHNOLOGY ON BIACORE T200

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1 INTRODUCTION

¹ Triplitt and Chiquette, *J of the Am Pharma Assn*, **46** (2006), pp.44-55.

² Thorkildsen C, Neve S, Larsen BD, Meier E, Petersen JS (2003): Glucagon-like peptide 1 receptor agonist ZP10A increases insulin mRNA expression and prevents diabetic progression in db/db mice. *J Pharmacol Exp Ther.* 307(2):490-6.

³ Christensen M, Knop FK, Holst JJ, Vilsboll T. (2009): Lixisenatide, a novel GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus. *IDrugs*, Aug;12(8):503-13.

⁴ Elkinson S and Keating GM (2013): Lixisenatide: First Global Approval, *Drugs*, 73:383-391.

⁵ Iltz JL, Baker DE, Setter SM, and Campbell RK (2006): Exenatide: An Incretin Mimetic for the Treatment of Type 2 Diabetes Mellitus. *Clinical Therapeutics*, Vol. 28, No. 5, 652-665.

2 MATERIALS AND METHODS

3 ASSAY PROCEDURES

4 RESULTS AND DISCUSSION

- 4.1 SCREENING CUT POINT
- 4.2 CONFIRMATORY CUT POINT
- 4.3 INTER-ASSAY PRECISION AND ACCURACY
- 4.4 ASSAY SENSITIVITY
- 4.5 MATRIX INTERFERENCES
- 4.6 DILUTION EFFECT
- 4.7 DRUG TOLERANCE AND CROSS-REACTIVITY
- 4.8 CROSS-REACTIVITY
- 4.9 STABILITIES

5 IMMUNOGENICITY RESULTS OF PHASE I, II & III STUDIES

1 INTRODUCTION:

AVE0010 or lixisenatide, is an exendin-4 analog for the treatment of type II diabetes. The Sponsor used Surface Plasmon Resonance (SPR) based methods using a Biacore T200 for anti-drug antibody (ADA) binding/ screening assay.

This report outlines the validation of the SPR method using Biacore T200 for the detection and confirmation of anti-AVE0010 antibodies in human K3-EDTA plasma from obese subjects to support clinical development of AVE0010 in this specific population.

Reviewer's Comment: Surface Plasmon Resonance (SPR), an optical-based detection technology has emerged as a powerful technique in the study of molecular binding processes in life science research and development. Although, several technologies exist that are utilized for molecular binding studies, the SPR is gaining popularity for several reasons such as, high sensitivity, label-free detection, real-time monitoring, low volume sample consumption, quantitative evaluation, and determination of kinetic rate constants.

2 MATERIALS AND METHODS

2.1 Reference Antibody:



4. RESULTS AND DISCUSSION:

4.1 SCREENING CUT POINT (SCP)

The Sponsor stated that one hundred (100) individual obese human plasma samples (before treatment with AVE0010 - study TDR11215) were used to determine SCP and confirmatory cut point (CCP) at MRD 1:10. The assay was performed on 6 different occasions over 3 weeks by 3 different analysts, on 2 Biacore T200 leading to 12 runs.

Two sets of individual obese human plasma samples were performed as listed in the following table:

Set	Run	Subject numbers
A	01 - 02 - 07 - 08 - 11 - 12	1 to 50
B	03 - 05 - 06 - 09 - 10 - 13	51 to 100

The CCP was determined according to Shankar et al. (2008) that includes 5% false positive. A floating cut point was used.

Calculations: For a floating cut point, the sponsor stated that all individual samples were first normalized by the mean of NC per Flow Cell, FC_i (i = 1, 2, 3, 4) and per experiment over the 12 runs. The calculation was performed for both binding and enhancement values as follows:

$$\text{Normalized FC}_i = \text{FC}_i \text{ sample} - \text{mean}(\text{FC}_i \text{ INC})$$

Then normalized samples were referenced with the corresponding FCref as follows:

$$\begin{aligned}\text{Referenced FC2}_{\text{sample}} &= \text{Normalized FC2}_{\text{sample}} - \text{Normalized FC1}_{\text{sample}} \\ \text{Referenced FC4}_{\text{sample}} &= \text{Normalized FC4}_{\text{sample}} - \text{Normalized FC3}_{\text{sample}}\end{aligned}$$

Finally, the referenced FC2 or FC4 sample values were used for the statistical analysis.

- **The screening cut point (SCP) derived from the calculation was 11.9.**
- **The confirmatory cut point (CCP) derived from the calculation was 19.1.**

(b) (4)

Reviewer's Comment: The Sponsor stated that 12 out of total 600 measurements were removed from the assay as they were analytically invalid. Individual study data for 600 measurements for FC1, FC2, FC3 and FC4 were provided that I reviewed but did not reproduce in this review. The Sponsor stated that the distribution analyses on both binding and enhancement values had many outliers that did not fit a normal distribution. Therefore, they used a non-parametric approach using 95th percentile from the empirical distribution for the determination of both cut-points. The calculation process indicated that the Sponsor initially calculated the referenced FC using normalized sample values which were then used for the statistical analyses to derive cut points. The cut-point for the screening assay was lower (11.942) than expected for a biological assay. Per FDA draft guidance the sponsor used a suitable number of samples and appropriately incorporated sources of variability into these studies. Therefore, the results are acceptable.

4.2 CONFIRMATORY CUT POINT

The strategy used in this method was the injection of an enhancement reagent directed against common antibody structures after the sample injection. The enhancement reagent contains rabbit anti-human IgG. This step reveals whether the response was

derived from an IgG anti-AVE0010 antibody.

Table 3 –Cut-Points Values

Method	SCP	CCP
Cut-Point NON Parametric (95th percentile)	11.942	19.108

31 mai 2011, 18:34

Reviewer's Comment: *The sponsor used SPR technology to determine confirmatory cut point which is specific for IgG only (used anti-human IgG). This assay format theoretically can detect only IgG classes of immunoglobulins which may obscure the presence of other Ig subtype. It may be okay since samples collected later are expected to be predominantly IgG type.*

The confirmatory cut-point is generally determined based upon a 0.1% false positive rate. The Sponsor used 95th percentile for the calculation of CCP which is 19.1 and seemed very good for this type of biological assay.

4.3 INTER-ASSAY PRECISION AND ACCURACY:

According to the Sponsor the evaluation of precision and accuracy consisted of the analysis of 3 replicates of 2 levels of performance controls (PC2 & PC3) at 0.833, 3.33 and 16.7nM (125ng/mL, 500ng/mL and 2500ng/mL) in 6 runs.

PC2 and PC3 were first prepared at 333nM and 3333nM concentrations in pool of human EDTA plasma diluted at 3 different dilutions to achieve 16.7, 3.33 and 0.833nM concentration at the MRD. Results are presented in the [Table below](#) (reproduced from the original submission).

Table 2 -Inter-assay-precision (nM)

Assay date	File name	Measured Concentrations nM PC2 (333 nM)			Measured Concentrations nM PC3 (3333 nM)		
		Dilution in Pool of plasma			Dilution in Pool of plasma		
		dil 1:20	dil 1:100	dil 1:400	dil 1:200	dil 1:1000	dil 1:4000
	With MRD Nominal concentrations	16.7	3.33	0.833	16.7	3.33	0.833
02-Aug-11	110802_DOH1133_CFCA_BC1_01.blr	12.5	2.50	0.725	11.5	2.20	0.700
		12.5	2.40	0.600	11.5	2.30	0.475
		12.0	2.40	0.700	11.5	2.40	0.700
02-Aug-11	110802_DOH1133_CFCA_BC2_01.blr	11.0	2.10	0.500	10.5	2.00	0.425
		11.0	2.20	0.550	10.5	2.00	0.525
		11.0	2.10	NR	10.5	2.00	0.425
02-Aug-11	110802_DOH1133_CFCA_BC3_01.blr	10.5	2.20	0.675	10.0	1.90	0.400
		11.0	1.90	0.425	10.0	1.90	0.400
		10.5	2.00	0.450	10.0	1.90	0.425
03-Aug-11	110803_DOH1133_CFCA_BC2_02.blr	11.5	2.10	0.450	10.5	2.00	0.500
		11.0	2.20	0.550	10.5	2.00	0.475
		11.5	2.20	0.550	10.5	2.10	0.525
03-Aug-11	110803_DOH1133_CFCA_BC3_02.blr	11.0	2.50	0.600	9.50	1.90	0.500
		11.0	2.50	0.600	9.50	1.70	0.400
		11.0	2.30	0.600	9.50	1.70	0.400
04-Aug-11	110804_DOH1133_CFCA_BC3_03.blr	11.5	2.10	NR	11.5	2.30	0.550
		12.0	2.30	0.575	11.5	2.30	0.550
		12.0	2.30	0.750	11.5	2.30	0.575
	Mean	11.4	2.24	0.581	10.6	2.05	0.497
	SD	0.6	0.18	0.097	0.8	0.21	0.094
	CV%	5.4	7.8	17	7.1	10	19
	%Diff	-32	-33	-30	-37	-38	-40
	Recovery	68	67	70	63	62	60

NR: Not Reported due to bad fitting (evaluation software)

The precision and accuracy were also evaluated at the low limit of the CFCA test (Calibration-free concentration analysis), using the PC1 (6.67nM) and analyzed in 2 replicates in 6 separate runs. The results are provided below.

Table 6 - Overall anti-AVE0010 antibodies Performance Controls summary (nM)

Assay No.	Assay date	File name	Nominal concentration of PC (nM)		
			PC1 6.67	PC2 333	PC3 3333
1	02-Aug-11	110802_DOH1133_CFCA_BC1_01.blr	5.40	250	2300
			6.00	250	2400
2	02-Aug-11	110802_DOH1133_CFCA_BC2_01.blr	4.40	220	2000
			4.80	210	1900
3	02-Aug-11	110802_DOH1133_CFCA_BC3_01.blr	4.60	220	2100
			3.70	210	2000
5	03-Aug-11	110803_DOH1133_CFCA_BC2_02.blr	4.30	230	2200
			4.40	210	2200
6	03-Aug-11	110803_DOH1133_CFCA_BC3_02.blr	4.30	240	2400
			4.00	240	2300
7	04-Aug-11	110804_DOH1133_CFCA_BC3_03.blr	NR	210	2000
			3.70	230	2200
		Mean	4.51	227	2167
		S.D.	0.69	16	167
		CV %	15	6.9	7.7
		n	11	12	12
		min	3.70	210	1900
		max	6.00	250	2400
		%Diff	-32	-32	-35
		Recovery	68	68	65

NR: Not Reported

Reviewer's Comment: The precision (%CV) of the assay was 5.4, 7.8 and 17 at dilutions 1:20, 1:100 and 1:400 respectively for PC2. The precision (%CV) for PC3 was also good. Therefore the assay fulfils the acceptance criteria for intra-assay precision.

The %CV for inter-assay precision of PC1 prepared at 6.67nM measured after dilution at MRD was no more than 15% in human K3-EDTA plasma; therefore, the assay fulfils the acceptance criteria for intra-assay precision. This level of precision is acceptable for the technology used.

The % Diff or the accuracy calculated on PC1, PC2 and PC3 were between -40% and -30% of the nominal concentrations. The recovery was only from 60% to 70% for all PCs. The Sponsor argued that the CFCA method measures only the active concentrations, therefore 100% recovery could not be achieved. Although the issue could be arguable, the recovery was stable in all assays including the assays described below. Therefore, this assay for accuracy was accepted.

4.5 SENSITIVITY

The Sponsor stated that the sensitivity assay consisted of the analysis of 3 replicates of the LLPC, LoPC, MiPC and HiPC in 6 separate runs. The LLPC defined by the lowest concentration for all replicates provided positive signal in the assay (above SCP in human EDTA plasma). The sensitivity, defined as the lowest concentration at which a positive control provides a positive signal was evaluated at 300 ng/mL using the batch SPRAVEDAB-2 of positive control. The results are provided in table below (reproduced from the original).

Table 6 - Sensitivity and screening assay precision evaluated in human EDTA plasma

Assay date	File name	Replicate	Binding (RU Resonance Unit)																											
			Mean binding (NC)		NC			LLPC			LoPC			MiPC			HiPC													
			FC Ref	FC Act	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	
13-May-11	110513_DOH1038_BC2_15.br	1	46.3	32.5	38.6	24.9	-7.7	-7.6	0.1	35.6	56.8	-10.7	24.3	35.0	37.8	110	-8.5	77.5	86.0	38.0	219	-8.3	187	195	37.9	1249	-8.4	1217	1225	
		2			37.7	22.7	-8.6	-9.8	-1.2	35.4	57.2	-10.9	24.7	35.6	37.6	111	-8.7	78.5	87.2	37.4	221	-8.9	189	197	37.5	1267	-8.8	1235	1243	
		3			37.8	24.3	-8.5	-8.2	0.3	35.3	57.4	-11.0	24.9	35.9	37.7	112	-8.6	79.5	88.1	36.9	215	-9.4	183	192	37.0	1254	-9.3	1222	1231	
16-May-11	110516_DOH1038_BC2_16.br	1	57.5	44.1	48.7	34.4	-8.8	-9.7	-0.9	48.2	76.3	-9.3	32.2	41.5	47.5	130	-10.0	85.9	95.9	48.1	247	-9.4	203	212	48.0	1426	-9.5	1382	1391	
		2			47.4	31.7	-10.1	-12.4	-2.3	46.0	74.1	-11.5	30.0	41.5	46.2	128	-11.3	83.9	95.2	46.4	246	-11.1	202	213	46.3	1433	-11.2	1389	1400	
		3			47.5	33.5	-10.0	-10.6	-0.6	47.0	76	-10.5	31.9	42.4	45.6	128	-11.9	83.9	95.8	45.6	246	-11.9	202	214	44.8	1438	-12.7	1394	1407	
17-May-11	110517_DOH1038_BC2_17.br	1	57.2	44.2	50.5	36.1	-6.7	-8.1	-1.4	49.2	79.9	-8.0	35.8	43.7	49.2	136	-8.0	91.9	99.8	49.5	262	-7.7	218	226	50.1	1523	-7.1	1479	1486	
		2			48.0	32.0	-9.2	-12.2	-3.0	47.5	76.6	-9.7	32.5	42.1	47.7	131	-9.5	86.9	96.3	48.1	253	-9.1	209	218	48.5	1483	-8.7	1439	1448	
		3			46.6	32.7	-10.6	-11.5	-0.9	46.1	74.3	-11.1	30.2	41.2	45.9	126	-11.3	81.9	80.1	46.7	244	-10.5	200	210	46.9	1436	-10.3	1392	1402	
18-May-11	110518_DOH1038_BC2_18.br	1	55.8	43.6	47.7	34.6	-8.1	-9.0	-1.0	47.4	77.2	-8.4	33.6	41.9	47.1	131	-8.7	87.4	83.9	46.9	248	-8.9	204	213	47.8	1465	-8.0	1421	1429	
		2			47.3	32.1	-8.5	-11.5	-3.1	46.8	75.9	-9.0	32.3	41.2	46.7	130	-9.1	86.4	83.3	46.8	247	-9.0	203	212	47.4	1459	-8.4	1415	1424	
		3			47.2	33.5	-8.6	-10.1	-1.6	46.9	76.3	-8.9	32.7	41.5	46.9	130	-8.9	86.4	83.1	46.8	248	-9.0	204	213	47.5	1455	-8.3	1411	1420	
19-May-11	110519_DOH1038_BC2_19.br	1	44.3	29.5	37.8	22.5	-6.5	-7.0	-0.4	37.7	56.5	-6.6	27.0	33.7	38.0	102	-6.3	72.5	64.0	37.4	196	-6.9	167	173	39.1	1195	-5.2	1166	1171	
		2			37.0	21.2	-7.3	-8.3	-0.9	36.4	56.1	-7.9	26.6	34.6	36.8	102	-7.5	72.5	65.2	36.7	199	-7.6	170	177	38.3	1222	-6.0	1193	1199	
		3			36.6	21.6	-7.7	-7.9	-0.1	36.0	56.5	-8.3	27.0	35.4	36.1	102	-8.2	72.5	65.9	36.1	201	-8.2	172	180	38.0	1242	-6.3	1213	1219	
20-May-11	110520_DOH1038_BC2_20.br	1	43.9	30.7	37.5	23.3	-6.4	-7.4	-1.0	36.4	59.0	-7.5	28.3	35.8	36.8	106	-7.1	75.3	69.2	36.5	209	-7.4	178	186	37.3	1237	-6.6	1206	1213	
		2			37.2	21.8	-6.7	-8.9	-2.2	36.0	58.4	-7.9	27.7	35.6	36.4	106	-7.5	75.3	69.6	36.3	210	-7.6	179	187	37.0	1250	-6.9	1219	1226	
		3			36.9	22.2	-7.0	-8.5	-1.5	36.0	58.8	-7.9	28.1	36.0	36.3	107	-7.6	76.3	70.7	36.3	211	-7.6	180	188	36.8	1261	-7.1	1230	1237	
		Mean			42.7	28.1	-	-	-1.2	41.7	66.9	-	-	38.6	42.0	118	-	-	82.2	42.0	229	-	-	200	42.6	1350	-	-	1321	
		SD			5.4	5.6	-	-	1.0	5.8	9.8	-	-	3.5	5.2	13	-	-	12.1	5.4	22	-	-	16	5.2	114	-	-	108	
		%CV			13	20	-	-	-80	14	15	-	-	9.1	12	11	-	-	15	13	9.5	-	-	8.0	12	8.4	-	-	8.2	
		Min			36.6	21.2	-	-	-3.1	35.3	56.1	-	-	33.7	36.1	102	-	-	64.0	36.1	196	-	-	173	36.8	1195	-	-	1171	
		Max			50.5	36.1	-	-	0.3	49.2	79.9	-	-	43.7	49.2	136	-	-	99.8	49.5	262	-	-	226	50.1	1523	-	-	1486	
		n			18	18	-	-	18	18	18	-	-	18	18	18	-	-	18	18	18	-	-	18	18	18	18	-	-	18

--: Not Applicable SCP - 11.942

Reviewer's Comment: The sensitivity of the assay is defined by the lowest concentration at which a positive control antibody preparation consistently provides a positive signal in the assay and generally deduced by assaying few serial dilution of the positive

control spanning the assay cut-point. It is expected that the assay result be above the screening cut point at least 95% of the time. However, the Sponsor used LLPC which were above the SCP in human EDTA plasma that allowed confirming the sensitivity at 300ng/mL. Assay sensitivity is not a regulatory requirement but it provides a good sense of the relevance of the assay and it is considered to be within about 500ng/mL for a good assay. Although the assay is slightly lacking in accuracy, the sensitivity is low enough to detect low positive samples. Therefore, this assay is acceptable.

4.5 MATRIX COMPONENT INTERFERENCES

The following assay results were reproduced from the original submission.

Matrix recovery with hemolyzed samples:

Table 10 - Matrix component interferences

Table 10a: Matrix recovery with hemolyzed samples

Assay date	File name	Matrix ID	Binding (RU Resonance Unit)																			
			Mean binding (NC)		NC in plasma				LoPC in plasma				HiPC in plasma									
			FC Ref	FC Act	Status	FC Act	FC Ref:NC	FC Act:NC	Act-Ref	Status	FC Ref	FC Act	FC Ref:NC	FC Act:NC	Act-Ref	Status	FC Ref	FC Act	FC Ref:NC	FC Act:NC	Act-Ref	Status
25-May-11	110525_DOH1038_BC1_23.blr	Normal Pool (Control sample)	46.9	38.9	48.3	40.7	1.4	1.8	0.33	Neg	40.2	111	-6.7	72.1	78.7	Pos	40.3	1246	-6.6	1207	1214	Pos
			-	-	47.6	39.2	0.7	0.3	-0.47	Neg	39.4	111	-7.5	72.1	79.5	Pos	40.4	1232	-6.5	1193	1200	Pos
		Hemolysed Pool	46.9	38.9	46.3	38.1	-0.6	-0.8	-0.27	Neg	38.6	113	-8.3	74.1	82.3	Pos	39.3	1218	-7.6	1179	1187	Pos
			-	-	39.8	29.6	-7.1	-9.3	-2.3	Neg	39.8	109	-7.1	70.1	77.1	Pos	39.5	1208	-7.4	1169	1176	Pos
			-	-	39.1	28.9	-7.8	-10.0	-2.3	Neg	39.0	109	-7.9	70.1	77.9	Pos	39.1	1223	-7.8	1184	1192	Pos
			-	-	39.6	29.3	-7.3	-9.6	-2.4	Neg	39.4	109	-7.5	70.1	77.5	Pos	39.3	1209	-7.6	1170	1178	Pos

Matrix recovery with lipidic content samples:

Table 10b: Matrix recovery with lipidic content samples

Assay date	Lipidic plasma File name	Matrix ID	Binding (RU Resonance Unit)																			
			Mean binding FC (NC)		Lipidic plasma				Lipidic plasma at LoPC Level				Lipidic plasma at HiPC Level									
			FC Ref	FC Act	FC Ref	FC Act	FC Ref:NC	FC Act:NC	Act-Ref	Status	FC Ref	FC Act	FC Ref:NC	FC Act:NC	Act-Ref	Status	FC Ref	FC Act	FC Ref:NC	FC Act:NC	Act-Ref	Status
25-May-11	110525_DOH1038_BC1_23.blr	L1	46.9	38.9	25.9	22.0	-21.0	-16.9	4.0	Neg	27.3	104	-19.6	65.1	84.6	Pos	27.2	1257	-19.7	1218	1238	Pos
		L2	-	-	21.1	18.0	-25.8	-20.9	4.8	Neg	22.7	98.9	-24.2	60.0	84.1	Pos	22.7	1214	-24.2	1175	1199	Pos
		L3	-	-	23.5	18.6	-23.4	-20.3	3.0	Neg	25.3	106	-21.6	67.1	88.6	Pos	24.7	1214	-22.2	1175	1197	Pos
		L4	-	-	38.2	28.8	-8.7	-10.1	-1.5	Neg	39.6	110	-7.3	71.1	78.3	Pos	39.6	1241	-7.3	1202	1209	Pos
		L5	-	-	27.3	25.9	-19.6	-13.0	6.5	Neg	27.3	82.6	-19.6	43.7	63.2	Pos	27.6	1142	-19.3	1103	1122	Pos

Matrix recovery with diabetic type II subject samples:

Table 10c: Matrix recovery with diabetic type II subject samples

Assay date	Diabetic plasma (type II) File name	Matrix ID	Binding (RU Resonance Unit)																			
			Mean binding FC (NC)		Diabetic plasma						Diabetic plasma at LoPC level						Diabetic plasma at HiPC level					
			FC Ref	FC Act	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	Status	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	Status	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	Status
25-May-11	110525_DOH1038_BC1_23.blr	D1	46.9	38.9	30.0	21.8	-16.9	-17.1	-0.3	Neg	31.7	98.9	-15.2	60.0	75.1	Pos	34.1	1135	-12.8	1096	1109	Pos
		D2	-	-	25.8	21.5	-21.1	-17.4	3.6	Neg	26.7	99.4	-20.2	60.5	80.6	Pos	26.9	1200	-20.0	1161	1181	Pos
		D3	-	-	12.5	9.3	-34.4	-29.6	4.7	Neg	15.0	88.9	-31.9	50.0	81.8	Pos	15.3	1197	-31.6	1158	1190	Pos
		D4	-	-	54.1	42.2	7.2	3.3	-4.0	Neg	51.7	103	4.8	64.1	59.2	Pos	52.2	1079	5.3	1040	1035	Pos
		D5	-	-	69.8	46.6	22.9	7.7	-15	Neg	68.3	126	21.4	87.1	65.6	Pos	68.8	1259	21.9	1220	1198	Pos

Reviewer’s Comment: The results provided in table 10a from hemolysed samples on NC, LoPC and HiPC indicated that there was no hemolysed serum effect on PC which could produce false negative results below the SCP.

Similarly, the results provided in Table 10b and 10c from plasma sample containing lipidic substances (Table 10b) and from diabetic type II subjects (Table 10c) on NC, LoPC and HiPC indicated that there was no effect on PC which could produce false negative results below the SCP. These results indicated that the matrix components do not interfere with this SPR assay.

4.6 DILUTION EFFECT:

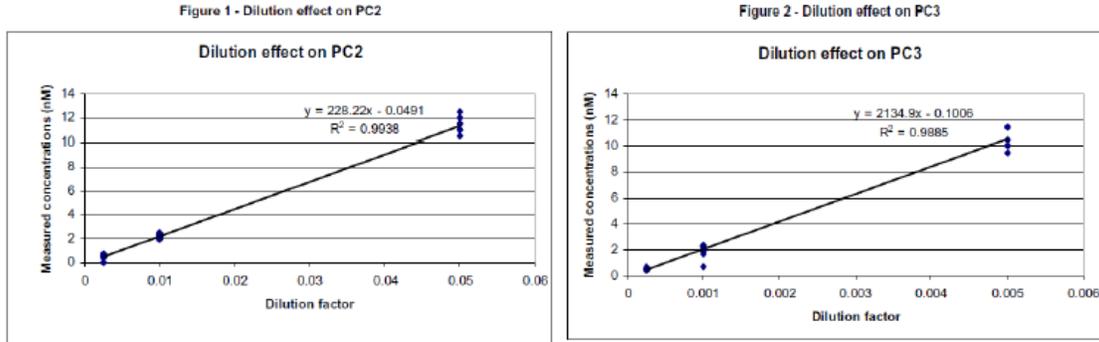
The data indicated that the dilution effect was studied on PC2 and PC3. PC2 spiked at 333nM, and PC3 spiked at 3333nM, diluted 2, 10 and 40-fold with or without MRD in a pool of human K3-EDTA plasma into the quantitation range and analyzed over 6 different runs. The results are shown below.

Table 4 - Dilution effect (nM)

Assay date	File name	Concentrations nM PC2 (333 nM)			Concentrations nM PC3 (3333 nM)		
		Dilution in Pool of plasma			Dilution in Pool of plasma		
		Without MRD dil 1:2	Without MRD dil 1:10	Without MRD dil 1:40	Without MRD dil 1:20	Without MRD dil 1:100	Without MRD dil 1:400
	With MRD dil 1:20	With MRD dil 1:100	With MRD dil 1:400	With MRD dil 1:200	With MRD dil 1:1000	With MRD dil 1:4000	
02-Aug-11	110802_DOH1133_CFCA_BC1_01.blr	250	250	290	2300	2200	2800
		250	240	240	2300	2300	1900
		240	240	280	2300	2400	2800
02-Aug-11	110802_DOH1133_CFCA_BC2_01.blr	220	210	200	2100	2000	1700
		220	220	220	2100	2000	2100
		220	210	NR	2100	2000	1700
02-Aug-11	110802_DOH1133_CFCA_BC3_01.blr	210	220	270	2000	1900	1600
		220	190	170	2000	1900	1600
		210	200	180	2000	1900	1700
03-Aug-11	110803_DOH1133_CFCA_BC2_02.blr	230	210	180	2100	2000	2000
		220	220	220	2100	2000	1900
		230	220	220	2100	2100	2100
03-Aug-11	110803_DOH1133_CFCA_BC3_02.blr	220	250	240	1900	1900	2000
		220	250	240	1900	1700	1600
		220	230	240	1900	1700	1600
04-Aug-11	110804_DOH1133_CFCA_BC3_03.blr	230	210	NR	2300	2300	2200
		240	230	230	2300	2300	2200
		240	230	300	2300	2300	2300
	Mean	227	224	233	2117	2050	1989
	SD	12	18	39	150	209	376
	CV%	5.4	7.8	17	7.1	10	19
	%Diff	-32	-33	-30	-36	-38	-40
	Recovery	68	67	70	64	62	60

NR: Not Reported due to bad fitting (evaluation software)

Reviewer’s Comment: The precision was within acceptance criteria: %CV from 5.4% to 17%. The % Diff or the accuracy was from -33% to -30%. For PC3, the precision (%CV) was within the acceptable criteria (from 7.1% to 19%). As we have seen in previous studies, the accuracy achieved only 60-70% of the nominal values, in this case the % Diff was from -40% to -36%. The linearity of the dilution was highlighted on [Figure 1](#) with $R^2 = 0.994$ for PC2 and on [Figure 2](#) with $R^2 = 0.989$ for PC3 demonstrating the absence of dilution effect from 1:20 to 1:4000. The figures were reproduced from the original.



4.7 DRUG TOLERANCE AND CROSS-REACTIVITY

The Sponsor assessed that anti-AVE0010 antibody detection assay was tolerant to the presence of AVE0010 for up to 200ng/mL at low and high level of positive controls (LoPC and HiPC). The results are provided below (taken from the original).

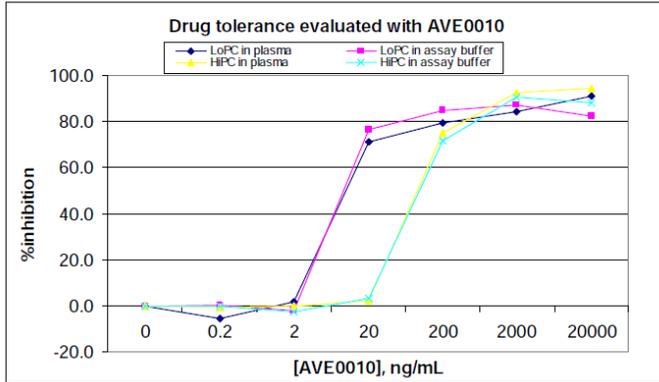
Table 11 - Drug tolerance evaluated with AVE0010

Assay date	File name	Concentrations of product added in PC (ng/mL)	Binding values (RU)																					
			Mean binding (NC)		LoPC in plasma				LoPC in assay buffer				HiPC in plasma				HiPC in assay buffer							
			FC Ref	FC Act	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	% Inhibition	FC Ref	FC Act	Act-Ref	% Inhibition	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	% Inhibition	FC Ref	FC Act	Act-Ref	% Inhibition
14-Jun-11	110614_DOH1038_BC2_29.bir	0 AVE0010	55.0	44.3	30.1	86.3	-24.9	42.0	66.9	0.0	5.1	75.9	70.8	0.0	35.5	1135	-19.5	1091	1110	0.0	6.7	1143	1136	0.0
		0.2 AVE0010	-	-	31.5	91.2	-23.5	46.9	70.4	-5.2	5.2	75.8	70.6	0.28	35.5	1141	-19.5	1097	1116	-0.54	6.6	1146	1139	-0.27
		2 AVE0010	-	-	31.1	86.2	-23.9	41.9	65.8	1.6	5.0	77.1	72.1	-1.8	34.9	1136	-20.1	1092	1112	-0.14	6.4	1173	1167	-2.7
		20 AVE0010	-	-	28.5	36.8	-26.5	-7.5	19.0	72	5.1	21.5	16.4	77	35.9	1111	-19.1	1067	1086	2.2	6.5	1103	1097	3.5
		200 AVE0010	-	-	28.9	31.7	-26.1	-12.6	13.5	80	4.8	15.6	10.8	85	34.5	298	-20.5	254	274	75	7.3	329	322	72
		2000 AVE0010	-	-	30.6	30.4	-24.4	-13.9	10.5	84	5.2	14.0	8.8	88	33.6	103	-21.4	58.7	80	93	6.6	113	106	91
		20 000 AVE0010	-	-	30.7	25.7	-24.3	-18.6	5.7	91	5.3	17.8	12.5	82	32.8	80.4	-22.2	36.1	58	95	6.7	140	133	88

%Inhibition = 100x(1-(signal of spiked samples with AVE0010 / signal of unspiked samples))

Reviewer’s Comment: The % Inhibition for the LoPC was up to 91% in human EDTA plasma and 82% in assay buffer for up to 20 000 ng/mL of AVE0010. The LoPC stayed positive in plasma in the presence of up to 20 ng/mL of AVE0010. The inhibition was even higher with HiPC. The % Inhibition for the HiPC was up to 95% in human EDTA plasma and up to 88% in assay buffer for up to 20 000 ng/mL of AVE0010. The sample remained positive in plasma and in assay buffer for up to 200ng/mL of AVE0010. Using 0, 0.2 and 2ng/mL of AVE00100 did not affect assay results. Therefore, these results demonstrated that the assay is tolerant to AVE0010 for up to 200ng/mL because all PC samples were still detected as positive in presence of these concentrations of AVE0010.

Figure 4 - Drug tolerance evaluated with AVE0010



4.8 CROSS-REACTIVITY

The Sponsor assessed cross-reactivity of the anti-AVE0010 antibody at low and high level of positive controls (LoPC and HiPC) against the GLP-1, Glucagon, recombinant human insulin (rh-insulin) and Lantus up to 20µg/mL. No-crossreactivity was found against GLP-1, Glucagon, rh-insulin and Lantus up to 20µg/mL.

However, cross-reactivity of the anti-AVE0010 antibody was found against exendin-4 and oxidized AVE0010 at low and high levels (LoPC and HiPC). The results are shown below.

a) GLP-1, glucagon, rh-insulin, Lantus

Figure 5 - Cross-reactivity evaluated with GLP-1

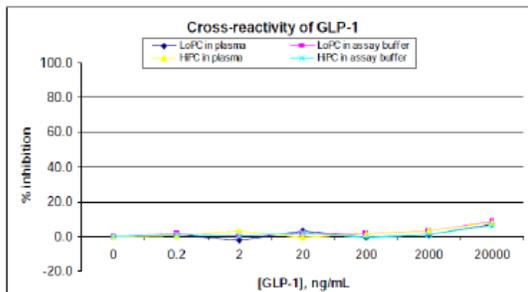


Figure 6 - Cross-reactivity evaluated with Glucagon

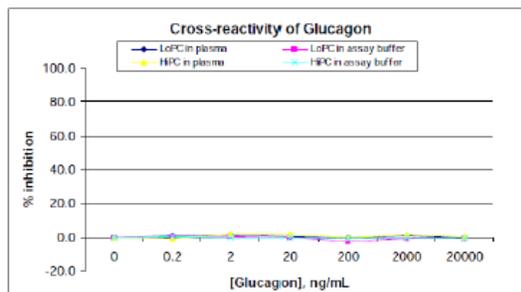


Figure 7 - Cross-reactivity evaluated with rh-insulin

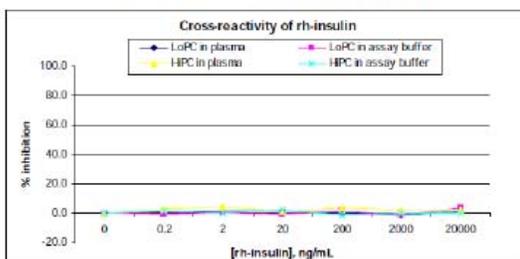


Figure 8 - Cross-reactivity evaluated with Lantus

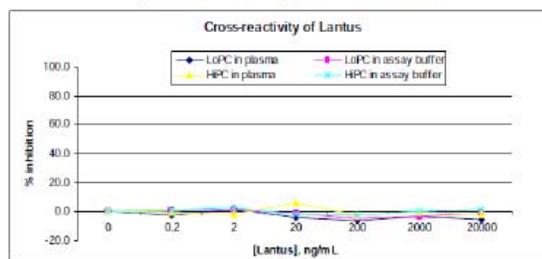


Table 12 - Cross-reactivity

Table 12a: Cross-reactivity evaluated with GLP-1 and Glucagon

Assay date	File name	Concentrations of product added in PC (ng/mL)	Binding (RU)																					
			Mean binding (NC)		LoPC in plasma					LoPC in assay buffer				HiPC in plasma				HiPC in assay buffer						
			FC Ref	FC Act	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	% Inhibition	FC Ref	FC Act	Act-Ref	% Inhibition	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	% Inhibition	FC Ref	FC Act	Act-Ref	% Inhibition
24-May-11	110524_DOH1038_BC1_21.blr	0 GLP-1	45.6	38.6	29.8	85.8	-15.8	47.2	63.0	0.0	5.2	68.4	63.2	0.0	30.9	959	-14.7	920	935	0.0	6.0	966	960	0.0
		0.2 GLP-1	-	-	29.7	85.2	-15.9	46.6	62.5	0.79	5.2	67.4	62.2	1.6	30.6	956	-15.0	917	932	0.29	6.3	956	950	1.1
		2 GLP-1	-	-	30.1	87.4	-15.5	48.8	64.3	-2.1	5.3	68.2	62.9	0.47	30.1	929	-15.5	890	906	3.1	6.3	961	955	0.55
		20 GLP-1	-	-	28.9	83.0	-16.7	44.4	61.1	3.0	5.4	67.6	62.2	1.6	31.0	963	-14.6	924	939	-0.42	6.6	947	940	2.0
		200 GLP-1	-	-	30	86.5	-15.6	47.9	63.5	-0.79	5.5	67.7	62.2	1.6	30.5	942	-15.1	903	918	1.8	6.4	970	964	-0.37
		2000 GLP-1	-	-	29.5	84.8	-16.1	46.2	62.3	1.1	5.3	66.4	61.1	3.3	30.6	931	-15.0	892	907	3.0	7.1	959	952	0.84
		20 000 GLP-1	-	-	30.2	81.8	-15.4	43.2	58.6	7.0	5.5	63.2	57.7	8.7	30.7	884	-14.9	845	860	8.0	6.9	904	897	6.6
24-May-11	110524_DOH1038_BC1_21.blr	0 Glucagon	45.6	38.6	31.1	89.7	-14.5	51.1	65.6	0.0	6.4	71.2	64.8	0.0	31.1	996	-14.5	957	972	0.0	6.9	999	992	0.0
		0.2 Glucagon	-	-	30.7	88.6	-14.9	50.0	64.9	1.1	6.7	71.1	64.4	0.62	31.3	1002	-14.3	963	978	-0.60	6.7	995	988	0.38
		2 Glucagon	-	-	30.6	88.4	-15.0	49.8	64.8	1.2	6.8	71.2	64.4	0.62	30.7	978	-14.9	939	954	1.8	7.0	1000	993	-0.09
		20 Glucagon	-	-	30.6	88.8	-15.0	50.2	65.2	0.61	6.8	71.7	64.9	-0.15	31.3	976	-14.3	937	952	2.1	8.2	1001	993	-0.07
		200 Glucagon	-	-	31.2	89.9	-14.4	51.3	65.7	-0.15	6.9	73.4	66.5	-2.6	30.7	996	-14.9	957	972	-0.04	7.2	1006	999	-0.68
		2000 Glucagon	-	-	30.8	88.8	-14.8	50.2	65.0	0.91	6.7	72.0	65.3	-0.77	30.7	981	-14.9	942	957	1.5	7.0	1001	994	-0.19
		20 000 Glucagon	-	-	30.7	89.5	-14.9	50.9	65.8	-0.30	6.7	71.8	65.1	-0.46	30.8	992	-14.8	953	968	0.4	6.9	1005	998	-0.60

% inhibition = 100x(1-(signal of spiked samples with GLP-1 or Glucagon / signal of unspiked samples))

Table 12 - Cross-reactivity (Continued)

Table 12b: Cross-reactivity evaluated with rh-insulin and Lantus

Assay date	File name	Concentrations of product added in PC (ng/mL)	Binding (RU)																					
			Mean binding (NC)		LoPC in plasma					LoPC in assay buffer				HiPC in plasma				HiPC in assay buffer						
			FC Ref	FC Act	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	% Inhibition	FC Ref	FC Act	Act-Ref	% Inhibition	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	% Inhibition	FC Ref	FC Act	Act-Ref	% Inhibition
24-May-11	110524_DOH1038_BC2_22.blr	0 rh-Insulin	45.5	33.9	29.7	90.4	-15.8	56.5	72.3	0.0	9.8	77.6	67.8	0.0	30.6	1082	-14.9	1048	1063	0.0	9.9	1107	1097	0.0
		0.2 rh-Insulin	-	-	30.7	90.7	-14.8	56.8	71.6	0.97	9.3	77.8	68.5	-1.0	30.1	1051	-15.4	1017	1033	2.9	9.8	1095	1085	1.1
		2 rh-Insulin	-	-	30.3	89.9	-15.2	56.0	71.2	1.5	9.2	77.2	68.0	-0.29	30.2	1036	-15.3	1002	1017	4.3	9.5	1103	1094	0.33
		20 rh-Insulin	-	-	29.9	89.8	-15.6	55.9	71.5	1.1	9.2	77.4	68.2	-0.59	30.2	1065	-15.3	1031	1046	1.6	9.7	1084	1074	2.1
		200 rh-Insulin	-	-	30.2	90.8	-15.3	56.9	72.2	0.14	9.4	76.2	66.8	1.5	30.0	1043	-15.5	1009	1025	3.6	9.5	1119	1110	-1.1
		2000 rh-Insulin	-	-	30.1	91.9	-15.4	58.0	73.4	-1.5	9.1	78.1	69.0	-1.8	29.7	1057	-15.8	1023	1039	2.3	10.0	1107	1097	0.01
		20 000 rh-Insulin	-	-	28.6	88.5	-16.9	54.6	71.5	1.1	9.0	74.2	65.2	3.8	28.7	1058	-16.8	1024	1041	2.1	9.7	1110	1100	-0.29
24-May-11	110524_DOH1038_BC2_22.blr	0 Lantus	45.5	33.9	28.0	84.8	-17.5	50.9	68.4	0.0	9.4	77.7	68.3	0.0	28.6	1052	-16.9	1018	1035	0.0	9.4	1122	1113	0.0
		0.2 Lantus	-	-	29.1	87.9	-16.4	54.0	70.4	-2.9	9.0	76.8	67.8	0.73	29.0	1055	-16.5	1021	1038	-0.25	9.4	1113	1104	0.81
		2 Lantus	-	-	28.6	84.3	-16.9	50.4	67.3	1.6	8.9	76.5	67.6	1.0	29.4	1076	-16.1	1042	1058	-2.2	9.6	1099	1089	2.1
		20 Lantus	-	-	29.2	88.6	-16.3	54.7	71.0	-3.8	9.3	78.3	69.0	-1.0	27.6	989	-17.9	955	973	6.0	10.2	1144	1134	-1.9
		200 Lantus	-	-	29.4	90.3	-16.1	56.4	72.5	-6.0	9.4	80.8	71.4	-4.5	29.5	1078	-16.0	1044	1060	-2.4	9.3	1151	1142	-2.6
		2000 Lantus	-	-	28.6	87.8	-16.9	53.9	70.8	-3.5	9.4	80.1	70.7	-3.5	28.5	1058	-17.0	1024	1041	-0.59	9.5	1116	1107	0.55
		20 000 Lantus	-	-	27.9	88.6	-17.6	54.7	72.3	-5.7	8.9	78.1	69.2	-1.3	27.8	1072	-17.7	1038	1056	-2.0	8.6	1106	1097	1.4

%inhibition = 100x(1-(signal of spiked samples with rh-insulin or Lantus / signal of unspiked samples))

Reviewer's Comment: The results provided demonstrated that no cross-reactivity of the anti-AVE0010 antibodies was observed at low and high levels against GLP-1, glucagon, rh-insulin and Lantus. The % inhibition was no more than 8.0% in human EDTA plasma (see table 12 above) and no more than 8.7% in assay buffer for up to 20 000 ng/mL.

b) Exendin-4, oxidized-AVE0010

Figure 9 - Cross-reactivity evaluated with Exendin-4

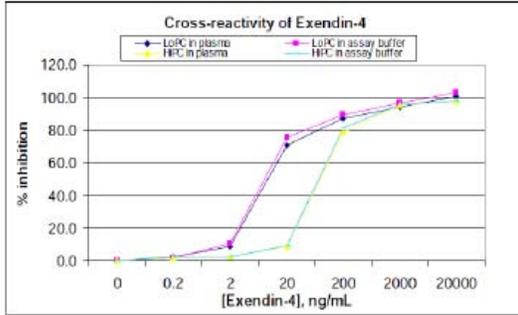


Figure 10 - Cross-reactivity evaluated with Oxidized AVE0010

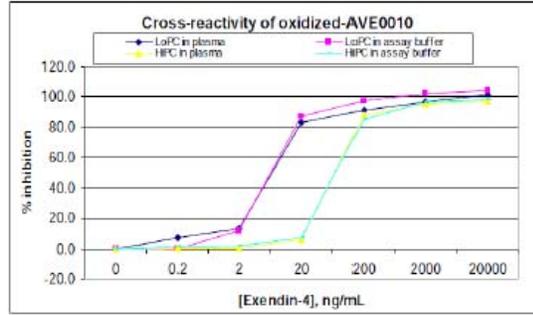


Table 12c: Cross-reactivity evaluated with exendin-4 and oxidized AVE0010

Assay date	File name	Concentrations of product added in PC (ng/mL)	Binding (RU)																					
			Mean binding (NC)		LoPC in plasma				LoPC in assay buffer				HiPC in plasma				HiPC in assay buffer							
			FC	Act	FC	Act	FC Ref-NC	FC Act-NC	Act-Ref	% Inhibition	FC	Act	FC Ref	Act-Ref	FC	Act	FC Ref-NC	FC Act-NC	Act-Ref	% Inhibition	FC	Act	FC Ref	Act-Ref
27-May-11	110527_DOH1038_BC2_26.blr	0 Exendin-4	47.6	37.8	33.4	103	-14.2	65.2	79.4	0.0	9.9	89.6	79.7	0.0	34.6	1246	-13.0	1208	1221	0.0	10.3	1271	1261	0.0
		0.2 Exendin-4	-	-	33.1	101	-14.5	63.2	77.7	2.1	9.7	88.2	78.5	1.5	34.0	1222	-13.6	1184	1198	1.9	10.2	1245	1235	2.1
		2 Exendin-4	-	-	33.4	96.2	-14.2	58.4	72.6	8.6	9.7	80.9	71.2	11	34.0	1219	-13.6	1181	1195	2.2	10.3	1241	1231	2.4
		20 Exendin-4	-	-	33.5	46.9	-14.1	9.1	23.2	71	9.8	29.4	19.6	75	34.1	1139	-13.5	1101	1115	8.7	10.0	1152	1142	9.4
		200 Exendin-4	-	-	33.2	33.7	-14.4	-4.1	10.3	87	9.6	18.0	8.4	89	33.8	279	-13.8	241	255	79	9.90	244	234	81
		2000 Exendin-4	-	-	33.0	28.0	-14.6	-9.8	4.8	94	9.8	12.6	2.8	96	33.8	77.7	-13.8	39.9	53.7	96	10.0	62.2	52.2	96
		20000 Exendin-4	-	-	32.8	22.4	-14.8	-15.4	-0.6	101	9.7	7.3	-2.4	103	33.0	49.0	-14.6	11.2	25.8	98	10.1	35.3	25.2	98
27-May-11	110527_DOH1038_BC2_26.blr	0 Oxidized AVE0010	47.6	37.8	32.2	103	-15.4	65.2	80.6	0.0	9.8	86.2	76.4	0.0	33.2	1185	-14.4	1147	1162	0.0	10.3	1250	1240	0.0
		0.2 Oxidized AVE0010	-	-	34.4	99.6	-13.2	61.8	75.0	7.0	7.9	84.5	76.6	-0.26	33.2	1184	-14.4	1146	1161	0.09	9.90	1236	1226	1.1
		2 Oxidized AVE0010	-	-	32.5	92.4	-15.1	54.6	69.7	14	8.7	75.9	67.2	12	33.2	1179	-14.4	1141	1156	0.52	10.2	1229	1219	1.7
		20 Oxidized AVE0010	-	-	34.0	37.6	-13.6	-0.2	13.4	83	9.8	19.3	9.5	88	33.9	1121	-13.7	1083	1097	5.6	10.2	1157	1147	7.5
		200 Oxidized AVE0010	-	-	33.4	30.3	-14.2	-7.5	6.7	92	10.3	12.0	1.7	98	33.0	163	-14.6	125	140	88	10.0	194	184	85
		2000 Oxidized AVE0010	-	-	33.7	26.7	-13.9	-11.1	2.8	97	10.4	8.7	-1.7	102	32.9	76.0	-14.7	38.2	52.9	95	9.6	48.9	39.3	97
		20000 Oxidized AVE0010	-	-	33.1	22.0	-14.5	-15.8	-1.3	102	10.4	6.9	-3.5	105	33.0	49.4	-14.6	11.6	26.2	98	9.7	31.2	21.5	98

*Inhibition = 100x(1-(signal of spiked samples with exendin-4 or oxidized AVE0010 / signal of unspiked samples))

Reviewer’s Comment: The results provided demonstrated that cross-reactivity of the anti-AVE0010 antibodies was observed at low and high level against exendin-4 and oxidized-AVE0010 with a % inhibition for up to 102% in human EDTA plasma and 105% in assay buffer for up to 20 000 ng/mL (Table 12C). This cross reactivity was expected because AVE0010 was derived from exendin-4 peptide, sharing about 90 or higher percentage of sequence homology.

C. Cross-reactivity by RIP:

4 ANTI-AVE0010 RIP – CROSS-REACTIVITY RESULTS

Test sample: Subject: 1045 Follow-up Dilution: 1:20 with blank plasma

Peptide concentrations for testing: 2, 20, 200, 2000, 20000, 200000 and 2000000 pM

Table 1 Summary of cross-reactivity results

	Cross-reactivity at						Peptide sequence
	IC80	IC50	IC20	IC80	IC50	IC20	
	[pM]	[pM]	[pM]	%	%	%	
AVE0010	20980	3483	578	100	100.0	100.00	HGEGTFTSDLKQMEEEAVRLFIEWLKNGGPSSGAPPSKSKKKKK amide
Peptide 325387	51967	5747	636	40.4	60.6	91.0	FIEWLKNGGPSSGAPPSKSKKKKK amide
Peptide 325388	9.00E+37	1.39E+09	1.18E+07	0.0000	0.0003	0.0049	HGEGTFTSDLKQMEEEA
Peptide 325389	N/AP	N/AP	N/AP	< 0.0001	< 0.0001	< 0.0001	HGEGTFTSDLKQM
Peptide 325390	N/AP	N/AP	N/AP	< 0.0001	< 0.0001	< 0.0001	TSDLKQMEEEA
Exendin-4	48874	6229	794	42.9	55.9	72.8	HGEGTFTSDLKQMEEEAVRLFIEWLKNGGPSSGAPPS amide
GLP-1	N/AP	N/AP	N/AP	< 0.0001	< 0.0001	< 0.0001	HDEFERHAEFTSDVSSYLEGQAAKEFIAWLKGRG

Reviewer’s Comment: Exendin-4 and Peptide 325387 showed high cross-reactivity with AVE0010 on antibodies developed in the investigated subject. Cross-reactivity with Exendin-4 was 55.9% and with the C-terminal sequence Peptide 325387 60.6 % if IC50 values were compared. Very low (0.0003%) cross-reactivity was detected with the N terminal Peptide 325388 of AVE0010, GLP-1, Peptide 325389 and Peptide 325390 do not cross-react with AVE0010 on antibodies developed in the investigated subject.

4.9 STABILITY.

The Sponsor assessed stability of anti-AVE0010 antibody at room temperature, at +4°C and at -20°C/-80°C for three months. The stability was assessed on LoPC and HiPC. The results are provided below.

a) Stability at room temperature and at +4°C up to 72 hours

Table 13 - Evaluation of anti-AVE0010 antibody stability at room temperature up to 72 hours

Assay date	File name	Number of cycles	Binding (RU)											
			Mean binding (NC)		LoPC in plasma					HiPC in plasma				
			FC Ref	FC Act	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref
26-May-11	110526_DOH1038_BC1_25.blr	T0	46.9	38.1	41.1	116	-5.8	77.9	83.7	41.1	1276	-5.8	1238	1244
			-	-	40.7	115	-6.2	76.9	83.1	40.9	1277	-6.0	1239	1245
			-	-	40.8	116	-6.1	77.9	84.0	41.3	1279	-5.6	1241	1247
				Mean	-	-	-	-	83.6	-	-	-	-	1245
				SD	-	-	-	-	0.5	-	-	-	-	1
				%CV	-	-	-	-	0.55	-	-	-	-	0.11
				n	-	-	-	-	3	-	-	-	-	3
		2 h room temperature	46.9	38.1	40.1	115	-6.8	76.9	83.7	39.6	1246	-7.3	1208	1215
			-	-	39.7	114	-7.2	75.9	83.1	39.5	1246	-7.4	1208	1215
			-	-	40.0	115	-6.9	76.9	83.8	39.5	1246	-7.4	1208	1215
				Mean	-	-	-	-	83.5	-	-	-	-	1215
				SD	-	-	-	-	0.4	-	-	-	-	0
		%CV	-	-	-	-	0.45	-	-	-	-	0.00		
		% diff	-	-	-	-	-0.08	-	-	-	-	-2.4		
		n	-	-	-	-	3	-	-	-	-	3		
24 h room temperature	46.9	38.1	37.4	109	-9.5	70.9	80.4	37.9	1239	-9.0	1201	1210		
	-	-	37.2	109	-9.7	70.9	80.6	37.8	1239	-9.1	1201	1210		
	-	-	37.5	109	-9.4	70.9	80.3	38.0	1239	-8.9	1201	1210		
		Mean	-	-	-	-	80.4	-	-	-	-	1210		
		SD	-	-	-	-	0.2	-	-	-	-	0		
		%CV	-	-	-	-	0.19	-	-	-	-	0.01		
		% diff	-	-	-	-	-3.8	-	-	-	-	-2.8		
		n	-	-	-	-	3	-	-	-	-	3		

	48 h room temperature	46.9	38.1	35.6	107	-11.3	68.9	80.2	36.3	1238	-10.6	1200	1211	
		-	-	35.6	108	-11.3	68.9	81.2	36.2	1238	-10.7	1200	1211	
		-	-	35.8	108	-11.1	69.9	81.0	36.3	1241	-10.6	1203	1214	
		Mean	-	-	-	-	-	80.8	-	-	-	-	-	1212
		SD	-	-	-	-	-	0.5	-	-	-	-	-	2
		%CV	-	-	-	-	-	0.65	-	-	-	-	-	0.14
		% diff	-	-	-	-	-	-3.3	-	-	-	-	-	-2.7
		n	-	-	-	-	-	3	-	-	-	-	-	3
		46.9	38.1	33.1	107	-13.8	68.9	82.7	33.0	1237	-13.9	1199	1213	
		-	-	33.0	107	-13.9	68.9	82.8	33.0	1237	-13.9	1199	1213	
		-	-	32.9	107	-14.0	68.9	82.9	33.2	1238	-13.7	1200	1214	
		Mean	-	-	-	-	-	82.8	-	-	-	-	-	1213
SD	-	-	-	-	-	0.1	-	-	-	-	-	0		
%CV	-	-	-	-	-	0.12	-	-	-	-	-	0.04		
% diff	-	-	-	-	-	-0.96	-	-	-	-	-	-2.6		
n	-	-	-	-	-	3	-	-	-	-	-	3		

%Diff calculated in relation to value at T0

Reviewer’s Comment: The result demonstrated that anti-AVE0010 antibody was stable in human EDTA plasma for up to 72 hours at room temperature with a % Diff between -3.8% and -0.08%. The Sponsor also assessed stability of anti-AVE0010 antibody stored at +4°C for 72 hours (data not shown in this review). I reviewed the data. The results demonstrated the stability of ADA in these conditions.

b) Freeze-thawed stability

Table 15 - Evaluation of anti-AVE0010 antibody freeze/thaw stability in human EDTA plasma

Assay date	File name	Number of cycles	Binding (RU)											
			Mean binding (NC)			LoPC in plasma				HIPC in plasma				
			FC Ref	FC Act	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref
26-May-11	110526_D0H1038_BC2_24.tif	T0	47.0	35.1	39.5	120	-7.5	84.9	92.4	39.9	1378	-7.1	1343	1350
			-	-	39.8	119	-7.2	83.9	91.1	40.0	1379	-7.0	1344	1351
			-	-	39.2	119	-7.8	83.9	91.7	39.7	1378	-7.3	1343	1350
		Mean	-	-	-	-	-	91.6	-	-	-	-	-	1350
		SD	-	-	-	-	-	0.7	-	-	-	-	-	0
		%CV	-	-	-	-	-	0.71	-	-	-	-	-	0.03
		n	-	-	-	-	-	3	-	-	-	-	-	3
		C1	47.0	35.1	38.2	116	-8.8	80.9	89.7	38.9	1358	-8.1	1323	1331
			-	-	38.9	117	-8.1	81.9	90.0	38.7	1358	-8.3	1323	1331
			-	-	38.7	117	-8.3	81.9	90.2	38.4	1352	-8.6	1317	1326
		Mean	-	-	-	-	-	90.0	-	-	-	-	-	1329
		SD	-	-	-	-	-	0.3	-	-	-	-	-	3
%CV	-	-	-	-	-	0.28	-	-	-	-	-	0.24		
% diff	-	-	-	-	-	-1.9	-	-	-	-	-	-1.6		
n	-	-	-	-	-	3	-	-	-	-	-	3		
C2	47.0	35.1	38.1	115	-8.9	79.9	88.8	38.3	1357	-8.7	1322	1331		
	-	-	37.7	115	-9.3	79.9	89.2	38.1	1359	-8.9	1324	1333		
	-	-	37.8	115	-9.2	79.9	89.1	38.1	1357	-8.9	1322	1331		
Mean	-	-	-	-	-	89.1	-	-	-	-	-	1331		
SD	-	-	-	-	-	0.2	-	-	-	-	-	1		
%CV	-	-	-	-	-	0.23	-	-	-	-	-	0.09		
% diff	-	-	-	-	-	-2.9	-	-	-	-	-	-1.4		
n	-	-	-	-	-	3	-	-	-	-	-	3		
C3	47.0	35.1	37.7	116	-9.3	80.9	90.2	37.6	1360	-9.4	1325	1334		
	-	-	38.1	116	-8.9	80.9	89.8	37.2	1356	-9.8	1321	1331		
	-	-	37.9	115	-9.1	79.9	89.0	37.0	1357	-10.0	1322	1332		
Mean	-	-	-	-	-	89.7	-	-	-	-	-	1332		
SD	-	-	-	-	-	0.6	-	-	-	-	-	2		
%CV	-	-	-	-	-	0.68	-	-	-	-	-	0.14		
% diff	-	-	-	-	-	-2.3	-	-	-	-	-	-1.3		
n	-	-	-	-	-	3	-	-	-	-	-	3		
		C4	47.0	35.1	37.5	116	-9.5	80.9	90.4	37.4	1381	-9.6	1346	1356
			-	-	37.9	116	-9.1	80.9	90.0	37.9	1384	-9.1	1349	1358
			-	-	37.4	116	-9.6	80.9	90.5	37.8	1382	-9.2	1347	1356
		Mean	-	-	-	-	-	90.3	-	-	-	-	-	1357
		SD	-	-	-	-	-	0.3	-	-	-	-	-	1
		%CV	-	-	-	-	-	0.29	-	-	-	-	-	0.10
		% diff	-	-	-	-	-	-1.6	-	-	-	-	-	0.46
		n	-	-	-	-	-	3	-	-	-	-	-	3
		C5	47.0	35.1	37.3	116	-9.7	80.9	90.6	36.3	1361	-10.7	1326	1337
			-	-	37.0	116	-10.0	80.9	90.9	36.0	1361	-11.0	1326	1337
			-	-	36.8	115	-10.2	79.9	90.1	36.0	1361	-11.0	1326	1337
		Mean	-	-	-	-	-	90.6	-	-	-	-	-	1337
SD	-	-	-	-	-	0.4	-	-	-	-	-	0		
%CV	-	-	-	-	-	0.45	-	-	-	-	-	0.01		
% diff	-	-	-	-	-	-1.3	-	-	-	-	-	-1.0		
n	-	-	-	-	-	3	-	-	-	-	-	3		
C6	47.0	35.1	36.8	115	-10.2	79.9	90.1	37.6	1376	-9.4	1341	1350		
	-	-	37.1	114	-9.9	78.9	88.8	37.5	1377	-9.5	1342	1351		
	-	-	37.1	114	-9.9	78.9	88.8	37.4	1378	-9.6	1343	1353		
Mean	-	-	-	-	-	89.3	-	-	-	-	-	1351		
SD	-	-	-	-	-	0.8	-	-	-	-	-	1		
%CV	-	-	-	-	-	0.84	-	-	-	-	-	0.08		
% diff	-	-	-	-	-	-2.7	-	-	-	-	-	0.08		
n	-	-	-	-	-	3	-	-	-	-	-	3		

%Diff calculated in relation to value at T0

Reviewer's Comment: Freeze (-80°C) / thaw cycles (n=6) did not impact the detection of anti-AVE0010 antibody in human EDTA plasma with % Diff between -2.7% and 0.08%.

c) Stability at -20°C/-80°C

The Sponsor provided 1 month, 2 month and 3 month stability data of anti-AVE0010 antibody in human EDTA plasma at -20°C and also -80°C. I reviewed all data, presented only the stability data of anti-AVE0010 stored at -20°C and -80°C for three months.

Table 16e: 3 month stability data of anti-AVE0010 antibody at -20°C

Assay date	File name	Reference	Binding (RU)													
			Mean binding FC (NC)		LoPC in plasma					HiPC in plasma						
			FC Ref	FC Act	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref		
17-Aug-11	110817_DOH1038_BC3_32.bir	T0	61.9	43.1	48.4	105	-13.5	61.9	75.4	49.8	1209	-12.1	1166	1178		
			-	-	48.4	105	-13.5	61.9	75.4	49.8	1212	-12.1	1169	1181		
			-	-	48.2	105	-13.7	61.9	75.6	49.8	1209	-12.1	1166	1178		
				Mean	-	-	-	-	75.5	-	-	-	-	-	1179	
				SD	-	-	-	-	0.1	-	-	-	-	-	2	
				%CV	-	-	-	-	0.15	-	-	-	-	-	0.15	
				n	-	-	-	-	3	-	-	-	-	-	3	
				3 months at -20°C	61.9	43.1	47.9	111	-14.0	67.9	81.9	49.1	1238	-12.8	1195	1208
				-	-	47.7	111	-14.2	67.9	82.1	49.1	1239	-12.8	1196	1209	
				-	-	47.1	111	-14.8	67.9	82.7	49.3	1240	-12.6	1197	1210	
		Mean	-	-	-	-	82.2	-	-	-	-	-	1209			
		SD	-	-	-	-	0.4	-	-	-	-	-	1			
		%CV	-	-	-	-	0.51	-	-	-	-	-	0.07			
		% diff	-	-	-	-	9.0	-	-	-	-	-	2.5			
		n	-	-	-	-	3	-	-	-	-	-	3			

%Diff calculated in relation to value at T0 (fresh preparation)

Table 16f: 3 month stability data of anti-AVE0010 antibody at -80°C

Assay date	File name	Reference	Binding (RU)													
			Mean binding FC (NC)		LoPC in plasma					HiPC in plasma						
			FC Ref	FC Act	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref	FC Ref	FC Act	FC Ref-NC	FC Act-NC	Act-Ref		
17-Aug-11	110817_DOH1038_BC3_32.bir	T0	61.9	43.1	48.4	105	-13.5	61.9	75.4	49.8	1209	-12.1	1166	1178		
			-	-	48.4	105	-13.5	61.9	75.4	49.8	1212	-12.1	1169	1181		
			-	-	48.2	105	-13.7	61.9	75.6	49.8	1209	-12.1	1166	1178		
				Mean	-	-	-	-	75.5	-	-	-	-	-	1179	
				SD	-	-	-	-	0.1	-	-	-	-	-	2	
				%CV	-	-	-	-	0.15	-	-	-	-	-	0.15	
				n	-	-	-	-	3	-	-	-	-	-	3	
				3 months at -80°C	61.9	43.1	47.6	112	-14.3	68.9	83.2	49.2	1232	-12.7	1189	1202
				-	-	47.5	112	-14.4	68.9	83.3	48.9	1234	-13.0	1191	1204	
				-	-	47.5	112	-14.4	68.9	83.3	48.9	1232	-13.0	1189	1202	
		Mean	-	-	-	-	83.3	-	-	-	-	-	1202			
		SD	-	-	-	-	0.1	-	-	-	-	-	1			
		%CV	-	-	-	-	0.07	-	-	-	-	-	0.10			
		% diff	-	-	-	-	10	-	-	-	-	-	2.0			
		n	-	-	-	-	3	-	-	-	-	-	3			

%Diff calculated in relation to value at T0 (fresh preparation)

Reviewer's Comment: The data demonstrated that anti-AVE0010 antibody was found to be stable after 3 months at -20°C and at -80°C in human EDTA plasma with % Diff between 2.0% and 10%.

IMMUNOGENICITY RESULTS FROM CLINICAL STUDY SAMPLES:

1) *In multiple dose studies, the incidence of antibody formation was comparable between Phase 1 studies in healthy subjects (49.3%) and Phase 2 studies in patients with T2DM (46.2%) (2.7.2 [Section 4.2]).*

2) *In the placebo-controlled Phase 3 studies, which were longer in duration than the Phase 2 studies and included follow-up measurements of antibody status after discontinuation of treatment. The incidence of patients with T2DM who had a positive anti-lixisenatide antibody status increased during treatment with lixisenatide, reaching 69.6% after 24 weeks of treatment and 71.5% after 76 weeks of treatment (5.3.5.3 iss [Section 4.3.6.1]).*

Phase 3 study results:

The immunogenicity assessment was based on anti-lixisenatide antibody data collected in the Phase 3 placebo-controlled study pool (EFC6014, EFC6015, EFC6016, EFC6017, EFC6018, EFC10743, EFC10781, EFC10887, and EFC11321).

Study details:

EFC6015 (n=574): multiple, 28 days (14 days 10ug, 14 days 20ug)

EFC6016 (n=484): antibodies determined at baseline, and at weeks 2, 4, 24, 76, and 100. At baseline, 8 patients (3.3%) treated with lixisenatide and 3 patients (2.3%) treated with placebo were antibody-positive. The percentage increased with time and was 77.2% at Week 100.

EFC6018 (n=239): multiple, 12 wks. At Baseline, a few patients were antibody-positive, with concentrations below the LLOQ. This percentage increased with time, and was 55% to 60% after 12 weeks of lixisenatide treatment.

EFC6019: At Week 24, of those lixisenatide patients with an evaluable anti-lixisenatide antibody status, 30.4% were negative. The concentration was <LLOQ for 885 (67.6%) patients and ≥LLOQ for 424 (32.4%) patients

EFC10887 (n=154): multiple 24 wks.

EFC10743 (n=322): A randomized, double-blind, placebo-controlled, parallel-group, multicenter, 24-week study followed by an extension assessing the efficacy and safety of AVE0010 in 2 titration regimens on top of metformin in patients with type 2 diabetes not adequately controlled with metformin.

EFC10781 (n=223): 24-weeks.

EFC11321 (n=196): 24-weeks

Antibody status summary in Phase 3 studies:

The immunogenicity data from nine phase III studies (by visit) are summarized in the following table.

Table 148 - Number (%) of patients with anti-lixisenatide antibody status by visit in Phase 3 placebo-controlled studies: entire treatment period - safety population

Visit	Antibody Status, n/N1 (%)	Placebo (N=1639)	Lixisenatide (N=2869)
Baseline	Positive	52/1484 (3.5%)	129/2515 (5.1%)
	Negative	1432/1484 (96.5%)	2386/2515 (94.9%)
Week 2	Positive	59/1419 (4.2%)	232/2406 (9.6%)
	Negative	1360/1419 (95.8%)	2174/2406 (90.4%)
Week 4	Positive	67/1409 (4.8%)	879/2354 (37.3%)
	Negative	1342/1409 (95.2%)	1475/2354 (62.7%)
Week 12	Positive	3/93 (3.2%)	101/175 (57.7%)
	Negative	90/93 (96.8%)	74/175 (42.3%)
Week 24	Positive	103/1318 (7.8%)	1370/1968 (69.6%)
	Negative	1215/1318 (92.2%)	598/1968 (30.4%)
Week 76	Positive	29/596 (4.9%)	913/1277 (71.5%)
	Negative	567/596 (95.1%)	364/1277 (28.5%)
Week 100	Positive	3/133 (2.3%)	226/322 (70.2%)
	Negative	130/133 (97.7%)	96/322 (29.8%)

N = the number of patients in the safety population.

N1 = the number of patients with evaluable anti-lixisenatide antibody status in the safety population at the respective visit.

Studies included: EFC6014, EFC6015, EFC6016, EFC6017, EFC6018, EFC10743, EFC10781, EFC10887 and EFC11321.

PGM=PRODOPS/AVE0010/OVERALL/CTD_2012_01/REPORT/PGM/ab_statusbyvisit_t.sas
OUT=REPORT/OUTPUT/i_ab_statusbyvisit_t_i.rtf (10SEP2012 - 23:42)

At Week 24, of those lixisenatide patients, 30.4% were anti-lixisenatide antibody **negative**, and at Week 76, 28.5% were **negative**.

At Week 24, out of the antibody-positive patients, the concentration was <LLOQ for 885 (67.6%) patients and \geq LLOQ for 424 (32.4%) patients.

At Week 76, out of the antibody-positive patients (n=907), the value was <LLOQ for 502 (55.3%) patients and \geq LLOQ for 405 patients (44.7%).

The antibody concentration was available at baseline in the Phase 3 placebo-controlled studies for 65/2869 lixisenatide patients, 90.8% had <LLOQ. The antibody concentration during the study increased with a concentration \geq LLOQ initially over the first 12 weeks, then returned to starting levels by Week 24 (34.9% at Week 2, 57.9% at Week 4, and 32.4% at Week 24) and was 44.7% at Week 76.

In the placebo group, 96.6% of patients were classified as <LLOQ at baseline and the incidence of those patients who had an antibody concentration <LLOQ remained stable up to the end of the treatment; concentration levels of \geq LLOQ fluctuated between 0% and 7% of patients over the entire treatment period.

METHOD USED: Biacore–based competition assay.

Lower limit of quantification (LLOQ) = 3.21nmol/L.

Phase 2 study results:

Multiple dose - patients with T2DM using Radioimmuno-precipitation assay: Phase 2 Studies DRI6012 (n=73/433), PDY6797 (n=22/80), and ACT6011 (n=19/42).

Multiple, QD or BID,

ACT6011: up to 20µg for 28 days Starting dose: 5µg, increased in increments of 2.5µg at 4-day intervals up to 28 days.

DRI6012: Multiple, QD or BID 5, 10, 20, or 30 µg for 13 weeks. For dose levels 20 and 30 µg: Starting dose: 10 µg, to be increased in weekly increments of 5 µg.

PDY6797: Single, 5 or 10 µg and Multiple, QD or BID, up to 30 µg for 6 weeks. Starting dose: 5 or 10 µg to be increased in weekly increments of 5 µg.

Reviewer's Comment: *Three multiple-dose Phase 2 studies in patients with T2DM, 114 out of 247 subjects (46.2%) were recorded as having anti-lixisenatide antibodies.*

Cross-reactivity:

Cross-reactivity of the antibodies with endogenous GLP1 and glucagon was determined in 3 Phase 3 studies (EFC6015, EFC10781, and EFC11321) using Biacore method and in one phase 2 study (DRI6012) using radioimmuno-precipitation assay. The cross-reactivity study data from Study DRI6012 were reviewed.

CSR-EFC6015-14.2.5.4.1-EN

Page 1 of 1

Project Code / Study Number / Analysis: AVE0010 / EFC6015 / CSR_01

14.2.5.4.1 Descriptive statistics for pharmacokinetic data

14.2.5.4.1.8 Number (%) of patients with cross-reactivity at Week 24 during on-treatment period - Safety population

Cross-reactivity	Cross-reactivity Status, n/N1(%)	Lixisenatide (N=574)
Cross-reactivity to endogenous GLP-1	Yes ^(a)	0/209
	No ^(b)	209/209 (100%)
Cross-reactivity to glucagon	Yes ^(a)	0/209
	No ^(b)	209/209 (100%)

^(a) Cross reactivity exists (Yes) [signal suppression \geq 50%].

^(b) Cross reactivity does not exist (No) [signal suppression < 50%].

N – the number of patients in the Safety population. Only patients in the lixisenatide group in the safety population were included.

N1 – the number of patients with positive anti-lixisenatide antibody at Week 24 and having a cross reactivity assessment at Week 24 in the Safety population.

On-treatment period for cross-reactivity and anti-lixisenatide antibody – the time from the first dose of double-blind study medication up to 28 days after the last dose administration.

FCM-PP03095(AVE0010/EFC6015/CSR_01)/REPORT/PGM/cdc_crossreactivity_t.sas OUT-REPORT/OUTPUT/a_cdc_crossreactivity_t.x.rtf (14JUL2011 - 0:46)

Reviewer's Comment: *The Sponsor provided cross-reactivity data from clinical study DRI6012 (week 13, visit 11) on samples who were positive for the presence of anti-lixisenatide antibody. The cross-reactivity testing results are available for 236 subjects with GLP-1 (7-37) and for 229 subjects with Glucagon (1-29). The anti-AVE0010 antibodies in the investigated samples showed no detectable cross-reactivity with the test compounds GLP-1 (7-37) for 236 subjects and with Glucagon (1-29) for 229 subjects. I*

reviewed the cross-reactivity data provided on Phase 2 clinical studies with GLP-1 and Glucagon (data are not included in this review memo). The data provided showed that all calculated depletion rates were lower or negative ((b) (4) PS Study AA43038CH-EB – Bioanalytical Report AAA43038CH-EB Sponsor Reference No.: DRI6012 [AVE0010-2002]) indicating that endogenous GLP-1 (7-37) and Glucagon (1-29) did not cross-react with anti-lixisenatide antibodies in clinical samples.

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/s/

FARUK G SHEIKH
09/13/2013

SUSAN L KIRSHNER
09/13/2013

NDA 204961

**TRADENAME
(lixisenatide injection)**

Sanofi-Aventis LLC

**Joseph Leginus, PhD
Office of New Drug Quality Assessment
Division III, Branch VII**

**For the Division of
Metabolism and Endocrinology Products**

CHEMISTRY REVIEW #2

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Chemistry Review Data Sheet

1. NDA 204961
2. REVIEW #: 2
3. REVIEW DATE: 25-Jul-2013
4. REVIEWER: Joseph Leginus, PhD
5. PREVIOUS DOCUMENTS:

Previous Documents

Original NDA

Document Date

20-Dec-2012

6. SUBMISSION(S) BEING REVIEWED:

Submission(s) Reviewed

NDA Amendment

Document Date

27-Jun-2013

7. NAME & ADDRESS OF APPLICANT:

Name:	Sanofi-Aventis LLC
Address:	55 Corporate Drive, Bridgewater, NJ 08807
Representative:	Ayse Baker, Director
Telephone:	908-981-4799

8. DRUG PRODUCT NAME/CODE/TYPE:

- a) Proprietary Name: N/A
- b) Non-Proprietary Name (INN): Lixisenatide
- c) Code Name/# (ONDC only): Laboratory Code: AVE0010; (CAS): 320367-13-3.
- d) Chem. Type/Submission Priority (ONDC only):
 - Chem. Type: 1
 - Submission Priority: Standard

9. LEGAL BASIS FOR SUBMISSION: This NDA is submitted as a 505(b)(1) application.

Chemistry Review Data Sheet

10. PHARMACOL. CATEGORY:

Lixisenatide is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

11. DOSAGE FORM: Solution for Injection

12. STRENGTH/POTENCY: a) 10 mcg per 0.2 mL of 0.05 mg/mL
b) 20 mcg per 0.2 mL of 0.1 mg/mL

13. ROUTE OF ADMINISTRATION: Subcutaneous Injection

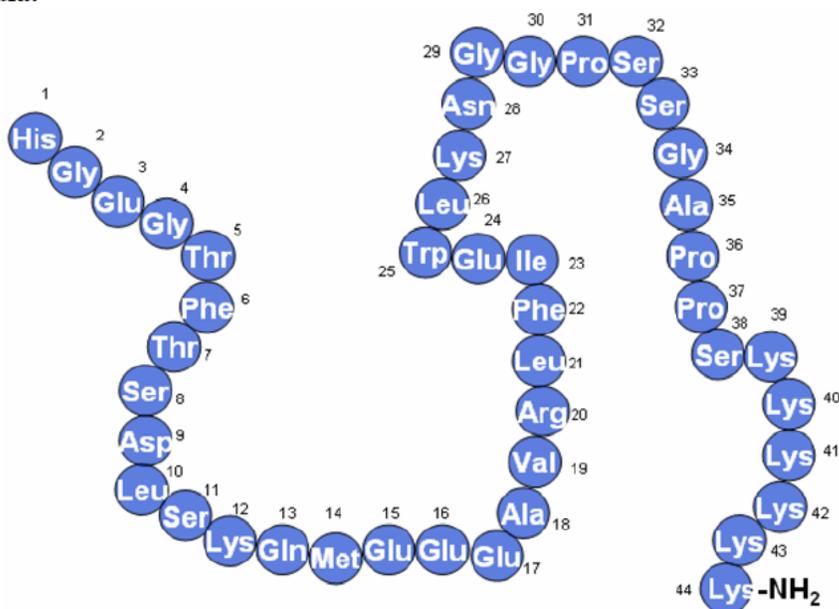
14. Rx/OTC DISPENSED: Rx OTC15. [SPOTS \(SPECIAL PRODUCTS ON-LINE TRACKING SYSTEM\)](#):

SPOTS product – Form Completed
 Not a SPOTS product

16. CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOLECULAR WEIGHT:

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

Structural Formula:



Chemistry Review Data Sheet

Molecular Formula: C₂₁₅H₃₄₇N₆₁O₆₅S

Molecular Weight: (b) (4) 4858.5 g/mol (average).

17. RELATED/SUPPORTING DOCUMENTS:

A. DMFs:

DMF #	Type	Holder	Item Referenced	Code ¹	Status ²	Date Review Completed
(b) (4)	III	(b) (4)	(b) (4)	1	Adequate	6-Feb-2003
	III			1	Adequate	28-Jan-2011
	III			1	Adequate	25-Jan-2013
	III			1	Adequate	6-Sep-2011

¹ Action codes for DMF Table:

1 – DMF Reviewed.

Other codes indicate why the DMF was not reviewed, as follows:

2 – Type 1 DMF

3 – Reviewed previously and no revision since last review

4 – Sufficient information in application

5 – Authority to reference not granted

6 – DMF not available

7 – Other (explain under "Comments")

² Adequate, Inadequate, or N/A (There is enough data in the application, therefore the DMF did not need to be reviewed)

B. Other Documents:

DOCUMENT	APPLICATION NUMBER	DESCRIPTION
IND	62724	Lixisenatide Injection

Chemistry Review Data Sheet

18. STATUS:

ONDC:

CONSULTS/ CMC RELATED REVIEWS	RECOMMENDATION	DATE	REVIEWER
EES	Pending.		
Pharm/Tox	Proposed limits for impurities in drug substance specifications are acceptable.	17-Jul-2013	Tim Hummer
Biopharm	Not applicable. This is an injectable product, and the commercial formulation was used in Phase 3 studies.		
Methods Validation	Not required.		
EA	Categorical exclusion granted by this CMC reviewer. See IR comment 11.	25-Jul-2013	Joseph Leginus
Microbiology	Review of 1) microbiology controls proposed for the drug product, and 2) and (b) (4) processing validation for the drug product.	Pending	Jessica Cole

19. ORDER OF REVIEW: N/A

The Chemistry Review for NDA 204961

The Executive Summary

I. Recommendations

A. Recommendation and Conclusion on Approvability

NDA 204961 is recommended for Approval from the standpoint of chemistry, manufacturing and controls pending acceptable microbiology and cGMP recommendations.

The microbiology review has not yet been finalized in DARRTS.

At this time, the Office of Compliance has not issued an acceptable cGMP recommendation for 1) the drug substance manufacturer/finished dosage manufacturer (Sanofi Aventis GmbH), and 2) the drug substance release tester (b) (4)

B. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable

Not applicable.

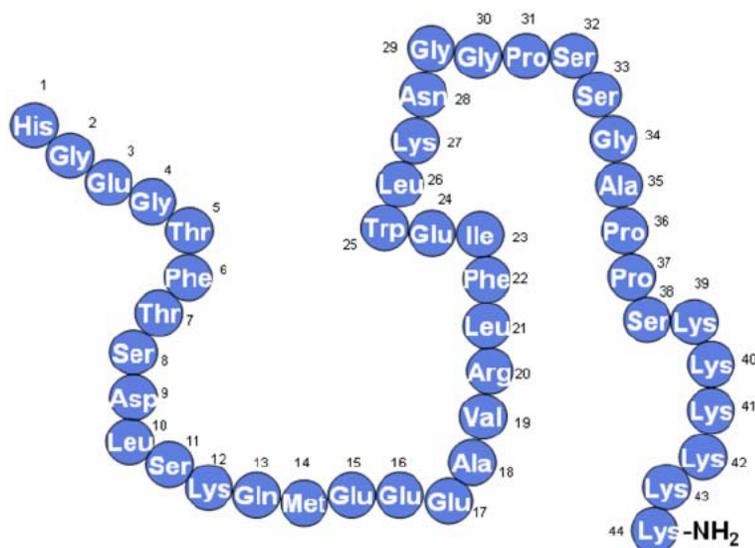
II. Summary of Chemistry Assessments

A. Description of the Drug Product(s) and Drug Substance(s)

DRUG SUBSTANCE

Lixisenatide, a human glucagon-like peptide-1 (GLP-1) receptor agonist, is a synthetic peptide containing 44 amino acids, which is amidated at the C-terminal amino acid (position 44). The amino acid sequence of lixisenatide is shown below.

Executive Summary Section



The structure of lixisenatide is based on that of exendin-4, a hormone found in the saliva of the Gila monster that displays biological properties similar to GLP-1. Lixisenatide has a modified C-terminal with six lysine residues which makes it more able to withstand physiological degradation by dipeptidyl peptidase IV.

The amino acid sequence of lixisenatide is shown alongside that of exendin-4 below.

GLP-1 (7–37)	H_2N-H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R G $-COOH$
GLP-1 (7–36) amide	H_2N-H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R $-CONH_2$
Exendin-4	H_2N-H G E G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P P S $-CONH_2$
Lixisenatide	H_2N-H G E G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P S K K K K K K K $-CONH_2$

Amino acid sequences of both exendin-4 and lixisenatide partially overlap that of human GLP-1. Amino acids highlighted in black above show elements of lixisenatide and exendin-4 that differ from human GLP-1. Amino acids highlighted in gray show elements unique to lixisenatide.

The manufacturing process for lixisenatide is (b) (4)

(b) (4)

(b) (4). The final lixisenatide drug substance is (b) (4) (b) (4) having a molecular formula of $C_{215}H_{347}N_{61}O_{65}S$ and a molecular weight of 4858.5 g/mol.

Executive Summary Section

(b) (4)

The structure of lixisenatide was elucidated by a variety of analytical and spectrophotometric techniques, including N-terminal amino acid analysis and sequencing, amino acid composition analysis, mass spectrometry, peptide mapping, UV, (b) (4) and bioactivity.

Specifications for lixisenatide drug substance include appearance, identification by 1) amino acid sequencing, and 2) mass spectrometry, assay (HPLC), chiral purity (amino acid analysis – GC), impurity profile (HPLC), (b) (4) content (HPLC), residual solvents (GC), (b) (4) bioburden and endotoxin. The applicant has provided data showing a correlation between the lixisenatide cell-based bioassay and assay as determined by HPLC. As a result, assay determination of lixisenatide drug substance by HPLC is sufficient for routine release testing and stability assessment precluding the need for inclusion of a cell-based bioassay as part of the drug substance specifications. Descriptions of analytical methods and validation of these methods are appropriately described and justified. Information on batch analyses, reference standards and container closure system is acceptable. Confirmation was received from Pharmacology/Toxicology that drug substance (and drug product) impurities have been adequately qualified at or above the proposed limits found in the drug substance specifications. The Toxicology review has not yet been entered in DARRTS.

Thirty months of stability data are available on three pilot-scale batches of lixisenatide stored at the proposed long term storage condition of (b) (4) and 6 months at an accelerated condition of (b) (4). Based on these data, a retest period of (b) (4) months at (b) (4) °C is appropriate for the drug substance when stored in the primary packaging.

DRUG PRODUCT

Lixisenatide injection is a sterile, clear, colorless aqueous solution for subcutaneous injection. It is available at dosage strengths of 0.05 mg/mL and 0.1 mg/mL. The compositions of the two formulations are identical with the exception of the amount of the active ingredient. The solution contains the excipients 85% glycerol (b) (4) sodium acetate trihydrate (b) (4) methionine (b) (4), metacresol (b) (4) and water for injection. Hydrochloric acid and/or sodium hydroxide may be added to adjust pH to 4.5. All excipients listed in the USP comply with the respective compendial requirements.

The drug product solution is filled into a 3 mL cartridge that is closed with a plunger stopper on one end and a (b) (4) cap on the other end. This cartridge is irreversibly integrated in a disposable pen-injector.

Two disposable pen-injectors are available. The pen-injectors have the same external shape and identical mechanical components. To differentiate the two dosage strengths,

Executive Summary Section

the pens have different color, tactile features and label design. The 10 µg dose (0.2 mL of a 0.05 mg/mL solution) will be supplied in a green pen and the 20 µg dose (0.2 mL of a 0.1 mg/mL solution) in a (b) (4) pen as shown below.

Concentration per dose (0.2 mL) 10 µg



Concentration per dose (0.2 mL) 20 µg



Each pen-injector delivers 14 fixed doses. A dose counter is not included as a feature on these pens.

The manufacturing process of the drug product is the standard common process for this type of dosage form: (b) (4)

Two alternative manufacturing process variants exist, (b) (4)

- (b) (4)
- (b) (4)

The manufacturing process (b) (4) was implemented for the primary stability batches as well as for clinical trials. For commercial distribution only drug product manufactured according to manufacturing process (b) (4) will be shipped to the United States. Batch analysis and stability data through 12 months indicate that products manufactured using the two process variants are comparable.

The proposed release specifications include appearance (visual, clarity and color), lixisenatide identity (HPLC and SEC), metacresol identity and content, lixisenatide and methionine assay (HPLC), individual and total impurities (HPLC), high molecular weight proteins (SEC), volume of injection in container, pH, particulate matter, endotoxin and sterility. The analytical procedures have been properly described and the proposed regulatory methods have been validated. Batch analysis data from 21 lots show that the drug products meet the specifications proposed.

The container closure for both strengths of lixisenatide injection is a clear, colorless 3 mL cartridge (b) (4) closed with a (b) (4) plunger stopper on one side and a (b) (4) cap (b) (4). The cartridge is (b) (4) integrated into a disposable pen-injector which is used to dispense multiple fixed doses of lixisenatide. The pen-injector housing also serves to protect the lixisenatide injection cartridges from light which is important since lixisenatide was determined to be photosensitive when exposed to intense light.

Executive Summary Section

Results from stability studies of the pen injector show that the drug product at both 0.1 mg/mL and 0.05 mg/mL remain stable through a) 36 months at the long-term storage condition of $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$, and b) 3 months at the accelerated condition of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Based on these data, and following the recommendations outlined in ICH QE1 Evaluation of Stability Data, a shelf-life of ^{(b)(4)} months is granted for lixisenatide injection 0.1 mg/mL and 0.05 mg/mL strengths when stored at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$. This is in agreement with the Applicant's proposed expiry period for the drug product. Due to the findings in the photostability study, labeling will include a statement that the product should be kept in its secondary package with the cap attached during storage and when not in use in order to minimize its exposure to light.

In-use stability studies suggest the quality of lixisenatide injection will not be compromised by conditions anticipated during patient use of the drug product (daily usage of the product for 14 days maintained at room temperature) throughout expiry.

B. Description of How the Drug Product is Intended to be Used

Lixisenatide is intended as an adjunct to diet and exercise to achieve glycemic control in patients with type 2 diabetes mellitus. It acts as a glucagon-like peptide-1 (GLP-1) receptor agonist to stimulate insulin release from the pancreatic islets, suppress glucagon secretion, delay gastric emptying and reduce body weight.

Lixisenatide has been designed to be resistant to physiological degradation by dipeptidyl peptidase-4 (DPP4), an enzyme responsible for degradation of GLP-1. This is accomplished by synthetic modification of the C-terminal of the peptide with six lysine residues which slows its degradation. The half-life of lixisenatide is 2 – 4 hours, and it is classified as a short-acting GLP-1-receptor agonist. Despite its relatively short half-life, lixisenatide is supplied as a solution for subcutaneous injection to be taken once daily within one hour prior to either the first meal of the day or the evening meal. It is supplied in a reusable disposable pen injector in two strengths: A "Starting Dose" of 10 μg once daily for 14 days, and a "Maintenance Dose" of 20 μg once daily starting on Day 14. Lixisenatide should be injected into the abdomen, thigh region, or outer area of the upper arm.

C. Basis for Approvability or Not-Approval Recommendation

This is a 505(b)(1) application where the drug substance, lixisenatide, is a New Molecular Entity (NME). IND 62724 for lixisenatide injection was received on 6/8/2001. A pre-NDA meeting was held on 11/28/2012. The original NDA was submitted on 12/20/2012.

All items in the List of Deficiencies from Chemistry Review #1 have been satisfactorily addressed in the 27-Jun-2013 amendment to the original NDA. See Chemistry Assessment section below for details.

Executive Summary Section

Lixisenatide drug substance has been adequately characterized and manufactured reproducibly. The two manufacturing processes for the drug product are equivalent. Process (b) (4) will be the commercial process. The drug substance and product are supported by a sufficient body of stability data from pilot and production scale batches.

Confirmation was received from Pharmacology/Toxicology that drug substance and drug product impurities have been adequately qualified at or above the proposed limits found in the drug substance and drug product specifications, respectively.

At this time, inspections of all manufacturing and testing facilities have not been completed. As a result, an overall recommendation has not yet been received from the Office of Compliance.

The drug substance will be manufactured for commercial use by Sanofi-Aventis Deutschland GmbH located in Frankfurt, Germany. The drug product, lixisenatide injection 0.1 mg/mL and 0.05 mg/mL, will be manufactured as a sterile, aqueous solution intended for delivery of 0.2 mL by subcutaneous injection. The drug product will be available in two multi-use, disposable pen injectors for delivery of 20 mcg and 10 mcg doses. The drug product will be manufactured and assembled at the same location as was used in the manufacture of the drug substance (Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany).

The final recommendation from the microbiology product quality standpoint is pending.

III. Administrative

A. Reviewer's Signature: in DARRTS

B. Endorsement Block: in DARRTS

C. CC Block: in DARRTS

9 Page(s) have been Withheld in Full as b4 (CCI/TS) immediately following this page

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

/s/

JOSEPH LEGINUS
07/25/2013

DANAE D CHRISTODOULOU
07/25/2013

I concur with the reviewer's conclusions and recommendations

NDA 204961

**TRADENAME
(lixisenatide injection)**

Sanofi-Aventis LLC

**Joseph Leginus, PhD
Office of New Drug Quality Assessment
Division III, Branch VII**

**For the Division of
Metabolism and Endocrinology Products**

CHEMISTRY REVIEW #1

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Chemistry Review Data Sheet

1. NDA 204961
2. REVIEW #: 1
3. REVIEW DATE: 31-May-2013
4. REVIEWER: Joseph Leginus, PhD
5. PREVIOUS DOCUMENTS:

Previous Documents

N/A

Document Date

6. SUBMISSION(S) BEING REVIEWED:

Submission(s) Reviewed

Original NDA

Document Date

20-Dec-2012

7. NAME & ADDRESS OF APPLICANT:

Name:	Sanofi-Aventis LLC
Address:	55 Corporate Drive, Bridgewater, NJ 08807
Representative:	Ayse Baker, Director
Telephone:	908-981-4799

8. DRUG PRODUCT NAME/CODE/TYPE:

- a) Proprietary Name: N/A
- b) Non-Proprietary Name (INN): Lixisenatide
- c) Code Name/# (ONDC only): Laboratory Code: AVE0010; (CAS): 320367-13-3.
- d) Chem. Type/Submission Priority (ONDC only):
 - Chem. Type: 1
 - Submission Priority: Standard

9. LEGAL BASIS FOR SUBMISSION: This NDA is submitted as a 505(b)(1) application.

Chemistry Review Data Sheet

10. PHARMACOL. CATEGORY:

Lixisenatide is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

11. DOSAGE FORM: Solution for Injection

12. STRENGTH/POTENCY: a) 10 mcg per 0.2 mL of 0.05 mg/mL
b) 20 mcg per 0.2 mL of 0.1 mg/mL

13. ROUTE OF ADMINISTRATION: Subcutaneous Injection

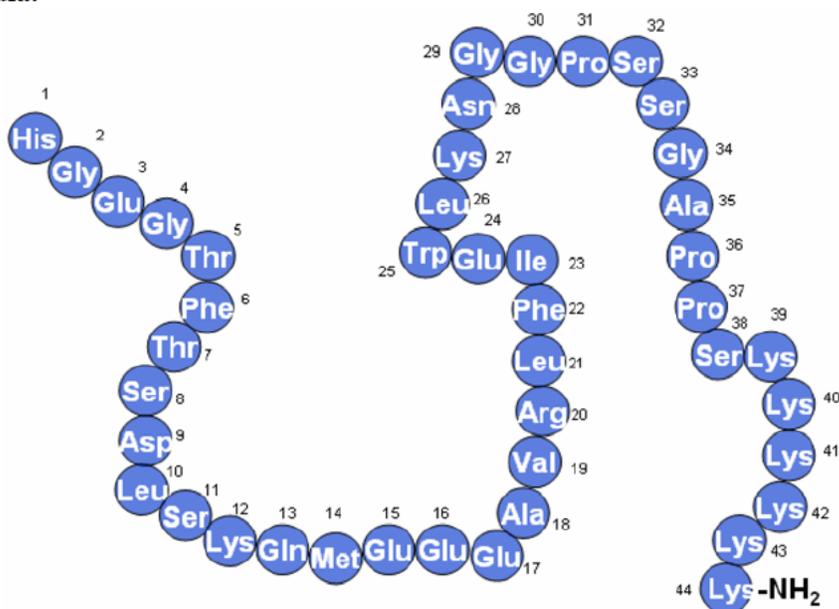
14. Rx/OTC DISPENSED: Rx OTC15. [SPOTS \(SPECIAL PRODUCTS ON-LINE TRACKING SYSTEM\)](#):

SPOTS product – Form Completed
 Not a SPOTS product

16. CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOLECULAR WEIGHT:

Chemical Name: H-His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-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

Structural Formula:



Chemistry Review Data Sheet

Molecular Formula: C₂₁₅H₃₄₇N₆₁O₆₅S

Molecular Weight: (b) (4) 4858.5 g/mol (average).

17. RELATED/SUPPORTING DOCUMENTS:

A. DMFs:

DMF #	Type	Holder	Item Referenced	Code ¹	Status ²	Date Review Completed
(b) (4)	III	(b) (4)	(b) (4)	1	Adequate	6-Feb-2003
	III			1	Adequate	28-Jan-2011
	III			1	Adequate	25-Jan-2013
	III			1	Adequate	6-Sep-2011

¹ Action codes for DMF Table:

1 – DMF Reviewed.

Other codes indicate why the DMF was not reviewed, as follows:

2 – Type 1 DMF

3 – Reviewed previously and no revision since last review

4 – Sufficient information in application

5 – Authority to reference not granted

6 – DMF not available

7 – Other (explain under "Comments")

² Adequate, Inadequate, or N/A (There is enough data in the application, therefore the DMF did not need to be reviewed)

B. Other Documents:

DOCUMENT	APPLICATION NUMBER	DESCRIPTION
IND	62724	Lixisenatide Injection

18. STATUS:

ONDC:

CONSULTS/ CMC RELATED REVIEWS	RECOMMENDATION	DATE	REVIEWER
EES	Pending.		
Pharm/Tox	A request for the safety evaluation of impurities was made.	Pending	Tim Hummer

Chemistry Review Data Sheet

Biopharm	Not applicable. This is an injectable product, and the commercial formulation was used in Phase 3 studies.		
Methods Validation	Validation may be requested of FDA labs after test methods are finalized.		
EA	Conducted by CMC reviewer. See IR comment 11.	Pending	Joseph Leginus
Microbiology	Review of 1) microbiology controls proposed for the drug product, and 2) and ^{(b) (4)} processing validation for the drug product.	Pending	Jessica Cole

19. ORDER OF REVIEW: N/A

The Chemistry Review for NDA 204961

The Executive Summary

I. Recommendations

A. Recommendation and Conclusion on Approvability

The recommendation from a CMC perspective is pending a) satisfactory responses to the deficiencies identified in Review #1, and b) confirmation from Pharmacology/Toxicology that drug substance impurities have been adequately qualified at or above the proposed limits found in the drug substance specifications.

At this time, the Office of Compliance has not issued an acceptable cGMP recommendation for 1) the drug substance manufacturer/finished dosage manufacturer (Sanofi Aventis GmbH), and 2) the drug substance release tester (b) (4). An Overall Compliance recommendation is pending as of 9-May-2013. In addition, an assessment of the acceptability of microbiology information regarding sterility assurance of the drug product has not been provided.

B. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable

Not applicable.

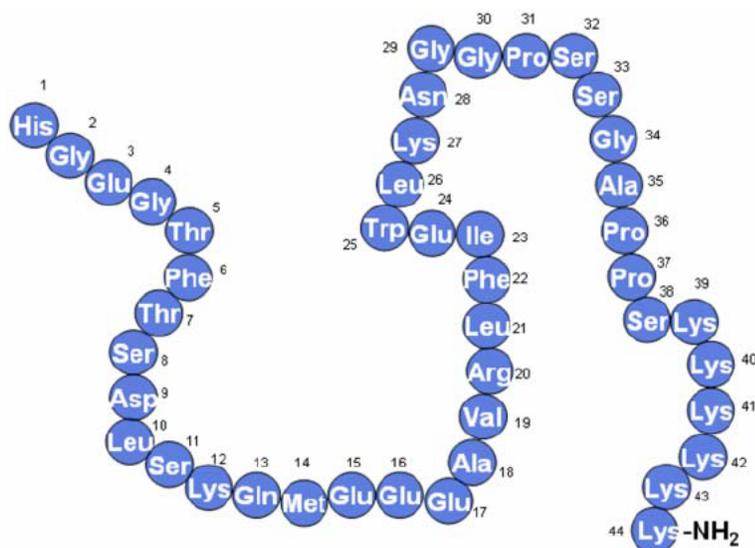
II. Summary of Chemistry Assessments

A. Description of the Drug Product(s) and Drug Substance(s)

DRUG SUBSTANCE

Lixisenatide, a human glucagon-like peptide-1 (GLP-1) receptor agonist, is a synthetic peptide containing 44 amino acids, which is amidated at the C-terminal amino acid (position 44). The amino acid sequence of lixisenatide is shown below.

Executive Summary Section



The structure of lixisenatide is based on that of exendin-4, a hormone found in the saliva of the Gila monster that displays biological properties similar to GLP-1. Lixisenatide has a modified C-terminal with six lysine residues which makes it more able to withstand physiological degradation by dipeptidyl peptidase IV.

The amino acid sequence of lixisenatide is shown alongside that of exendin-4 below.

GLP-1 (7–37)	H_2N-H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R G $-COOH$
GLP-1 (7–36) amide	H_2N-H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R $-CONH_2$
Exendin-4	H_2N-H G E G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P P S $-CONH_2$
Lixisenatide	H_2N-H G E G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P S K K K K K K $-CONH_2$

Amino acid sequences of both exendin-4 and lixisenatide partially overlap that of human GLP-1. Amino acids highlighted in black above show elements of lixisenatide and exendin-4 that differ from human GLP-1. Amino acids highlighted in gray show elements unique to lixisenatide.

The manufacturing process for lixisenatide is (b) (4)
 (b) (4)
 (b) (4)
 (b) (4) having a molecular formula of $C_{215}H_{347}N_{61}O_{65}S$ and a molecular weight of 4858.5 g/mol.

Executive Summary Section

(b) (4)

The structure of lixisenatide was elucidated by a variety of analytical and spectrophotometric techniques, including N-terminal amino acid analysis and sequencing, amino acid composition analysis, mass spectrometry, peptide mapping, UV, (b) (4) and bioactivity.

Specifications for lixisenatide drug substance include appearance, identification by 1) amino acid sequencing, and 2) mass spectrometry, assay (HPLC), chiral purity (amino acid analysis – GC), impurity profile (HPLC), (b) (4) content (HPLC), residual solvents (GC), (b) (4), bioburden and endotoxin. Descriptions of analytical methods and validation of these methods are appropriately described and justified. Information on batch analyses, reference standards and container closure system is acceptable. Input from Pharmacology/Toxicology reviewer, T. Hummer, was requested regarding the adequacy of non-clinical studies for qualifying the process impurities/degradation products at the proposed limits found in the drug substance specifications. Additional comment was requested on the applicant's conclusion that none of the impurities found in the drug substance pose a significant genotoxicity risk.

Thirty months of stability data are available on three pilot-scale batches of lixisenatide stored at the proposed (b) (4). Based on these data, a retest period of (b) (4) months at (b) (4) °C is appropriate for the drug substance when stored in the primary packaging.

DRUG PRODUCT

Lixisenatide injection is a sterile, clear, colorless aqueous solution for subcutaneous injection. It is available at dosage strengths of 0.05 mg/mL and 0.1 mg/mL. The compositions of the two formulations are identical with the exception of the amount of the active ingredient. The solution contains the excipients 85% glycerol (b) (4), sodium acetate trihydrate (b) (4), methionine (b) (4), metacresol (b) (4) and water for injection. Hydrochloric acid and/or sodium hydroxide may be added to adjust pH to pH 4.5. All excipients listed in the USP comply with the respective compendial requirements.

The drug product solution is filled into a 3 mL cartridge that is closed with a plunger stopper on one end and a (b) (4) cap on the other end. This cartridge is irreversibly integrated in a disposable pen-injector.

Two disposable pen-injectors are available. The pen-injectors have the same external shape and identical mechanical components. To differentiate the two dosage strengths, the pens have different color, tactile features and label design. The 10 µg dose (0.2 mL of a 0.05 mg/mL solution) will be supplied in a green pen and the 20 µg dose (0.2 mL of a 0.1 mg/mL solution) in a (b) (4) pen. Each pen-injector delivers 14 fixed doses.

Executive Summary Section

The manufacturing process of the drug product is the standard common process for this type of dosage form: (b) (4)

Two alternative manufacturing process variants exist, (b) (4)

- (b) (4)
- (b) (4)

The manufacturing process (b) (4) was implemented for the primary stability batches as well as for clinical trials. For commercial distribution only drug product manufactured according to manufacturing process (b) (4) will be shipped to the United States. Batch analysis and stability data through 12 months indicate that products manufactured using the two process variants are comparable.

The proposed release specifications include appearance (visual, clarity and color), lixisenatide identity (HPLC and SEC), metacresol identity and content, lixisenatide and methionine assay (HPLC), individual and total impurities (HPLC), high molecular weight proteins (SEC), volume of injection in container, pH, particulate matter, endotoxin and sterility. The analytical procedures have been properly described and the proposed regulatory methods have been validated. Batch analysis data from 21 lots show that the drug products meet the specifications proposed.

The container closure for both strengths of lixisenatide injection is a clear, colorless 3 mL cartridge (b) (4) closed with a (b) (4) plunger stopper on one side and a (b) (4) cap (b) (4). The cartridge is (b) (4) integrated into a disposable pen-injector which is used to dispense multiple fixed doses of lixisenatide. The pen-injector housing also serves to protect the lixisenatide injection cartridges from light which is important since lixisenatide was determined to be photosensitive when exposed to intense light.

Results from stability studies show that the drug product at both 0.1 mg/mL and 0.05 mg/mL remain stable through a) 36 months at the long-term storage condition of $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$, and b) 3 months at the accelerated condition of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$. (An out-of-specification result is observed for Total Impurities/degradation products at the 6 month time point at this condition). Based on these data, and following the recommendations outlined in ICH QE1 Evaluation of Stability Data, a shelf-life of (b) (4) months is granted for lixisenatide injection 0.1 mg/mL and 0.05 mg/mL strengths when stored at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$. This is in agreement with the Applicant's proposed expiry period for the drug product. Due to the findings in the photostability study, labeling will include a statement that the product should be kept in its secondary package with the cap attached during storage and when not in use in order to minimize its exposure to light.

Executive Summary Section

In-use stability studies suggest the quality of lixisenatide injection will not be compromised by conditions anticipated during patient use of the drug product (daily usage of the product for 14 days maintained at (b) (4) throughout expiry.

B. Description of How the Drug Product is Intended to be Used

Lixisenatide is intended as an adjunct to diet and exercise to achieve glycemic control in patients with type 2 diabetes mellitus. It acts as a glucagon-like peptide-1 (GLP-1) receptor agonist to stimulate insulin release from the pancreatic islets, suppress glucagon secretion, delay gastric emptying and reduce body weight.

Lixisenatide has been designed to be resistant to physiological degradation by dipeptidyl peptidase-4 (DPP4), an enzyme responsible for degradation of GLP-1. This is accomplished by synthetic modification of the C-terminal of the peptide with six lysine residues which slows its degradation. The half-life of lixisenatide is 2 – 4 hours, and it is classified as a short-acting GLP-1-receptor agonist. Despite its relatively short half-life, lixisenatide is supplied as a solution for subcutaneous injection to be taken once daily within one hour prior to either the first meal of the day or the evening meal. It is supplied in a reusable disposable pen injector in two strengths: A “Starting Dose” of 10 µg once daily for 14 days, and a “Maintenance Dose” of 20 µg once daily starting on Day 14. Lixisenatide should be injected into the abdomen, thigh region, or outer area of the upper arm.

C. Basis for Approvability or Not-Approval Recommendation

The recommendation from a CMC perspective is pending a) satisfactory responses to the deficiencies identified in Review #1, b) acceptability of microbiology information regarding sterility assurance of the drug product, c) an Acceptable recommendation from the Office of Compliance for manufacturing facilities associated with this application, and d) confirmation from Pharmacology/Toxicology that drug substance and drug product impurities/degradation products have been adequately qualified at or above the proposed limits found in the drug substance and drug product specifications.

This is a 505(b)(1) application where the drug substance, lixisenatide, is a New Molecular Entity (NME). IND 62724 for lixisenatide injection was received on 6/8/2001. A pre-NDA meeting was held on 11/28/2012. The original NDA was submitted on 12/20/2012.

The drug substance (lixisenatide) will be manufactured for commercial use by Sanofi-Aventis Deutschland GmbH located in Frankfurt, Germany. The drug product, lixisenatide injection 0.1 mg/mL and 0.05 mg/mL, will be manufactured as a sterile, aqueous solution intended for delivery of 0.2 mL by subcutaneous injection. The drug product will be available in two multi-use, disposable pen injectors for delivery of 20 mcg and 10 mcg doses. The drug product will be manufactured and assembled at the

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same location as was used in the manufacture of the drug substance (Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany).

III. Administrative

A. Reviewer's Signature: in DAARTS

B. Endorsement Block: in DAARTS

C. CC Block: in DAARTS

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A APPENDICES**A.1 Facilities and Equipment (biotech only)**

Not applicable

A.2 Adventitious Agents Safety Evaluation

Neither the lixisenatide drug substance nor any of the excipients for lixisenatide injection are derived from human or animal sources.

A.3 Novel Excipients

No novel excipients were used in the drug product formulation.

R REGIONAL INFORMATION**R1 Executed Batch Records**

The applicant has submitted copies of executed (and translated) batch records for the drug product.

The drug product batch record for the lixisenatide solution 0.1 mg/mL concentration is batch C010 and that for the 0.05 mg/mL concentration is batch U001.

These are acceptable.

R2 Comparability Protocols

No comparability protocol has been submitted.

R3 Methods Validation Package

The methods validation package includes the analytical methods used in the analysis of lixisenatide drug substance and lixisenatide injection 0.1 mg/mL and 0.05 mg/mL. Detailed descriptions of each method of analysis for lixisenatide drug substance and drug product are referenced by identification of validation documents.

II. Review of Common Technical Document-Quality (Ctd-Q) Module 1**A. Labeling & Package Insert****1. Labeling**

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i) Pen Injector Label

The proposed labels for Lixisenatide (lixisenatide injection) 10 mcg and 20 mcg pen injector are reproduced below:



Item	Information Provided	Recommended Changes (highlighted)
Proprietary name, established name (font size and prominence (21 CFR 201.10(g)(2))	Lixisenatide (lixisenatide injection)	None
Dosage strength	10 and 20 mcg	None
Net contents	Not provided.	None. Acceptable for pen label.
“Rx only” displayed prominently on the main panel	Rx only	None
NDC number (21 CFR 207.35(b)(3)(i))	58468-0191-1	None
Lot number and expiration date (21 CFR 201.17)	Lot: Exp:	None
Storage conditions	Refrigerate prior to use. Discard 14 days after first use.	None
Bar code (21CFR 201.25)	Acceptable	None
Name of manufacturer/distributor	Sanofi-aventis US	None

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ii). Pen Injector Carton Label (2 Syringes)

The proposed Lixisenatide (lixisenatide injection) 10 mcg and 20 mcg pen injector carton label (2 pen/box) is shown below:

10 mcg Lixisenatide Injection Carton Label

(b) (4)

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20 mcg Lixisenatide Injection Carton Label

(b) (4)



Item	Information Provided	Recommended Changes
Proprietary name, established name (font size and prominence (21 CFR 201.10(g)(2))	Lixisenatide ¹ (lixisenatide injection)	None
Dosage strength	10 mcg and 20 mcg	None
Net contents	2 prefilled pens containing 10 mcg (or 20 mcg) per dose.	None

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<p>Ingredients</p>	<p>Each dose (0.2 mL) contains 10 mcg (or 20 mcg) lixisenatide.</p> <p>Excipients: water for injection, glycerol 85% (54 mg), sodium acetate trihydrate (10.5 mg), methionine (9.0 mg), metacresol (8 (b) (4) mg), and hydrochloric acid/sodium hydroxide solution for pH adjustment.</p>	<p>Ingredients Each 0.2 mL dose contains lixisenatide, 10 mcg (or 20 mcg), water for injection, glycerol 85% (54 mg), sodium acetate trihydrate (10.5 mg), methionine (9.0 mg), metacresol (8.0 mg), and may contain hydrochloric acid and sodium hydroxide for pH adjustment.</p>
<p>“Rx only” displayed prominently on the main panel</p>	<p>For subcutaneous injection only Rx only</p>	<p>None</p>
<p>NDC number (21 CFR 207.35(b)(3)(i))</p>	<p>NDC XXXX-XXXX-XX</p>	<p>None</p>
<p>Lot number and expiration date (21 CFR 201.17)</p>	<p>Lot Exp</p>	<p>None</p>
<p>Storage conditions</p>	<p>Before first use Store in a refrigerator 36°F – 46°F (2°C – 8°C). Do not freeze. Keep the prefilled pen in the original package to protect it from light.</p> <p>After first use Stored below 86° F (30°C). Replace the pen cap after each use to protect from light Discard pen 14 days after first use.</p>	<p>None</p>
<p>Bar code (21CFR 201.25)</p>	<p>Acceptable</p>	<p>None</p>
<p>Name of manufacturer/distributor</p>	<p>Sanofi-aventis U.S. LLC Bridgewater, NJ 08807 A SONOFI COMPANY</p>	<p>None</p>

Note: Carton labels for 1) a single 10 mcg pen injector, and 2) a starter carton containing one 10 mcg pen and one 20 mcg pen have also been included, but not reproduced here. Labels are identical to the 2 x 10 mcg and 2 x 20 mcg pen injector carton labels shown above, except for references to the number of pens per box.

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c. Labeling Text

CMC-Relevant Sections of the Package Insert.

Text	Recommended Changes (Highlighted)
HIGHLIGHTS OF PRESCRIBING INFORMATION	
TRADENAME (lixisenatide) injection (b) (4) for subcutaneous use	TRADENAME (lixisenatide injection) (b) (4) for subcutaneous use
DOSAGE FORMS AND STRENGTHS	
(b) (4)	(b) (4)
FULL PRESCRIBING INFORMATION	
3. DOSAGE FORMS AND STRENGTHS	
TRADENAME is (b) (4) a solution for subcutaneous injection available as: (b) (4)	TRADENAME is (b) (4) a solution for subcutaneous injection available as: (b) (4)
11. DESCRIPTION	

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<p>doses of 10 mcg, and (b) (4) maintenance pe (b) (4) deliver 14 doses of 20 mcg.</p> <p>The following packages are available:</p> <ul style="list-style-type: none"> • Starter Pack: For treatment initiation, Starter Pack of 1 pre-filled green pen of TRADENAME 10 mcg and 1 pre-filled (b) (4) pen of TRADENAME 20 mcg. NDC xxxx-xxxx-xx. • Maintenance Pack: 2 prefilled (b) (4) pens for TRADENAME 20 mcg. NDC xxxx-xxxxx. <p>(b) (4)</p>	<p>of 10 mcg per 0.2 mL dose, and the (b) (4) maintenance pen delivers 14 doses of 20 mcg per 0.2 mL dose.</p> <p>The following packages are available:</p> <ul style="list-style-type: none"> • Starter Pack: 1 prefilled green pen of TRADENAME 10 mcg and 1 prefilled (b) (4) pen of TRADENAME 20 mcg. NDC xxxx-xxxx-xx. • Maintenance Pack: 2 prefilled (b) (4) pens for TRADENAME 20 mcg. NDC xxxx-xxxxx. <p>(b) (4)</p> <p>Each TRADENAME pen is for use by a single patient. A TRADENAME pen should never be shared between patients, even if the needle is changed.</p>
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<p>16.2 Storage</p>	
<p>Prior to first use, TRADENAME should be stored in a refrigerator, 36°–46°F (2°C–8°C). Do not freeze. Keep the prefilled pen in the original package to protect it from light.</p> <p>After first use, store below 86°F (30°C). Replace the pen cap after each use to protect from light. Discard pen 14 days after first use.</p>	<p>Prior to first use, TRADENAME should be stored in a refrigerator, 36°–46°F (2°C–8°C). Do not freeze. Keep the prefilled pen in the original package to protect it from light.</p> <p>After first use, store below 86°F (30°C). Replace the pen cap after each use to protect from light. Discard pen 14 days after first use.</p> <p>Always remove and safely discard the needle after each injection and store the TRADENAME pen without an injection needle attached. This will reduce the potential for contamination, infection, and leakage.</p>

B. Environmental Assessment or Claim of Categorical Exclusion

Sanofi-aventis U.S. LLC is claiming Categorical Exclusion from preparation of an Environmental Assessment for the lixisenatide drug product based on regulations in 21 CFR Part 25, Subpart C, Categorical Exclusions, Section 25.31 (b), Human drugs and biologics, since approval of this action would result in a concentration of the active moiety lixisenatide in the aquatic environment of the United States below 1 part per billion. This claim is based upon marketing estimates for sales of lixisenatide during the first five years of sales after approval of this Application and environmental fate data.

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Comment to the Applicant:

For your claim of Categorical Exclusion from preparation of an Environmental Assessment for the lixisenatide drug product, provide an estimate for the concentration of lixisenatide drug substance at the point of entry into the aquatic environment that supports your assertion that the concentration of the active moiety lixisenatide in the aquatic environment of the United States would be below 1 part per billion.

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List of Deficiencies To Be Communicated

1. Provide a technical description of the (b) (4) and a supporting Certificate of Analysis from the manufacturer.
2. Revise the (b) (4) in Table 38 in Section 3.2.S.2.3 Control of Materials.
3. Justify the proposed limit of (b) (4) in the drug substance specifications.
4. Provide the validation report of the analytical procedure, Identification – Mass Spectrometry (QUA-FR-2010-25051).
5. Clarify any difference in drug substance manufacturing between the commercial process at the “Production Plant” and processes at the “Pilot Plant” and their impact (or lack thereof) on the comparability of the resulting drug substance.
6. Clarify if earlier development batches used in Toxicology have been adequately bridged to clinical and proposed commercial batches with respect to impurity profile(s).
7. (b) (4) in the drug substance specifications to a value that more accurately reflects analytical results obtained during development.
8. According to ICH Q1E (Evaluation of Stability Data, June 2004), “For drug substances or products intended for storage in a freezer, the retest period or shelf life should be based on long-term data”. Therefore, a retest period of (b) (4) months (not (b) (4) months as proposed) will be granted for the lixisenatide drug substance when stored (b) (4).
9. Provide the rationale for the change in the manufacturing process of the drug product from Process (b) (4).
10. Provide in-use stability results for lixisenatide injection samples (Batch 1F001 for 0.1 mg/mL and batch 1F002 for 0.05 mg/mL) assembled in pen-injectors for samples stored at 12 months under real-time storage conditions that were manufactured using Manufacturing Process (b) (4). Also, provide a summary of these data along with data obtained following similar in-use stability studies conducted on lixisenatide injection samples stored at 12 months under real-time storage conditions manufactured using Manufacturing Process (b) (4) in order to support a conclusion of comparability among the products manufactured using the two different manufacturing processes.
11. As part of the Postapproval Stability Protocol and Stability Commitment, updated stability results on the drug products should be included in the Annual Report pursuant to 21 CFR.314.81(b)(2).
12. For your claim of Categorical Exclusion from preparation of an Environmental Assessment for the lixisenatide drug product, provide an estimate for the concentration of lixisenatide drug substance

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at the point of entry into the aquatic environment that supports your assertion that the concentration of the active moiety lixisenatide in the aquatic environment of the United States would be below 1 part per billion.

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

/s/

JOSEPH LEGINUS
05/31/2013

DANAE D CHRISTODOULOU
05/31/2013

I concur with the reviewer's conclusions and recommendations