# CENTER FOR DRUG EVALUATION AND RESEARCH

***APPLICATION NUMBER:***

# 205692Orig1s000

# PHARMACOLOGY REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES**

**PUBLIC HEALTH SERVICE**
**FOOD AND DRUG ADMINISTRATION**
**CENTER FOR DRUG EVALUATION AND RESEARCH**

# PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

| | |
|---|---|
| Application number: | NDA 205692 |
| Supporting document/s: | Original Submission |
| Applicant's letter date: | October 18, 2013 |
| CDER stamp date: | October 18, 2013 |
| Product: | LY2963016 insulin glargine ( (b) (4) |
| Indication: | T1DM and T2DM |
| Applicant: | Lilly |
| Review Division: | DMEP |
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*Template Version: September 1, 2010*

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# 1 Executive Summary

## 1.1 Introduction

Lilly submitted NDA 205692 for LY2963016 under 505b2. LY2963016 is a similar version of LANTUS (insulin glargine injection; NDA 021081). Both have similar formulations. It is long acting insulin analog to improve glycemic control in adults and children with T1DM and in adults with T2DM.

It is administered as a SC injection and will be available in a 3ml cartridge sealed in a prefilled pen injector (KwikPen™) at the strength of 100 units/ml.

With regards to the clinical development program for LY2963016, the sponsor` used US- or EU-approved Lantus (trademark of Sanofi-Aventis) as the comparator product. It focused on providing supportive data that LY2963016 had similar safety and efficacy profile compared to Lantus. Phase I PK/PD and Phase III safety and efficacy trials were conducted.

The nonclinical development program for LY2963016 focused on demonstrating the similarity of LY2963016 to EU-approved or US-approved LANTUS® with regards to primary pharmacodynamics, pharmacokinetics, and animal toxicity.

## 1.2 Brief Discussion of Nonclinical Findings

The nonclinical development of Y2963016 demonstrated that LY2963016 and Lantus were similar with respect to in vitro (insulin and insulin life growth factor-1 receptor binding, metabolic potency, and mitogenic potential) and in vivo (PK, glucodynamics, local tolerability, and toxicity profile) characteristics.

Two in vitro pharmacology studies (Study No DBT93 and Study No DBT149) were conducted to show that both LY2963016 and US- and EU-approved Lantus were potent and functional insulin molecules. There were no biologically important difference were seen for receptor binding affinity at either the insulin or IGF-1 receptors nor in assays of functional or intrinsic activity. The mitogenic potential of two products was similar when tested in both insulin- and IGF-1 receptor dominant systems.

Two 4 week toxicity studies were conducted in non-diabetic rats administered subcutaneously once daily. These studies showed that LY2963013 was similar to US-approved Lantus (Study No 8229448) and EU-approved Lantus (Study No 8259267).

In one 4-week toxicity study (Study No 8229488), LY2963016 or US-approved LANTUS® was given to rats by daily subcutaneous injection at dose levels of 0, 0.3, 1, and 3/2 mg/kg for 4 weeks. It resulted in markedly reduced glucose levels with a dose-dependent duration at all dose levels and adverse axonal degeneration of the sciatic nerve for animals given 3/2 mg/kg of LY2963016 or US-approved LANTUS®. Findings were comparable whether animals were given LY2963016 or US-approved LANTUS® and were consistent with the pharmacological action of both compounds; there were no apparent off-target toxicities. Administration of LY2963016 exceeded the maximum-tolerated dose at >1 mg/kg due to clinical signs secondary to hypoglycemia and death. The NOAEL was 0.3 mg/kg in animals given LY2963016 or US-approved LANTUS®.



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In another 4-week toxicity study (Study No 8259267), LY2963016 or EU-approved LANTUS® was given to rats by daily subcutaneous injection at a dose level of 0, 0.3, 1, or 2 mg/kg for 4 weeks. It resulted in markedly reduced glucose levels with dose-dependent duration at all dose levels. There were increased body weight gain at 2 mg/kg; and microscopic findings in the axons in the sciatic nerve (minimal degeneration in females given 2 mg/kg LY2963016), skin/subcutis sections at subcutaneous injection sites (increased adipose tissue in males given 2 mg/kg LY2963016, females given >1 mg/kg LY2963016, and males and females given 2 mg/kg EU-approved LANTUS®), and pancreatic islet cells (decreased islet cell cytoplasm/vacuolation in males and females given >1.0 mg/kg LY2963016 or EU-approved LANTUS®). Administration of LY2963016 or EU-approved LANTUS® exceeded the maximum-tolerated dose at >1 mg/kg due to death and clinical observations secondary to hypoglycemia. The NOAEL was 0.3 mg/kg in animals given LY2963016 or EU-approved LANTUS®. TK analysis: After 29 days of dosing, a dose of 0.3 mg/kg LY2963016 corresponded to mean Cmax values of 11,227 (males) and 14,033 pM (females) and AUC0-24hr values of 58,558 (males) and 41,784 pM•hr (females). A dose of 0.3 mg/kg LANTUS® corresponded to mean Cmax values of 12,230 (males) and 9537 pM (females) and AUC0-24hr values of 44,819 (males) and 31,585 pM•hr (females).

All in all, in vitro and in vivo evaluations did not identify any significant difference between LY296316 and Lantus. The nonclinical data are adequate to support the use of LY2963016 in improving glycemic control in T1DM and T2DM patients.

## 1.3 Recommendations

### 1.3.1 Approvability

Yes

### 1.3.2 Additional Non Clinical Recommendations

No

### 1.3.3 Labeling

Sponsor's Proposed Labeling

**8 USE IN SPECIFIC POPULATIONS**

**8.1 Pregnancy**

Pregnancy Category C. Subcutaneous reproduction and teratology studies have been performed with another insulin glargine product and with regular human insulin in rats and Himalayan rabbits. (b) (4) insulin glargine product was given to female rats before mating, during mating, and throughout pregnancy at dose up to 0.36 mg/kg/day, which is approximately 7 times the recommended human subcutaneous starting dose of 10 units/day (0.008 mg/kg/day) based on mg/m$^2$. In rabbits, doses of 0.072 mg/kg/day, which is approximately 2 times the recommended human subcutaneous starting dose of 10 units/day (0.008 mg/kg/day), based on mg/m$^2$, were administered during organogenesis. The effects of (b) (4) insulin glargine product did not generally differ from those observed with regular human insulin in rats and rabbits. However, in rabbits, five fetuses from two litters of the high-dose group exhibited dilation of the cerebral ventricles. Fertility and early embryonic development appeared normal.

There are no well-controlled clinical studies of the use of insulin glargine in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. It is essential for patients with diabetes or a history of gestational diabetes to maintain good metabolic control before conception and throughout pregnancy. Insulin requirements may



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decrease during the first trimester, generally increase during the second and third trimesters, and rapidly decline after delivery. Careful monitoring of glucose control is essential in these patients.

**8.3 Nursing Mothers**

It is unknown whether insulin glargine is excreted in human milk. Because many drugs, including human insulin, are excreted in human milk, caution should be exercised when (b) (4) is administered to a nursing woman. Use of (b) (4) is compatible with breastfeeding, but women with diabetes who are lactating may require adjustments of their insulin doses.

**8.4 Pediatric Use**

The safety and effectiveness of (b) (4) have been established in pediatric patients (age 6 to 15 years) with type 1 diabetes *[see Clinical Studies (14)]*. (b) (4) in pediatric patients younger than 6 years of age with type 1 (b) (4) pediatric patients with type 2 diabetes.

(b) (4)

**13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**

In mice and rats, standard two-year carcinogenicity studies with another insulin glargine product were performed at doses up to 0.455 mg/kg, which was for the rat approximately 10 times and for the mouse approximately 5 times the recommended human subcutaneous starting dose of 10 units/day (0.008 mg/kg/day), based on mg/m$^2$. The findings in female mice were not conclusive due to excessive mortality in all dose groups during the study. Histiocytomas were found at injection sites in male rats (statistically significant) and male mice (not statistically significant) in acid vehicle containing groups. These tumors were not found in female animals, in saline control, or insulin comparator groups using a difference vehicle. The relevance of these findings to humans is unknown.

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

In a combined fertility and prenatal and postnatal study of another insulin glargine product in male and female rats at subcutaneous doses up to 0.36 mg/kg/day, which was approximately 7 times the recommended human subcutaneous starting dose of 10 units/day (0.008 mg/kg/day), based on mg/m$^2$, maternal toxicity due to dose-dependent hypoglycemia, including some deaths, was observed. Consequently, a reduction of the rearing rate occurred in the high-dose group only. Similar effects were observed with NPH insulin.

FDA's Recommendation for Labeling

Except for the tradename "(b) (4)" and notations to "(b) (4) insulin glargine", the proposed sponsor labeling is identical to Lantus. Therefore, this is acceptable without changes.

# 2 Drug Information

## 2.1 Drug

- CAS Registry Number: CAS-160337-95-1



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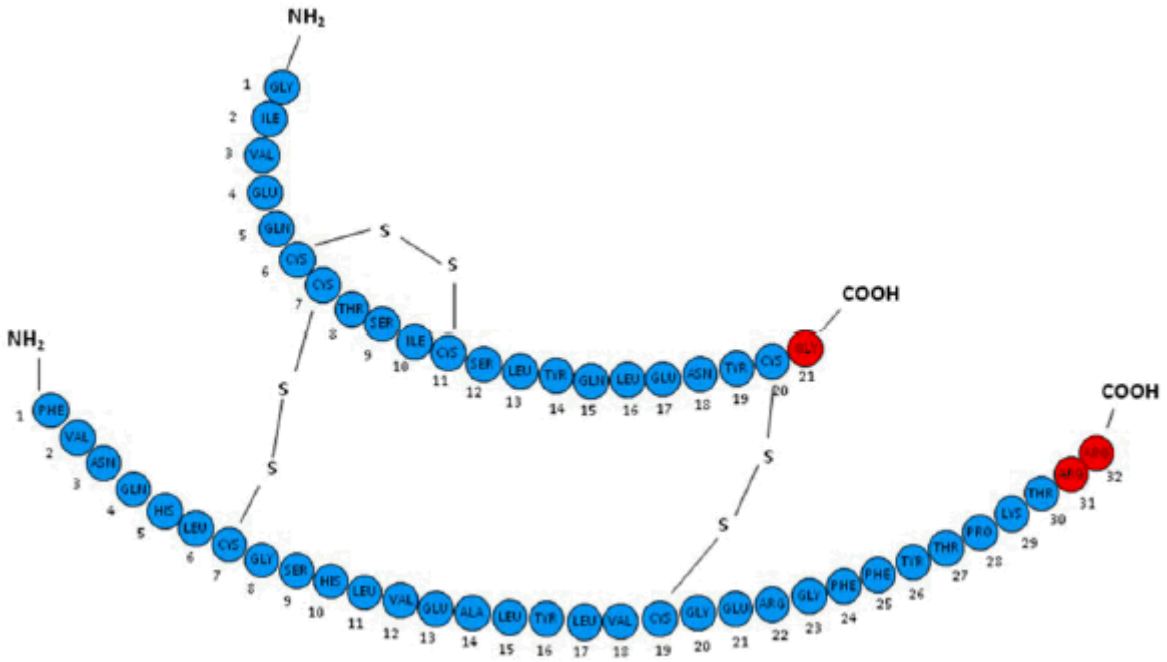
- Generic Name: Insulin glargine
- Code Name: LY2963016
- Chemical Name: 1) IUPAC: $21^{A}$-Gly-$30^{B}$a-L-Arg-$30^{B}$b-L-Arg insulin (human)
  2) USAN: Application pending
- Molecular Formula/Molecular Weight: $C_{267}H_{404}N_{72}O_{78}S_{6}$ and 6063 Da
- Structure or Biochemical Description: a 2-chain peptide containing 53 amino acids




- Pharmacologic Class: anti-diabetic agent

## 2.2 Relevant INDs, NDAs, BLAs and DMFs

NDA 021081

## 2.3 Drug Formulation

LY2963016 3ml cartridge for SC injection has an active ingredient of 100 Units LY2963016 and other ingredients including glycerin, metacresol, and zinc oxide.

**Table 1 LY2963016 Formulation**

**Unit Formula for LY2963016 Injection, 3 mL Cartridges**



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| Ingredient | Quantity/mL | Function | Reference to Standards |
|---|---|---|---|
| **Active Ingredient** | | | |
| LY2963016 | 100 Units (3.6378 mg[1]) | Active | In-house |
| **Other Ingredients[2]** | | | |
| Glycerin | 17 mg | (b) (4) | USP-NF/Ph.Eur. |
| Metacresol | 2.7 mg | | USP-NF/Ph.Eur. |
| Zinc Oxide[3] | (b) (4 | | USP-NF/Ph.Eur. |
| Hydrochloric Acid | (b) (4) | pH adjustment | In-house[4] |
| Sodium Hydroxide | | pH adjustment | In-house[4] |
| Water for Injection | q.s. to 1 mL | (b) (4) | USP-NF/Ph.Eur. |

(b) (4)

## 2.4 Comments on Novel Excipients

N/A

## 2.5 Comments on Impurities/Degradants of Concern

Drug substance impurities in final drug substance close or above the 0.1% were characterized.

**Table 2 Impurities: LY2963016 Drug Substance**

**Specifications for LY2963016 Drug Substance**

| Test | Analytical Procedure | Acceptance Criteria |
|---|---|---|
| **Identification Test** | | |
| Identity | B12882/ HPLC | The retention time of the main peak compares with that of the reference standard. |
| Identity | B12976/ HPLC-Peptide Map | Conforms to reference standard |
| Biological Identity | B12968/ (b) (4) | Identity is confirmed when the bioresponse of the sample is not less than (b) (4)% potency relative to the potency of the reference standard. |
| **Potency Test** | | |
| Assay | B12882/ HPLC | Not less than 95.0% and not more than 105.0% (Not less than (b) (4); and not more than (b) (4) on a volatiles-free (LOD) basis ) |
| **Purity Tests** | | |
| Impurities: Total | B12883/ HPLC | Not more than (b) (4)% |
| (b) (4) | B03622/ (b) (4) HPLC | Not more than (b) (4)% |
| **Other Tests** | | |
| Physical Appearance[1] | Visual | White or almost white solid |
| (b) (4) | B09980/ Atomic Absorption Spectroscopy | Not less than (b) (4)% and not more than (b) (4)% |
| Loss on Drying | USP <731> Ph.Eur. 2.2.32 | Not more than (b) (4)% |
| (b) (4) | G1324/ Immunoassay | Not more than (b) (4) |
| | B12977/ Immunoassay | Not more than (b) (4) |
| | B05416/ Atomic Absorption Spectroscopy | Not more than (b) (4) |

**(continued) Specifications for LY2963016 Drug Substance**

| Test | Analytical Procedure | Acceptance Criteria |
|---|---|---|
| Bacterial Endotoxins | USP <85> Ph.Eur. 2.6.14 | Not more than (b) (4) |
| Bioburden (Total Aerobic Microbial Count) | USP <61> Ph.Eur. 2.6.12 | Not more than (b) (4) |
| (b) (4) | B05054/ (b) (4) | Not more than (b) (4)% |

[1] "Physical Appearance" is equivalent to the ICH term "Description".

Drug product impurities for full identification were set at (b) (4)%. (b) (4) and unidentified modifications were observed.

Drug product impurities were quantified in 4 week repeat dose rat studies and were below specifications. Two lots were tested. Total impurities were (b) (4)% and (b) (4) was (b) (4)% (with specifications of (b) (4)% for total impurities and (b) (4)% for (b) (4)).

**Table 3 Impurities: LY2963016 Drug Product**

**Specifications for LY2963016 Injection**

| Test | Analytical Procedure | Acceptance Criteria |
|---|---|---|
| **Identification Test** | | |
| Identity | B12882/HPLC | The retention time of the main peak compares with that of the reference standard. |
| **Potency Tests** | | |
| Assay | B12882/HPLC | Not less than (b) (4)% and not more than (b) (4)% of the label claim[1] |
| (b) (4) | B03996/HPLC | Not less than (b) (4)% and not more than (b) (4)% of label claim (b) (4) |
| **Purity Tests** | | |
| Impurities: Total | B12883/HPLC | Not more than (b) (4)% |
| High Molecular Weight (b) (4) (b) (4) | B03622/ (b) (4) HPLC | Not more than (b) (4)% |

Communication with CMC reviewer in June 2014: There was very limited information (i.e. Lot# A889460 used in the 4 week toxicity study No 8259267 – (b) (4)% total impurities and (b) (4)%

(b) (4) were tested) provided to support the proposed limits for total product related impurities, (b) (4) and largest unspecified impurity (b) (4)

In June 2014, an IR request was sent to Lilly to clarify the specification limits sought. Lilly is proposing a total impurities spec of (b) (4)% & (b) (4) spec of (b) (4)% both of which exceeds the USP monograph level for Lantus as well as setting a novel impurity specification for (b) (4) % limit. While the tox profile of LY2963016 is comparable to Lantus the impurity levels tested in tox studies are far below these proposed specification limits.

**Table 4 Impurities: LY2963016 Used in Toxicity Studies**

**Toxicology: Drug Product**

| Lot Number | Purity (%) | Specified Impurities (%) | | | | | Document ID | Type of Study |
|---|---|---|---|---|---|---|---|---|
| | | Total Impurities | (b) (4) | | | | | |
| Specification: | | NMT | NMT (b) (4) | | | | | |
| (b) (4) | | | | | | | Study 8229488<br>Study 8259267 | Repeat dose, 4 week, rat<br>Repeat dose, 4 week, rat |

Abbreviation: NMT = not more than.

ONDQA indicates that they are in negotiations with the sponsor to reduce the specifications of these non-compendia impurities and those with higher levels than the USP monograph for Lantus.

## 2.6 Proposed Clinical Population and Dosing Regimen

T1DM and T2DM patients

## 2.7 Regulatory Background

- May 2011 - IND 105423 as a new IND submission (pharm/tox review in DARRTS)
- June 2012 – Type C mtg written response (no pharm/tox question)
- August 2012 – Annual report 01
- August 2013 – Annual report 02
- August 2013 – Type B mtg preNDA (no pharm/tox question; mtg minutes in DARRTS)
- Oct 2013 – NDA 205692 as a new NDA submission

**Clinical Studies Supporting US 505b2 Submission**



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| Study Alias | Objective | Study Population | Number of Subjects Randomized |
|---|---|---|---|
| Phase 1 Studies | | | |
| ABEO | Comparison of the PK and PD of LY2963016 and US-approved LANTUS® | Healthy subjects | 91 |
| ABEA | Comparison of the PK and PD of LY2963016 and EU-approved LANTUS® | Healthy subjects | 80 |
| ABEN[a] | Comparison of the PK and PD of EU- and US-approved LANTUS® | Healthy subjects | 40 |
| ABEI | Relative bioavailability of LY2963016 to EU-approved LANTUS® | Healthy subjects | 16 |
| ABEE | Comparison of the PD of LY2963016 and EU-approved LANTUS® | Patients with T1DM | 20 |
| ABEM | Relative bioavailability of LY2963016 to EU-approved LANTUS® | Healthy subjects | 24 |
| Phase 3 Studies | | | |
| ABEB | Comparison of LY2963016 with LANTUS® (EU- and US-approved), as measured by change in HbA1c, when each is used in combination with premeal insulin lispro | Patients with T1DM (open-label) | 536 |
| | | | LY2963016: 269<br>LANTUS®: 267<br>(US-approved: 96/<br>EU-approved: 171) |
| ABEC | Comparison of LY2963016 with LANTUS® (EU- and US-approved), as measured by change in HbA1c, when each is used in combination with OAMs | Patients with T2DM (double-blind) | 759 |
| | | | LY2963016: 379<br>LANTUS®: 380<br>(US-approved: 215/<br>EU-approved: 165) |

Abbreviations: EU = European Union; HbA1c = hemoglobin A1c; OAM = oral antihyperglycemic medication; PD = pharmacodynamics; PK = pharmacokinetic; T1DM = type 1 diabetes mellitus; T2DM = type 2 diabetes mellitus; US = United States.

[a] Study ABEN was a comparison of EU- and US-approved LANTUS®; no LY2963016 was administered.

Sources: ABEO, ABEA, ABEN, ABEI, ABEE, ABEM, ABEB, ABEC Clinical Study Reports; [ABEC_prd]/final/programs_stat/tfl_output/smbsca12.rtf; [ABEB_prd]/final/programs_stat/tfl_output/smbsca12.rtf.

# 3 Studies Submitted

## 3.1 Studies Reviewed

Primary PD: dbt93 and dbt149

- DBT93 (in vitro PD study vs. US Lantus) was reviewed under IND105423 original submission.
- DBT149 (in vitro PD study comparing batches with EU or US Lantus) was first submitted under IND105423 eCTD0032.

PK: 8225346

Tox: 8229488 and 8259267

- Study No 8229488 (4 week toxicity rat study vs. US Lantus) was reviewed under IND105423 original submission.



Reference ID: 3527465

- Study No 8259267 (4 week toxicity rat study vs. EU Lantus) was first submitted under IND105423 eCTD0023

### 3.2 Studies Not Reviewed

N/A

### 3.3 Previous Reviews Referenced

One review was done under IND 105423 and it can be found in DARRTS.

## 4 Pharmacology

The sponsor conducted one pharmacology study which included several in vitro assays to characterize the biological activity of LY2963016 and to show that LY2963016 is a functionally active insulin molecule and LY2963016 is comparable to Lanus.

### 4.1 Primary Pharmacology

No primary PD studies have been done with LY2963016, except the one study which is described below.

### Study Report: DBT93: PD study with US-Lantus

**In Vitro Receptor Binding, functional Activation, Lipogenesis, and Mitogenic Potency of LY2963016 in Comparison to US-Approved Lantus®, Human Insulin, Insulin AspB10, and IGF-1 (Final Report Amendment 01)**

Aim

This study was to compare LY2963016 (Lot# RS0671) to the commercial preparation of insulin glargine (US-Lantus; insulin glargine, Sanofi-Aventis, LSN2517641, Lot# 40N091). Comparative studies of in vitro assays (affinity, insulin- and IGF-1 receptor binding, intrinsic lipogenic activity, and mitogenicity) were conducted with both compounds to assess any differences between LY2963016 and US-Lantus. Other comparators included zinc-free biosynthetic human insulin (BHI, 2518346), AspB10 insulin (3001706), and wt-IGF1 (2518454).

Method

- In vitro binding potency of LY2963016 was done using membrane preparations of stably-transfected 293HEK-EBNA cells over-expressing both isoforms of human insulin receptors Type A and Type B (hIRA and hIRB), as well as human insulin-like growth factor 1 (hIGF-1) receptor.
- Functional activity was determined using the same insulin receptor over-expressing 293HEK cell lines and ELISA technique (KIRA: Kinase Insulin Receptor Assay) to determine $EC_{50}$ for inducing tyrosine auto-phosphorylation of the insulin receptor (hIR).
- Metabolic activity was also determined using a 3T3L1 adipocyte model to measure de novo lipogenesis of triglycerides from radioactivity labeled glucose.
- Mitogenic/proliferation potential was assessed two different cellular assays (H4IIe and SAOS cell lines) since both insulin receptor and IGF-1 receptor can induce proliferation and cell growth. The rat H4IIe hepatoma is deficient in IGF1-R expression and is dependent on insulin for proliferation in serum-free media. The human SAOS osteosarcoma cell line expresses 10-fold more IGF-1 than it does IR (insulin receptor) so it is more sensitive to IGF-1 stimulated mitogenicity.
- The commercially available Lantus (insulin glargine) and several insulin-like molecules (i.e. BHI, asp-B10-insulin, and IGF) were used in this study.



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Results

- The binding affinity assay in 293-HEK cells showed that LY2963016 had Ki values of 0.259 nM±0.00866 for hIR-A, 0.320±0.0464 nM for hIR-B, and 22.1±1.84 nM for hIGF1-R. LY2963016 and Lantus showed comparable binding potency.

**Table 5 DBT93: Binding Affinity**

**Binding of LY2963016 to the Human Insulin Receptor, Isoforms A and B, and the Human IGF-1 Receptor Compared to Other Insulin-like Molecules**

| Compound | Binding Affinity, hIR-A $K_i$ ± SEM (nM), n=3 | Binding Affinity, hIR-B $K_i$ ± SEM (nM), n=3 | Binding Affinity, hIGF-1R $K_i$ ± SEM (nM), n=3 | *Selectivity |
|---|---|---|---|---|
| 2963016 | 0.259 ± 0.00866 | 0.320 ± 0.0464 | 22.1 ± 1.84 | 85.3 |
| glargine | 0.351 ± 0.0538 | 0.352 ± 0.0255 | 24.8 ± 1.43 | 70.7 |
| BHI | 0.211 ± 0.0423 | 0.199 ± 0.0272 | 178 ± 4.16 | 844 |
| Asp-B10 | 0.0901 ± 0.0116 | 0.0889 ± 0.0179 | 56.3 ± 8.58 | 625 |
| wt-IGF1 | 24.5 ± 2.34 | 215 ± 63.4 | 0.626 ± 0.00948 | 0.0256 |

Binding Affinities are Expressed as $K_i$ Values in nM. Values are the Geometric Mean ± Standard Error of the Mean for the Number of Separate Experiments Indicated by n.
*selectivity determined as ratio of Ki for hIGF1-R divided by Ki for hIR-A

- The functional activity assay in 293-HEK cells showed LY2963016 had $EC_{50}$ of 8.13±0.930 nM (vs. 6.7±2.34 nM with Lantus) for IR-A and 3.96±0.524 nM (vs. 4.19±0.622 nM with Lanus) for IR-B. LY2963016 and Lantus showed comparable functional potency.

**Table 6 DBT93: Functional Activity - auto-phosphorylation**

**Functional Activity of LY2963016 and Other Insulin-like Molecules to Stimulate the Auto-phosphorylation of the Human Insulin Receptor, isoforms A and B**

| Compound | Autophos-IR-A, EC50±SEM, nM | Autophos-IR-B, EC50±SEM, nM |
|---|---|---|
| 2963016 | 8.13±0.930 (n=3) | 3.96±0.524 (n=3) |
| Lantus® | 6.70±2.34 (n=3) | 4.19±0.622 (n=3) |
| BHI | 4.13±0.621 (n=6) | 2.80±0.585 (n=8) |
| Asp-B10 | 1.79±0.0733 (n=3) | 0.903±0.083 (n=3) |
| wt-IGF1 | 1307±381 (n=2) | 2255±823 (n=4) |

Functional Activity Expressed as Half-maximal Effect ($EC_{50}$) in nM. Values are the Geometric Mean ± Standard Error of the Mean for the Number of Separate Experiments Indicated by n.

- The metabolic activity assay in 3T3-L1 adipocytes showed that LY2963016 had $EC_{50}$ of 6.85±4.63 nM for de novo lipogenesis. The lipogenic activity of LY2963016 is comparable to Lantus ($EC_{50}$ of 3.68±3.35 nM) in stimulating de novo lipogenesis.

**Table 7 DBT93: De novo Lipogenesis**

**Stimulation of *de novo* Lipogenesis of Triglycerides From [U-$^{14}$C]-Glucose in Differentiated 3T3-L1 Adipocytes by LY2963016 and Other Insulin-like Analogs**



Reference ID: 3527465

| Compound | de novo Lipogenesis, EC50±SEM, nM |
|---|---|
| 2963016 | 6.85±4.63 (n=4) |
| Lantus® | 3.68±3.35 (n=4) |
| BHI | 2.20±0.584 (n=8) |
| Asp-B10 | 0.18±0.102 (n=8) |
| wt-IGF1 | 1.99±0.715 (n=8) |

Half-maximal Effect ($EC_{50}$) for De Novo Lipogenesis is in nM. Values are Geometric Mean ± Standard Error of the Mean for the Number of Separate Experiments Indicated by "n".

- The mitogenic potency assay showed that LY2963016 had $EC_{50}$ of 0.223±0.0457 nM in rat H4II3 hepatomas (1st model with little to no IGF1-R expression) and 4.21±0.669 nM in human osteosarcoma SAOS-2 (2nd model with IGF1-R expression). There was no significant difference in the mitogenic activity seen for LY2963016 and the commercially available Lantus in either mitogenic assay model.

**Table 8 DBT93: Mitogenicity**

**Mitogenecity of LY2963016 and Other Insulin-like Analogs**

Half-maximal Effect ($EC_{50}$) for [$^3$H]-thymidine Incorporated Into the DNA is Expressed in nM. Values are Geometric Mean ± Standard Error of the Mean for the Number of Separate Experiments Indicated by "n".

| Compound | [a]Mitogenecity, SAOS-2, EC50±SEM, nM | *Selectivity (SAOS-2) | [b]Mitogenecity, H4IIe, EC50±SEM, nM | *Selectivity (H4IIe) |
|---|---|---|---|---|
| 2963016 | 4.21± 0.669 (n=5) | 7 | 0.223±0.0457 (n=6) | 409 |
| glargine | 4.34±0.650 (n=3) | 7 | 0.186±0.0719 (n=12) | 491 |
| BHI | 17.9±1.38 (n=15) | 31 | 0.128±0.00290 (n=23) | 712 |
| Asp-B10 | 2.92±0.346 (n=4) | 5 | 0.0735±0.0153 (n=10) | 1244 |
| wt-IGF1 | 0.579±0.032 (n=5) | 1 | 91.4±9.99 (n=3) | 1 |

[a]Stimulating [$^3$H]-thymidine Incorporation Into the DNA of the Human SAOS Osteosarcoma Cell Line
[b]Stimulation of Cell Growth of Rat H4IIe Hepatoma Cell Line
*Selectivity determined as the ratio of EC50 Values for the Test Compound Divided by the EC50 values for wt IGF-1

All in all, LY2963016 was a potent and functionally active insulin molecule. When compared these results of LY2963016 to Lantus, there was no statistically difference in either potency or efficacy in any assays tested. Therefore, the in vitro characteristics of LY2963016 were comparable to Lantus.

## Study Report: DBT149: PD Study with US-/EU-Lantus

### The In Vitro Pharmacology of LY2963016 Injection Batches Formulated at (b) (4) Lilly France Compared to US-Approved or EU-Approved Lantus® (Insulin Glargine)

Aim

This study was to compare LY2963016 from Lilly France (Lot# C187060, C187091, C187582) (b) (4) (CM3-094, CM3-095, CM3-096) to the commercial preparation of insulin glargine from Sanofi-Aventis (EU-Lantus - Lot# 2F0438, 2F086A, 1F164A or US-Lantus – Lot#

3F234A, 3F251A, 3F252A). Comparative studies of in vitro assays, including affinity, insulin- and IGF-1 receptor binding, intrinsic lipogenic activity, and mitogenicity, were conducted to assess any differences between LY2963016 and US/EU-Lantus. Other comparators included zinc-free biosynthetic human insulin (BHI, Lot# 010LX9, QA307X), AspB10 insulin, and hIGF1 (EJ8-01530-014).

Methods

- In vitro binding potency of LY2963016 was done using membrane preparations of stably-transfected 293HEK-EBNA cells over-expressing both isoforms of human insulin receptors Type A and Type B (hIRA and hIRB), as well as human insulin-like growth factor 1 (hIGF-1) receptor.
- Functional activity was determined using the same insulin receptor over-expressing 293HEK cell lines and ELISA technique (KIRA: Kinase Insulin Receptor Assay) to determine $EC_{50}$ for inducing tyrosine auto-phosphorylation of the insulin receptor (hIR).
- Metabolic activity was also determined using a 3T3L1 adipocyte model to measure de novo lipogenesis of triglycerides from radioactivity labeled glucose.
- Mitogenic/proliferation potential was assessed two different cellular assays (H4IIe and SAOS cell lines) since both insulin receptor and IGF-1 receptor can induce proliferation and cell growth. The rat H4IIe hepatoma is deficient in IGF1-R expression and is dependent on insulin for proliferation in serum-free media. The human SAOS osteosarcoma cell line expresses 10-fold more IGF-1 than it does IR (insulin receptor) so it is more sensitive to IGF-1 stimulated mitogenicity.
- The commercially available Lantus (insulin glargine) and several insulin-like molecules (i.e. BHI, asp-B10-insulin, and IGF) were used in this study.

Results

The **binding affinity assay** in 293-HEK cells showed that there was no significant difference in hIA, hIRB, or hIGF-1R B binding affinity for any of the comparisons evaluated (LY2964026 from Lilly France (b) (4) vs. EU or US-approved Lantus).

- With regards to hIRA, the Ki values for LY2963016 from Lilly France (b) (4) were 0.391±0.010 nM and 0.426±0.017 nM respectively. The Ki values for EU-approved or US-approved Lantus were 0.403±0.009 nM and 0.395±0.038 nM respectively. When all lots were compared, the Ki values for hIRA binding by either LY2963016 or Lantus were 0.408±0.012 nM and 0.399±0.018 mM respectively.
- With regards to hIRB, the Ki values for LY2963016 from Lilly France (b) (4) were 0.433±0.041 nM and 0.474±0.045 nM respectively. The Ki values for EU-approved or US-approved Lantus were 0.543±0.006 nM and 0.380±0.027 nM respectively. When all lots were compared, the Ki values for hIRA binding by either LY2963016 or Lantus were 0.453±0.028 nM and 0.450±0.037 mM respectively.
- With regards to hIGF-1R, the Ki values for LY2963016 from Lilly France (b) (4) were 16.0±0.6 nM and 15.9±0.8 nM respectively. The Ki values for EU-approved or US-approved Lantus were 15.0±1.1 nM and 16.1±0.2 nM respectively. When all lots were compared, the Ki values for hIRA binding by either LY2963016 or Lantus were 16.0±0.4 nM and 15.5±0.6 nM respectively.

**Table 9 DBT149: Binding Affinity – hIRA, hIRB, and hIGF1R**

**Comparisons of LY2963016 and LANTUS® for hIR-A Binding Analysis+**

| Comparator | Ki (nM) | $potency ratio | *MSR | ^p-value | ^Sidak | ^Holm-Bonferonni |
|---|---|---|---|---|---|---|
| #LY2963016 | 0.408 (0.012, 6) | 1.02 | 2.02 | 0.664 | NS | NS |
| #LANTUS® | 0.399 (0.018, 6) | | | | | |
| #LY2963016 (b) (4) | 0.426 (0.017, 3) | 1.09 | 2.02 | 0.299 | NS | NS |
| #LY2963016 (Lilly France) | 0.391 (0.010, 3) | | | | | |
| #LANTUS® US-Approved | 0.395 (0.038, 3) | 1.02 | 2.02 | 0.783 | NS | NS |
| #LANTUS® EU-Approved | 0.403 (0.009, 3) | | | | | |
| %BHI | 0.234 (0.018, 3) | 2.41 | 2.02 | | | |
| %insulin AspB10 | 0.097 (0.018, 3) | | | | | |

+Replicate Ki values for test sample and positive controls from each individual run are shown in Appendix A and B.
#Means are reported as the Geometric mean using the log transformed $K_i$ values (SEM, number lots analyzed)
%Geometric mean using the log transformed Ki values (SEM, number runs, single lot)
$ Potency Ratio represents the difference in potency calculated as a ratio of larger value over the smaller value
* MSR, Minimum Significant Ratio, the smallest $K_i$ ratio between two compounds tested in multiple runs that is statistically significant.
^ The p-values, Sidak, and Holm-Bonferroni multiple comparison adjustment, $\alpha$=0.05, results calculated from a mixed effect model using log transformed $K_i$ values

**Comparisons of LY2963016 and LANTUS® for hIR-B Binding Analysis+**

| Comparator | Ki (nM) | $potency ratio | *MSR | ^p-value | ^Sidak | ^Holm-Bonferonni |
|---|---|---|---|---|---|---|
| #LY2963016 | 0.453 (0.028, 6) | 1.01 | 1.89 | 0.952 | NS | NS |
| #LANTUS® | 0.450 (0.037, 6) | | | | | |
| #LY2963016 (b) (4) | 0.474 (0.045, 3) | 1.09 | 1.89 | 0.428 | NS | NS |
| #LY2963016 (Lilly France) | 0.433 (0.041, 3) | | | | | |
| #LANTUS® US-Approved | 0.380 (0.027, 3) | 1.41 | 1.89 | 0.0126 | NS | NS |
| #LANTUS® EU-Approved | 0.534 (0.006, 3) | | | | | |
| %BHI | 0.293 (0.030, 3) | 2.20 | 1.89 | | | |
| %insulin AspB10 | 0.133 (0.005, 3) | | | | | |

+Replicate Ki values for test sample and positive controls from each individual run are shown in Appendix C and D.
#Means are reported as the Geometric mean using the log transformed $K_i$ values (SEM, number lots analyzed)
%Geometric mean using the log transformed Ki values (SEM, number runs, single lot)
$ Potency Ratio represents the difference in potency calculated as a ratio of larger value over the smaller value
* MSR, Minimum Significant Ratio, the smallest $K_i$ ratio between two compounds tested in multiple runs that is statistically significant.
^ The p-values, Sidak, and Holm-Bonferroni multiple comparison adjustment, $\alpha$=0.05, results calculated from a mixed effect model using log transformed $K_i$ values

**Comparisons of LY2963016 and LANTUS® for hIGF-1R Binding Analysis+**

| Comparator | Ki (nM) | $potency ratio | *MSR | ^p-value | ^Sidak | ^Holm-Bonferonni |
|---|---|---|---|---|---|---|
| #LY2963016 | 16.0 (0.4, 6) | 1.03 | 1.90 | 0.564 | NS | NS |
| #LANTUS® | 15.5 (0.6, 6) | | | | | |
| #LY2963016 (b) (4) | 15.9 (0.8, 3) | 1.01 | 1.90 | 0.929 | NS | NS |
| #LY2963016 (Lilly France) | 16.0 (0.6, 3) | | | | | |
| #LANTUS® US-Approved | 16.1 (0.2, 3) | 1.07 | 1.90 | 0.328 | NS | NS |
| #LANTUS® EU-Approved | 15.0 (1.1, 3) | | | | | |
| %BHI | 78.5 (6.2, 3) | 2.57 | 1.90 | | | |
| %insulin AspB10 | 30.5 (7.1, 3) | | | | | |

+Replicate Ki values for test sample and positive controls from each individual run are shown in Appendix E and F.
#Means are reported as the Geometric mean using the log transformed $K_i$ values (SEM, number lots analyzed)
%Geometric mean using the log transformed Ki values (SEM, number runs, single lot)
$ Potency Ratio represents the difference in $K_i$ calculated as a ratio of larger value over the smaller value
* MSR, Minimum Significant Ratio, the smallest $K_i$ ratio between two compounds tested in multiple runs that is statistically significant.
^ The p-values, Sidak, and Holm-Bonferroni multiple comparison adjustment, α=0.05, results calculated from a mixed effect model using log transformed $K_i$ values

The **functional activity assay** in 293-HEK cells showed that there was no significant difference in hIRA or hIRB auto-phosphorylation (aka functional potency) for any of the comparisons evaluated (LY2964026 from Lilly France (b) (4) vs. EU or US-approved Lantus).

- With regards to hIRA, the $EC_{50}$ values for LY2963016 from Lilly France (b) (4) were 3.64±0.36 nM and 3.75±0.26 nM respectively. The $EC_{50}$ values for EU-approved or US-approved Lantus were 4.22±0.13 nM and 4.81±0.31 nM respectively. When all lots were compared, the $EC_{50}$ values for hIRA binding by either LY2963016 or Lantus were 0.408±0.012 nM and 0.399±0.018 nM respectively. When compared to all batches of LY2963016 and Lantus, independent of location, there was some difference. However, such difference was not likely to be biologically relevant given this difference is small (MSR = 2.27).
- With regards to hIRB, the $EC_{50}$ values for LY2963016 from Lilly France (b) (4) were 1.97±0.12 nM and 2.13±0.07 nM respectively. The $EC_{50}$ values for EU-approved or US-approved Lantus were 2.66±0.12 nM and 2.38±0.20 nM respectively. When all lots were compared, the $EC_{50}$ values for hIRA binding by either LY2963016 or Lantus was 2.05±0.07 nM and 2.52±0.12 nM respectively. However, When compared to all batches of LY2963016 and Lantus, independent of location, such difference was not likely to be biologically relevant given this difference is small (MSR = 1.93)

**Table 10 DBT149: Functional Activity (Autophosphorlylation)**

**Comparisons of LY2963016 and LANTUS® for hIR-A Receptor Tyrosine Autophosphorylation[+]**

| Comparator | $EC_{50}$ (nM) | [$]potency ratio | *MSR | ^p-value | ^Sidak | ^Holm-Bonferonni |
|---|---|---|---|---|---|---|
| [#]LY2963016 | 3.70 (0.20, 6) | 1.22 | 2.27 | 0.018 | NS | NS |
| [#]LANTUS® | 4.50 (0.20, 6) | | | | | |
| [#]LY2963016 (b) (4) | 3.75 (0.26, 3) | 1.03 | 2.27 | 0.781 | NS | NS |
| [#]LY2963016 (Lilly France) | 3.64 (0.36, 3) | | | | | |
| [#]LANTUS® US-Approved | 4.81 (0.31, 3) | 1.15 | 2.27 | 0.221 | NS | NS |
| [#]LANTUS® EU-Approved | 4.22 (0.13, 3) | | | | | |
| [%]BHI | 1.86 (0.23, 3) | 1.31 | 2.27 | | | |
| [%]insulin AspB10 | 1.42 (0.23, 3) | | | | | |

[+]Replicate $EC_{50}$ values for test sample and positive controls from each individual run are shown in Appendix G and H

[#]Means are reported as the Geometric mean using the log transformed potency values (SEM, number lots analyzed)

[%]Geometric mean using the log transformed potency values (SEM, number runs, single lot)

[$] Potency Ratio represents the difference in potency calculated as a ratio of larger value over the smaller value

* MSR, Minimum Significant Ratio, the smallest potency ratio between two compounds tested in multiple runs that is statistically significant.

^ The p-values, Sidak, and Holm-Bonferroni multiple comparison adjustment, $\alpha$=0.05, results calculated from a mixed effect model using log transformed $EC_{50}$ values

**Comparisons of LY2963016 and LANTUS® for hIR-B Receptor Tyrosine Autophosphorylation**

| Comparator | $EC_{50}$ (nM) | $potency ratio | *MSR | ^p-value | ^Sidak | ^Holm-Bonferonni |
|---|---|---|---|---|---|---|
| #LY2963016 | 2.05 (0.07, 6) | 1.23 | 1.93 | 0.0064 | NS | NS |
| #LANTUS® | 2.52 (0.12, 6) | | | | | |
| #LY2963016 (b) (4) | 2.13 (0.07, 3) | 1.08 | 1.93 | 0.349 | NS | NS |
| #LY2963016 (Lilly France) | 1.97 (0.12, 3) | | | | | |
| #LANTUS® US-Approved | 2.66 (0.12, 3) | 1.12 | 1.93 | 0.209 | NS | NS |
| #LANTUS® EU-Approved | 2.38 (0.20, 3) | | | | | |
| %BHI | 1.50 (0.03, 3) | 1.64 | 1.93 | | | |
| %insulin AspB10 | 0.916 (0.063, 3) | | | | | |

+Replicate $EC_{50}$ values for test sample and positive controls from each individual run are shown in Appendix I and J.
#Means are reported as the Geometric mean using the log transformed potency values (SEM, number lots analyzed)
%Geometric mean using the log transformed potency values (SEM, number runs, single lot)
$ Potency Ratio represents the difference in potency calculated as a ratio of larger value over the smaller value
* MSR, Minimum Significant Ratio, the smallest potency ratio between two compounds tested in multiple runs that is statistically significant.
^ The p-values, Sidak, and Holm-Bonferroni multiple comparison adjustment, α=0.05, results calculated from a mixed effect model using log transformed $EC_{50}$ values

The **metabolic activity assay** in 3T3-L1 adipocytes showed that there was no significant difference in de novo lipogenesis from glucose for any of the comparisons evaluated (LY2964026 from Lilly France (b) (4) vs. EU or US-approved Lantus).

- The $EC_{50}$ values for LY2963016 from Lilly France (b) (4) were 0.940±0.119 nM and 1.01±0.17 nM respectively. The $EC_{50}$ values for EU-approved or US-approved Lantus were 0.881±0.148 nM and 0.868±0.090 nM respectively. When all lots were compared, the $EC_{50}$ values for hIRA binding by either LY2963016 or Lantus were 0.973±0.092 nM and 0.874±0.077 nM respectively.

**Table 11 DBT149: De novo Lipogenesis**

**Comparisons of LY2963016 and LANTUS® for De Novo Lipogenesis in Mouse 3T3/L1 Adipocyte+**

| Comparator | $EC_{50}$ (nM) | $potency ratio | *MSR | ^p-value | ^Sidak | ^Holm-Bonferonni |
|---|---|---|---|---|---|---|
| #LY2963016 | 0.973 (0.092, 6) | 1.11 | 2.61 | 0.408 | NS | NS |
| #LANTUS® | 0.874 (0.077, 6) | | | | | |
| #LY2963016 (b) (4) | 1.01 (0.17, 3) | 1.07 | 2.61 | 0.725 | NS | NS |
| #LY2963016 (Lilly France) | 0.940 (0.119, 3) | | | | | |
| #LANTUS® US-Approved | 0.868 (0.090, 3) | 1.02 | 2.61 | 0.887 | NS | NS |
| #LANTUS® EU-Approved | 0.881 (0.148, 3) | | | | | |
| %BHI | 0.516 (0.078, 5) | 3.49 | 2.61 | | | |
| %insulin AspB10 | 0.148 (0.022, 4) | | | | | |

+Replicate $EC_{50}$ values for test sample and positive controls from each individual run are shown in Appendix K and L.

#Means are reported as the Geometric mean using the log transformed potency values (SEM, number lots analyzed)

%Geometric mean using the log transformed potency values (SEM, number runs, single lot)

$ Potency Ratio represents the difference in potency calculated as a ratio of larger value over the smaller value

* MSR, Minimum Significant Ratio, the smallest potency ratio between two compounds tested in multiple runs that is statistically significant.

^ The p-values, Sidak, and Holm-Bonferroni multiple comparison adjustment, α=0.05, results calculated from a mixed effect model using log transformed $EC_{50}$ values

The **mitogenic potency assay** showed that there was no significant difference in the mitogenic potential with human SAOS-2 and rat H4IIe hepatomas for any of the comparisons evaluated (LY2964026 from Lilly France (b) (4) vs. EU or US-approved Lantus).

- Human SAOS2 (1st model with IGF1-R expression): The $EC_{50}$ values for LY2963016 from Lilly France (b) (4) were 0.481±0.046 nM and 0.586±0.024 nM respectively. The $EC_{50}$ values for EU-approved or US-approved Lantus were 0.549±0. 058 nM and 0.513±0.012 nM, respectively. When all lots were compared, the $EC_{50}$ by either LY2963016 or Lantus was 0.531±0.034 nM and 0.530±0.027 nM, respectively.
- Rat H4II3 hepatoams (2nd model with little to no IGF1-R expression): The $EC_{50}$ values for LY2963016 from Lilly France (b) (4) were 8.67±0.42 nM and 9.28±0.25 nM respectively. The $EC_{50}$ values for EU-approved or US-approved Lantus were 7.92±0.20 nM and 8.88±0.63 nM respectively. When all lots were compared, the $EC_{50}$ by either LY2963016 or Lantus was 8.97±0.26 nM and 8.39±0.36 nM respectively.

**Table 12 DBT149: Mitogenicity**

**Comparisons of LY2963016 and LANTUS® Mitogenic Potential in the Human SAOS2 Osteosarcoma Cell Model by Thymidine Incorporation+**

| Comparator | $EC_{50}$ (nM) | $potency ratio | *MSR | ^p-value | ^Sidak | ^Holm-Bonferonni |
|---|---|---|---|---|---|---|
| #LY2963016 | 0.531 (0.034, 6) | 1.00 | 2.02 | 0.991 | NS | NS |
| #LANTUS® | 0.530 (0.027, 6) | | | | | |
| #LY2963016 ([redacted] (b) (4)) | 0.586 (0.024, 3) | 1.22 | 2.02 | 0.104 | NS | NS |
| #LY2963016 (Lilly France) | 0.481 (0.046, 3) | | | | | |
| #LANTUS® US-Approved | 0.513 (0.012, 3) | 1.07 | 2.02 | 0.543 | NS | NS |
| #LANTUS® EU-Approved | 0.549 (0.058, 3) | | | | | |
| %BHI | 1.47 (0.08, 3) | 3.37 | 2.02 | | | |
| %insulin AspB10 | 0.436 (0.019, 3) | | | | | |

+Replicate $EC_{50}$ values for test sample and positive controls from each individual run are shown in Appendix M and N.

#Means are reported as the Geometric mean using the log transformed potency values (SEM, number of source lots included)

$ Potency Ratio represents the difference in potency calculated as a ratio of larger value over the smaller value

* MSR, Minimum Significant Ratio, defined as the smallest potency ratio between two compounds tested in multiple runs that is statistically significant.

^ p-value, Sidak and Holm-Bonferroni results are from a mixed effect model using log transformed $EC_{50}$ values.

**Comparisons of LY2963016 and LANTUS® Mitogenic Potential in the Rat H4IIe Hepatoma Cell Model by Thymidine Incorporation+**

| Comparator | $EC_{50}$ (nM) | $potency ratio | *MSR | ^p-value | ^Sidak | ^Holm-Bonferonni |
|---|---|---|---|---|---|---|
| #LY2963016 | 8.97 (0.26, 6) | 1.07 | 2.12 | 0.265 | NS | NS |
| #LANTUS® | 8.39 (0.36, 6) | | | | | |
| #LY2963016 (b) (4) | 9.28 (0.25, 3) | 1.07 | 2.12 | 0.344 | NS | NS |
| #LY2963016 (Lilly France) | 8.67 (0.42, 3) | | | | | |
| #LANTUS® US-Approved | 8.88 (0.63, 3) | 1.12 | 2.12 | 0.126 | NS | NS |
| #LANTUS® EU-Approved | 7.92 (0.20, 3) | | | | | |
| %BHI | 1.60 (0.27, 5) | 2.21 | 2.12 | | | |
| %insulin AspB10 | 0.725 (0.124, 5) | | | | | |

+Replicate $EC_{50}$ values for test sample and positive controls from each individual run are shown in Appendix O and P.

#Means are reported as the Geometric mean using the log transformed potency values (SEM, number lots analyzed)

%Geometric mean using the log transformed potency values (SEM, number runs, single lot)

$ Potency Ratio represents the difference in potency calculated as a ratio of larger value over the smaller value

* MSR, Minimum Significant Ratio, the smallest potency ratio between two compounds tested in multiple runs that is statistically significant.

^ The p-values, Sidak, and Holm-Bonferroni multiple comparison adjustment, α=0.05, results calculated from a mixed effect model using log transformed $EC_{50}$ values

### Human insulin and insulin AspB10 controls

- Insulin Asp was 2.41, 2.20, 2.57-fold more potent than insulin for hIRA, hIRB and hIGF-1R binding respectively. With regards to functional aviation of the insulin receptor, insulin AspB10, was 1.31 and 1.64-fold more potent than insulin for activation of hIRA and hIRB respectively. With regards to de novo lipogenesis, insulin AspB10 was 3.49 fold more potent than human insulin. There were also 3.37 and 2.21 fold differences in potency when compared AspB10 to human insulin in the mitogenic assays, SAOS1 and H4IIe respectively. With regards to the hIRA and hRIB autophosphorylation assays, there were no distinguish between AspB10 and human insulin.

**Table 13 DBT149: In Vitro PD Profile with Other Insulins**
**Comparison of Asp-B10 Insulin and Human Insulin In Vitro Pharmacology**

| Assay | %Asp-B10 insulin | %human insulin | $Potency Ratio insulin/AspB10 |
|---|---|---|---|
| IR-A bindng, Ki, nM (SEM, n) | 0.097 (0.018, 3) | 0.234 (0.018, 3) | 2.41 |
| IR-B binding, Ki, nM (SEM, n) | 0.133 (0.005, 3) | 0.293 (0.030, 3) | 2.20 |
| IGF-1R Binding, Ki, nM (SEM, n) | 30.5 (7.1, 3) | 78.5 (6.2, 3) | 2.57 |
| IR-A phosphorylation, $EC_{50}$, nM (SEM, n) | 1.42 (0.23, 3) | 1.86 (0.23, 3) | 1.31 |
| IR-B phosphorylation$EC_{50}$, nM (SEM, n) | 0.916 (0.063, 3) | 1.50 (0.03, 3) | 1.64 |
| de novo Lipogenesis, $EC_{50}$, nM (SEM, n) | 0.148 (0.022, 4) | 0.516 (0.078, 5) | 3.49 |
| SAOS2 Thymidine, $EC_{50}$, nM (SEM, n) | 0.436 (0.019, 3) | 1.47 (0.08, 3) | 3.37 |
| H4IIe Thymidine, $EC_{50}$, nM (SEM, n) | 0.725 (0.124, 5) | 1.60 (0.27, 5) | 2.21 |

%Geometric mean using the log transformed Ki/$EC_{50}$ values (SEM, number runs, single lot)
$ Potency Ratio represents the difference in Ki/$EC_{50}$ calculated as a ratio of larger value over the smaller value

All in all, when combining results of all of the comparison of in vitro potency for LY2963016 from (b) (4) or Lilly France, EU-approved and US-approved Lantus, LY2963016 and Lantus, independent of location, showed that both were similar in their in vitro pharmacology as determined by IR and IGF1R binding affinity, IR receptor activation, de novo lipogenesis, and mitogenic potential.

### 4.2 Secondary Pharmacology

N/A

### 4.3 Safety Pharmacology

No safety pharmacology studies have been done with LY2963016.

## 5 Pharmacokinetics/ADME/Toxicokinetics

The TK data (from 28 day repeat dose rat toxicity study) showed exposure was similar for these two insulins (LY2963016 and Lantus) in each of the dose groups. No sex differences or accumulation was seen with daily dosing for 28 days in rats.

### 5.1 PK/ADME

N/A

### 5.2 Toxicokinetics

Note: TK parameters were included in their 28 day repeat dose toxicity rat study – Study No 8229488)

- Study No 8229488: LY2963016 levels were determined in serum samples with an RIA assay for insulin. Due to hemolysis and insufficient volume (attributed to multiple freeze-thaw cycles) of samples from Phase I TK animals, the data failed to meet acceptable criteria and to generate a valid result. This short-fall resulted in repeating the Day 1 TK analysis with Phase II TK animals. The sponsor justified that repeating the Day 1 TK analysis was sufficient enough to represent the daily exposure to LY2963016 throughout the 4 week study since there did not seem to have LY2963016 accumulation with repeated dosing in



Reference ID: 3527465

those Phase I TK animals. RESULTS: The TK of LY2963016 or US-Lantus were evaluated in rats after given LY2963016 or US-Lantus (0.3, 1, and 3 mg/kg) for > 4 weeks via sc injection. On Day 12, clinical signs and mortality (i.e. chronic hypoglycemia) were observed at 3 mg/kg. The dosing was on hold for Days 13 and 14 and was resumed on Day 15 at a lower dose of 2 mg/kg. The results showed that the duration of insulin exposure, above baseline, increased with increasing dose. On Day 1, exposure to LY2963016 and Lantus (AUC and $C_{max}$) generally increased with increasing dose, but were not consistently dose proportional.

# 6 General Toxicology

Two repeat dose toxicity rat study was conducted with LY2963016 and US- or EU-Lantus was used as a reference compound in this study. The dosing-limiting toxicity (e.g. hypoglycemia) was seen in rats with both LY2963016 and Lantus. A spectrum of effects (i.e. neuropathy and pancreatic islet cell atrophy) observed in these animals was associated with expected pharmacological effects of insulin in these animals.

## 6.1 Single-Dose Toxicity

There is no single-dose toxicity study with LY2963016. However, based on their repeat-dose toxicity rat study (Study No 8229488), the MTD of LY2963016 was 0.3 mg/kg in rats (same for Lantus).

## 6.2 Repeat-Dose Toxicity

The sponsor listed Lantus (NDA 021081) as their reference drug. The repeat-dose toxicity for Lantus was done in mice (daily sc injection for 3 months) and rats (daily sc injection for 3, 6, or 12 months).

**Table 14 Summary of Studies Conducted with Other Insulin Glargine**

**Summary of Repeat-Dose Toxicity Studies with Insulin Glargine (Stammberger et al. 2002)**

| Study description (duration/species) | Number | Treatment groups | Comments | Histologic examination | Tolerated dose (NOAEL) |
|---|---|---|---|---|---|
| 3 Months/mice | 10 M/10 F | 0 (vehicle control), 5, 10, 20 IU/kg/day insulin glargine | Slightly decreased body weight in females (20 IU/kg) 2 M in low-dose group and 3 F in high-dose group died | Pancreatic $\beta$-cell degranulation in the 10 and 20 IU/kg groups | MTD 10 IU/kg |
| 3 Months/rats | 15 M/15 F | 0 (vehicle control), 4, 12.5, 40 IU/kg/day insulin glargine | Severe hypoglycemia resulting in death of 19 animals at high dose, and 3 at median dose | Neuronal necrosis Pancreatic $\beta$-cell degranulation in 12.5 IU/kg group | MTD 12.5 IU/kg NOAEL 4.0 IU/kg |
| 6 Months/rats | 20 M/20 F | 0 (vehicle control), 2, 6.3, 20 IU/kg/day insulin glargine | Intercurrent deaths at high dose | Neuronal necrosis Dose-dependent pancreatic $\beta$-cell degranulation, reversible after the recovery period | NOAEL 6.3 IU/kg |
| 12 Months/rats | 30 M/30 F | 0 (control), 40 IU/kg/day, reduced to 20 IU/kg/day after day 111 | 20 M and 14 F rats were sacrificed or died intercurrently, attributed to compound-related hypoglycemia leading to cardiovascular failure | Pancreatic $\beta$-cell degranulation in insulin glargine–treated animals | MTD 12.5 IU/kg |

M = male; F = female; MTD = maximum tolerated dose; NOAEL = no-observable-adverse-effect level.
Note: LANTUS activity = 3.6378 mg/IU

In addition to published literature, the sponsor did conduct a bridging study and compare LY2963016 to Lantus. See below for details.

The sponsor did conduct a bridging study and compare LY2963016 to Lantus. See below for details.

## Study 8229488 – 4 wk rat study with US Lantus

**Study title: A Repeat-Dose Toxicity and Toxicokinetic Study in Rats Given LY2963016 or Lantus by Subcutaneous Injection for 4 Weeks**

| | |
|---|---|
| Study no.: | 8229488 |
| Study report location: | (b) (4) |
| Conducting laboratory and location: | |
| Date of study initiation: | July 12th 2010 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | LY2963016 (Lot MST006628-H), 98.4% purity |

**Key Study Findings**

- LY2963016 or Lantus given to rats at 0, 0.3, 1, and 3/2 mg/kg for 4 weeks via sc injection resulted in markedly reduced glucose levels with dose-dependent duration at all dose levels.
- Due to glucodynamics serum short-fall (severely hemolyzed Phase I TK samples), Phase II TK animals were added to repeat all Day 1 timepoints only in order to correct TK data.
- In general, lowest glucose levels were detected at 1-2 hrs postdose. The lowering glucose effect lasted approx. 2 hrs postdose at 0.3 mg/kg, 8 hrs postdose at 1.0 mg/kg, and 12-18 hrs postdose at 3/2 mg/kg LY2963016 or Lantus. Such effect is reversed approx. 24 hrs postdose.
- The LY2963016 administration exceeded the MTD at ≥ 1 mg/kg, due to clinical signs secondary to hypoglycemia and mortality.
- Hypoglycemia also occurred in the low-dose group for LY2963016 and US-Lantus but both compounds were tolerable and did not result in any mortality.
- Target organs were identified (occurred in low and mid dose groups): skin/subcutis, injection sites, sciatic nerve, and pancreas.
- Increased fat in the skin, subcutis, and at the injection sites (lipogenic pharmacological activity for insulins) were seen at all dose groups with LY2963016 and US-Lantus.
- Sciatic nerve degeneration (secondary to hypoglycemia) was seen in the LY2963016 and Lantus high dose groups.
- Islet cell degranulation or atrophy (i.e. decreased islet cell cytoplasm/vacuolation due to down-regulation of endogenous insulin synthesis, secondary to hypoglycemia) was seen in the LY2963016 and US-Lanus at ≥ 1 mg/kg.
- There were no off-target activities identified for either LY2963016 or US-Lantus.
- The NOAEL and MTD was 0.3 mg/kg/ for both LY2963016 and US-Lantus.



Reference ID: 3527465

Methods

| | |
|---|---|
| Doses: | 0.3, 1, 3/2 mg/kg for LY2963016 and Lantus |
| Frequency of dosing: | Daily |
| Route of administration: | Subcutaneous injection (rotating through 4 quadrants of the dorsal region – UL, UR, LL, LR) |
| Dose volume: | 3.64 mg/ml (100 IU/ml) |
| Formulation/Vehicle: | Vehicle control and diluent: glycerin, m-cresol,zinc oxide |
| Species/Strain: | Crl:CD(SD) rats |
| Number/Sex/Group: | Main: 10/sex/group, Phase I TK: 15/sex/group, Phase II TK: 19/sex/group |
| Age: | 10-11 weeks old (main and TK I), 13-14 weeks old (TK II) |
| Weight: | 333-484 g for males and 204-322 g for females (main and TK I)<br>355-445 g for males and 250-297 g for females (TK II) |
| Satellite groups: | Phase I TK group |
| Unique study design: | Phase II TK groups were to repeat all Day 1 timepoints in order to correct TK and glucodynamics serum short-fall. |
| Deviation from study protocol: | Yes but not affected the overall interpretation of study findings. |

**Table 15 Study No 8229488: Study Design**



Reference ID: 3527465

| Group | No. of Animals Male | No. of Animals Female | Dose Level (mg LY2963016 or LANTUS®/kg) | Dose Concentration (mg LY2963016 or LANTUS®/mL) |
|---|---|---|---|---|
| Toxicity Animals | | | | |
| LY2963016 | | | | |
| 1 (Control) | 10 | 10 | 0 | 0 |
| 2 (Low) | 10 | 10 | 0.3 | 0.3 |
| 3 (Mid) | 10 | 10 | 1 | 1 |
| 4 (High) | 10 | 10 | 3/2 | 3/2 |
| LANTUS® | | | | |
| 5 (Low) | 10 | 10 | 0.3 | 0.3 |
| 6 (Mid) | 10 | 10 | 1 | 1 |
| 7 (High) | 10 | 10 | 3/2 | 3/2 |
| Toxicokinetic Animals | | | | |
| LY2963016 | | | | |
| 8 (Control) | 15 | 15 | 0 | 0 |
| 9 (Low) | 15 | 15 | 0.3 | 0.3 |
| 10 (Mid) | 15 | 15 | 1 | 1 |
| 11(High) | 15 | 15 | 3/2 | 3/2 |
| LANTUS® | | | | |
| 12 (Low) | 15 | 15 | 0.3 | 0.3 |
| 13 (Mid) | 15 | 15 | 1 | 1 |
| 14 (High) | 15 | 15 | 3/2 | 3/2 |
| Phase 2 Toxicokinetic Animals | | | | |
| LY2963016 | | | | |
| 8 (Control) | 19 | 19 | 0 | 0 |
| 9 (Low) | 19 | 19 | 0.3 | 0.3 |
| 10 (Mid) | 19 | 19 | 1 | 1 |
| 11(High) | 19 | 19 | 2 | 2 |
| LANTUS® | | | | |
| 12 (Low) | 19 | 19 | 0.3 | 0.3 |
| 13 (Mid) | 19 | 19 | 1 | 1 |
| 14 (High) | 19 | 19 | 2 | 2 |

## Observations and Results

- Mortality (twice daily): There were 16 early deaths or unscheduled sacrifices occurred during the study. All deaths at ≥ 1 mg/kg of LY2963016 or US-Lantus were considered compound related.

**Table 16 Study No 8229488: Mortality**

**Unscheduled Deaths or Sacrifices**

| Total Number of animals sacrificed or found dead | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sex | Male | | | | | | Female | | | | | |
| Dose (mg LY2963016 or LANTUS®/kg) | 0 | 0.3 | 1 | 3[a] | 3/2[a] | 2[b] | 0 | 0.3 | 1 | 3[a] | 3/2[a] | 2[b] |
| Toxicity Animals | | | | | | | | | | | | |
| LY2963016 | - | - | 1 | - | - | NA | - | - | - | - | - | NA |
| LANTUS® | - | - | - | 1 | - | NA | - | - | - | 1 | - | NA |
| Toxicokinetic Animals (Phase 1 and 2) | | | | | | | | | | | | |
| LY2963016 | - | - | - | 1 | - | - | - | - | - | 1 | 3 | - |
| LANTUS® | - | 1 | - | 2 | - | - | - | 2[b] | - | 1 | 2 | - |

- = No deaths occurred; NA = not applicable.

a 7 animals given 3 mg/kg of LY2963016 or LANTUS® died or were sacrificed Days 1-14. Starting Day 15 of the dosing phase, dose level was reduced to 2 mg/kg and 5 females given 3/2 mg/kg of LY2963016 or LANTUS® died or were sacrificed Day 15 through the end of the dosing phase.

b Phase 2 Toxicokinetic animals were given a single dose of LY2963016 or LANTUS® at dose levels of 0, 0.3, 1, or 2 mg/kg. One Phase 2 Toxicokinetic female given 0.3 mg/kg of LANTUS® died following blood sampling.

- Clinical Signs (weekly): All compound related signs were seen in animals that died or were sacrificed early. These signs (i.e. hypoactivity, recumbency, irregular or labored respiration, cold to the touch, ataxia, and convulsion) were correlated with reduced glucose levels and were attributed to hypoglycemia.

- Body Weights (weekly) - Days 1, 8, 15, 22, 29, 30, and 33): There were compound-related increases in body weight gain (more pronounced in females) at 3/2 mg/kg, which were correlated with significantly increased food consumption probably to compensate reduced glucose levels.

**Table 17 Study No 8229488: Body Weight**

**Mean Body Weight Data**

| Test Article | Control | LY2963016 | | | LANTUS | | |
|---|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Level (mg/kg) | 0 | 0.3 | 1 | 3/2 | 0.3 | 1 | 3/2 |

| Day | | Mean body weights (g) for Group: 1M | 2M | 3M | 4M | Statistics |
|---|---|---|---|---|---|---|
| DSNG 1 | Mean | 405 | 406 | 405 | 403 | P |
| | SD | 19.6 | 27.6 | 28.5 | 27.1 | |
| | N | 10 | 10 | 10 | 10 | |
| DSNG 8 | Mean | 415 | 422 | 418 | 449 | P |
| | SD | 19.9 | 34.7 | 29.4 | 30.3 | |
| | N | 10 | 10 | 9 | 10 | |
| DSNG 15 | Mean | 445 | 453 | 454 | 459 | P |
| | SD | 22.9 | 36.7 | 33.8 | 31.1 | |
| | N | 10 | 10 | 9 | 10 | |
| DSNG 22 | Mean | 471 | 478 | 479 | 498 | P |
| | SD | 26.4 | 39.4 | 35.5 | 34.8 | |
| | N | 10 | 10 | 9 | 10 | |
| DSNG 29 | Mean | 491 | 501 | 503 | 526 | P |
| | SD | 26.9 | 40.7 | 42.3 | 36.4 | |
| | N | 10 | 10 | 9 | 10 | |
| DSNG 30 | Mean | 485 | 496 | 502 | - | P |
| | SD | 29.1 | 41.2 | 38.8 | - | |
| | N | 10 | 10 | 9 | 0 | |
| DSNG 33 | Mean | - | - | - | 545 | X |
| | SD | - | - | - | 42.2 | |
| | N | 0 | 0 | 0 | 10 | |

P = ANOVA (and Dunnett's, if applicable)
X = not analyzed

Mean Body Weight Data

| Test Article | Control | LY2963016 | | | LANTUS | | |
|---|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Level (mg/kg) | 0 | 0.3 | 1 | 3/2 | 0.3 | 1 | 3/2 |

| Day | | Mean body weights (g) for Group: 1F | 2F | 3F | 4F | Statistics |
|---|---|---|---|---|---|---|
| DSNG 1 | Mean | 254 | 253 | 253 | 253 | P |
| | SD | 20.2 | 27.9 | 14.7 | 18.8 | |
| | N | 10 | 10 | 10 | 10 | |
| DSNG 8 | Mean | 257 | 257 | 257 | 288* | P |
| | SD | 23.1 | 28.2 | 15.9 | 16.1 | |
| | N | 10 | 10 | 10 | 10 | |
| DSNG 15 | Mean | 266 | 269 | 271 | 279 | P |
| | SD | 25.2 | 26.7 | 18.2 | 18.4 | |
| | N | 10 | 10 | 10 | 10 | |
| DSNG 22 | Mean | 269 | 280 | 280 | 304* | P |
| | SD | 22.3 | 27.9 | 20.9 | 16.0 | |
| | N | 10 | 10 | 10 | 10 | |
| DSNG 29 | Mean | 282 | 291 | 289 | 321* | P |
| | SD | 25.2 | 30.2 | 20.4 | 19.4 | |
| | N | 10 | 10 | 10 | 10 | |
| DSNG 30 | Mean | 280 | 287 | 286 | - | P |
| | SD | 24.6 | 32.0 | 19.8 | - | |
| | N | 10 | 10 | 10 | 0 | |
| DSNG 33 | Mean | - | - | - | 332 | X |
| | SD | - | - | - | 19.4 | |
| | N | 0 | 0 | 0 | 10 | |

* P< or =0.05

P = ANOVA (and Dunnett's, if applicable)
X = not analyzed

Mean Body Weight Data

| Test Article | Control | LY2963016 | | | LANTUS | | |
|---|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Level (mg/kg) | 0 | 0.3 | 1 | 3/2 | 0.3 | 1 | 3/2 |

| Day | | Mean body weights (g) for Group: 5M | 6M | 7M | Statistics |
|---|---|---|---|---|---|
| DSNG 1 | Mean | 411 | 404 | 402 | P |
| | SD | 35.7 | 28.9 | 28.0 | |
| | N | 10 | 10 | 10 | |
| DSNG 8 | Mean | 432 | 422 | 456* | P |
| | SD | 42.1 | 27.3 | 32.5 | |
| | N | 10 | 10 | 9 | |
| DSNG 15 | Mean | 462 | 450 | 455 | P |
| | SD | 45.0 | 25.5 | 33.6 | |
| | N | 10 | 10 | 9 | |
| DSNG 22 | Mean | 491 | 477 | 488 | P |
| | SD | 47.8 | 22.6 | 38.8 | |
| | N | 10 | 10 | 9 | |
| DSNG 29 | Mean | 512 | 501 | 521 | P |
| | SD | 54.3 | 20.2 | 37.8 | |
| | N | 10 | 10 | 9 | |
| DSNG 30 | Mean | 513 | 500 | - | P |
| | SD | 49.8 | 23.3 | - | |
| | N | 10 | 10 | 0 | |
| DSNG 33 | Mean | - | - | 538 | X |
| | SD | - | - | 36.2 | |
| | N | 0 | 0 | 9 | |

* P< or =0.05

P = ANOVA (and Dunnett's, if applicable)
X = not analyzed



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Mean Body Weight Data

| Test Article | Control | LY2963016 | | | LANTUS | | |
|---|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Level (mg/kg) | 0 | 0.3 | 1 | 3/2 | 0.3 | 1 | 3/2 |

| Day | | Mean body weights (g) for Group: 5F | 6F | 7F | Statistics |
|---|---|---|---|---|---|
| DSNG 1 | Mean | 254 | 250 | 251 | P |
| | SD | 17.5 | 17.0 | 16.7 | |
| | N | 10 | 10 | 10 | |
| DSNG 8 | Mean | 259 | 258 | 287* | P |
| | SD | 18.7 | 17.9 | 21.1 | |
| | N | 10 | 10 | 10 | |
| DSNG 15 | Mean | 268 | 264 | 282 | P |
| | SD | 22.8 | 17.3 | 26.4 | |
| | N | 10 | 10 | 9 | |
| DSNG 22 | Mean | 282 | 276 | 305* | P |
| | SD | 23.4 | 18.9 | 29.3 | |
| | N | 10 | 10 | 9 | |
| DSNG 29 | Mean | 287 | 281 | 323* | P |
| | SD | 23.1 | 20.3 | 33.8 | |
| | N | 10 | 10 | 9 | |
| DSNG 30 | Mean | 285 | 283 | - | P |
| | SD | 25.0 | 18.9 | - | |
| | N | 10 | 10 | 0 | |
| DSNG 33 | Mean | - | - | 335 | X |
| | SD | - | - | 31.2 | |
| | N | 0 | 0 | 9 | |

* P< or =0.05

P = ANOVA (and Dunnett's, if applicable)
X = not analyzed

- Feed Consumption (twice weekly): The food consumption was significantly increased at 3/2 mg/kg LY2963016 or Lantus, correlating with effect on body weight.

- Ophthalmoscopy (Day 27): One male at 1 mg/kg Lantus had corneal dystrophy in the right eye (incidental). No other findings observed in other animals at any dose level.

- ECG: This was not done with this study.

- Hematology (Blood samples were collected on the day of scheduled sacrifice for Groups 1-4 and Groups 5-7- Day 30 or 33): No remarkable findings were noted in hematology and coagulation parameters in animals that survived until the end of the dosing phase.

- Clinical Chemistry (Blood samples were collected on the day of scheduled sacrifice for Groups 1-4 and Groups 5-7 - Day 30 or 33): No remarkable findings were noted (except glucose) in clinical chemistry parameters in animals that survived until the end of the dosing phase.

During Phase I, TK animals had low glucose levels (expected pharmacological effect of insulin) at all dose levels (≥ 0.3 mg/kg LY2963016 or Lantus). In these animals, lowest glucose levels occurred 1-2 hrs postdose (similar regardless of LY2963016 or Lantus). This appeared to be dose-dependent at later time points. Glucose levels decreased persisted for longer durations at higher doses. For LY2963016 or Lantus, lower glucose level generally lasted approx 2 hrs postdose at 0.3 mg/kg and approx 8 hrs postdose at 1 mg/kg, approx 12-18 hrs postdose at 3/2



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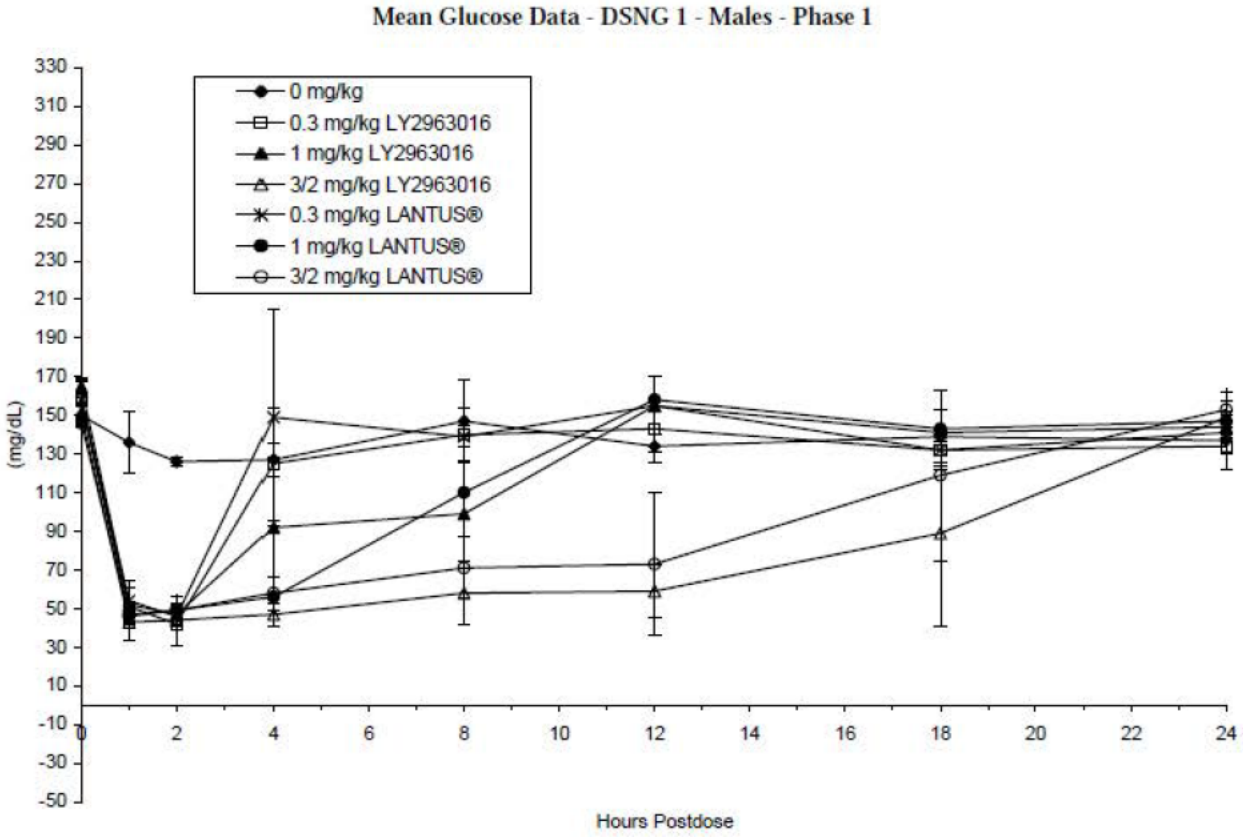
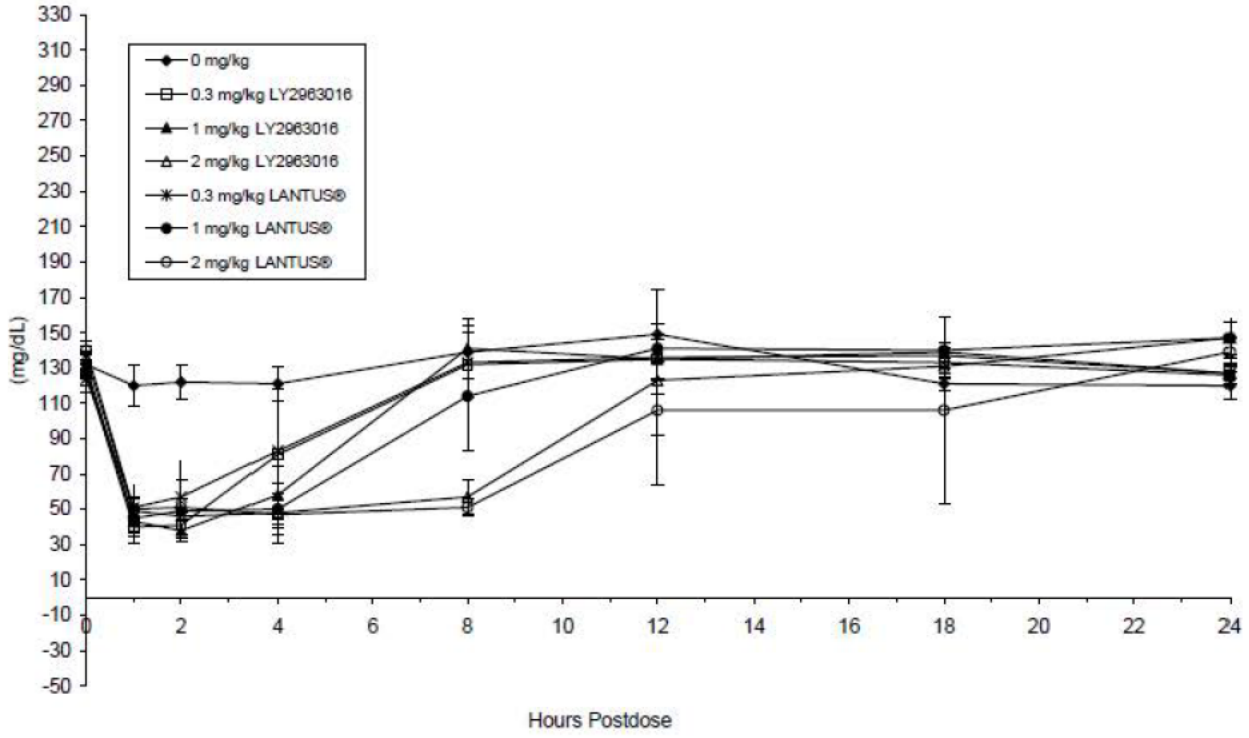
mg/kg. Such effect can be reversed by 24 hrs postdose. During Phase II, glucose data was only collected Day 1. These TK animals had similar patterns of lower glucose level as seen in Phase I (as described above).

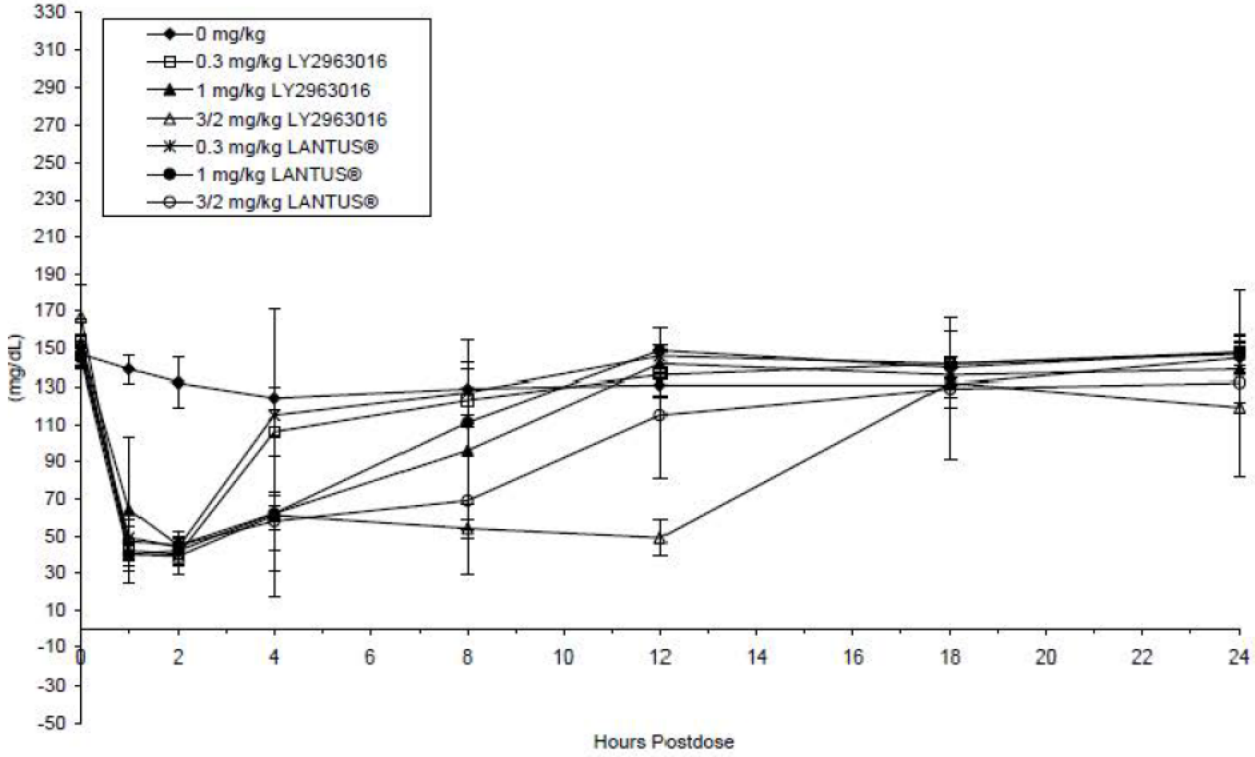
**Figure 1 Study No 8229488 – Glucose Data on Days 1, 15, and 29**



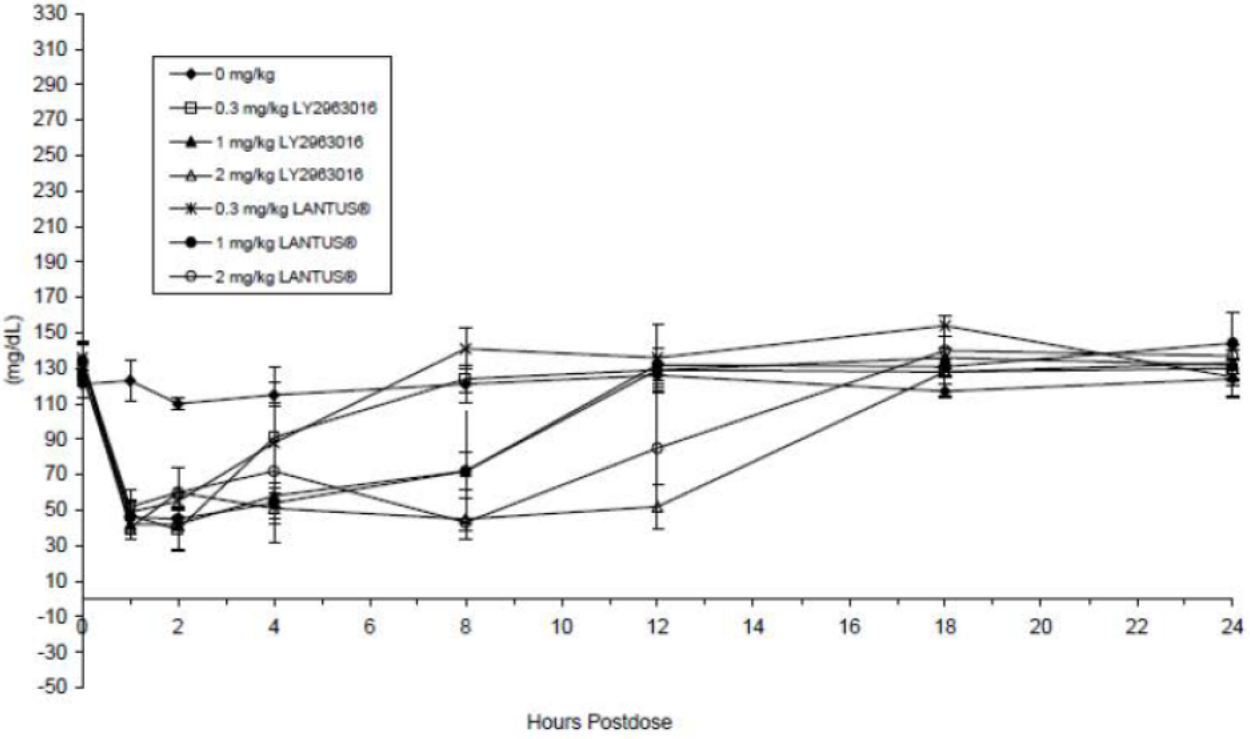
Mean Glucose Data - DSNG 1 - Males - Phase 2



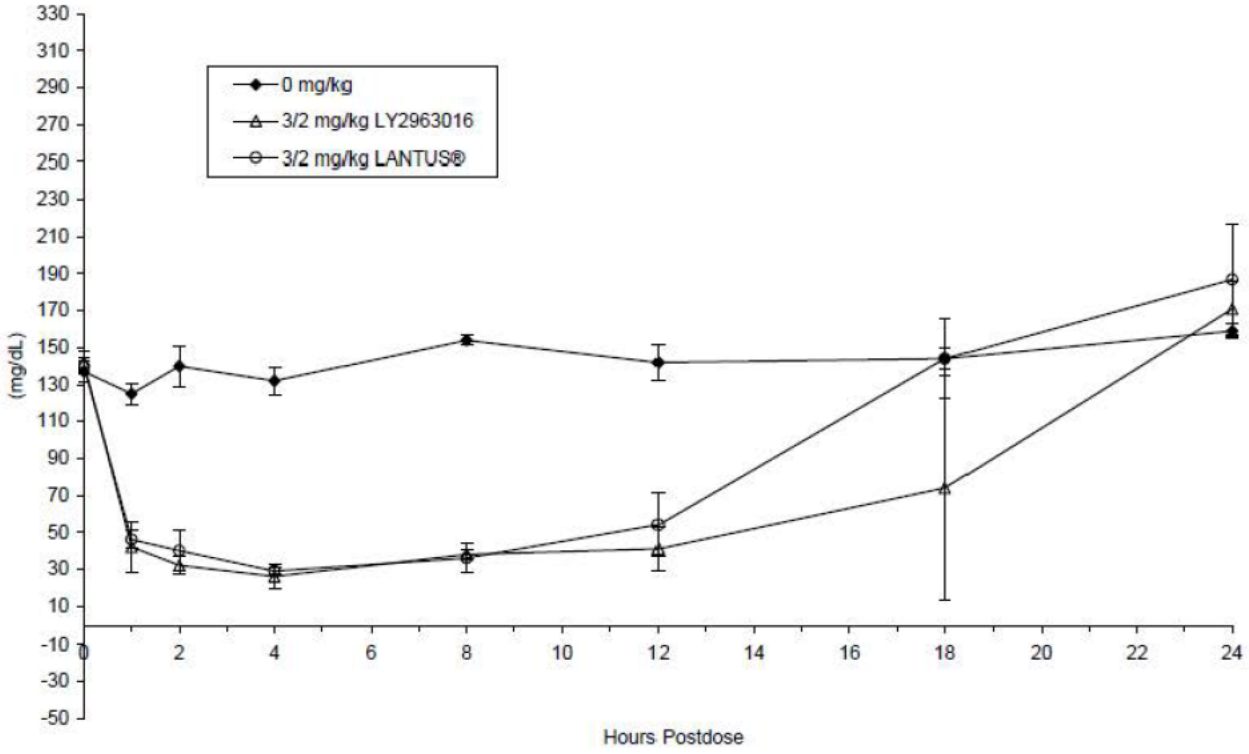

Mean Glucose Data - DSNG 1 - Females - Phase 1



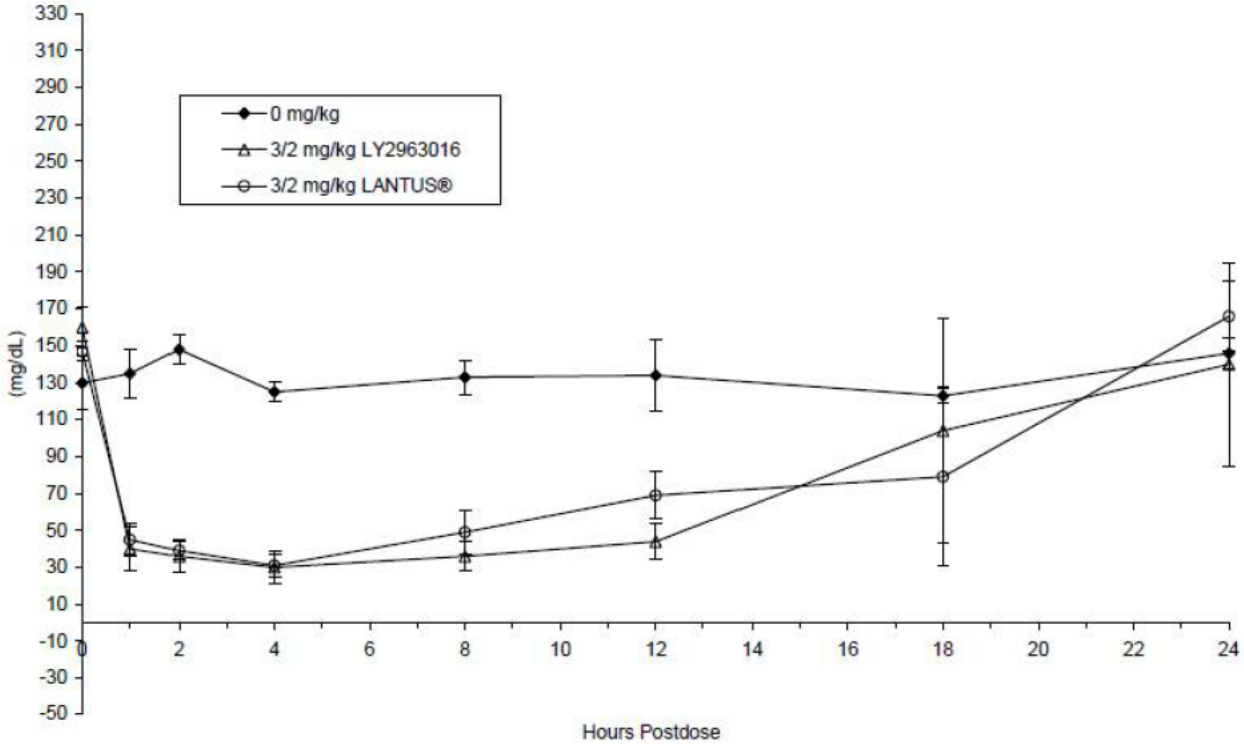
Mean Glucose Data - DSNG 1 - Females - Phase 2



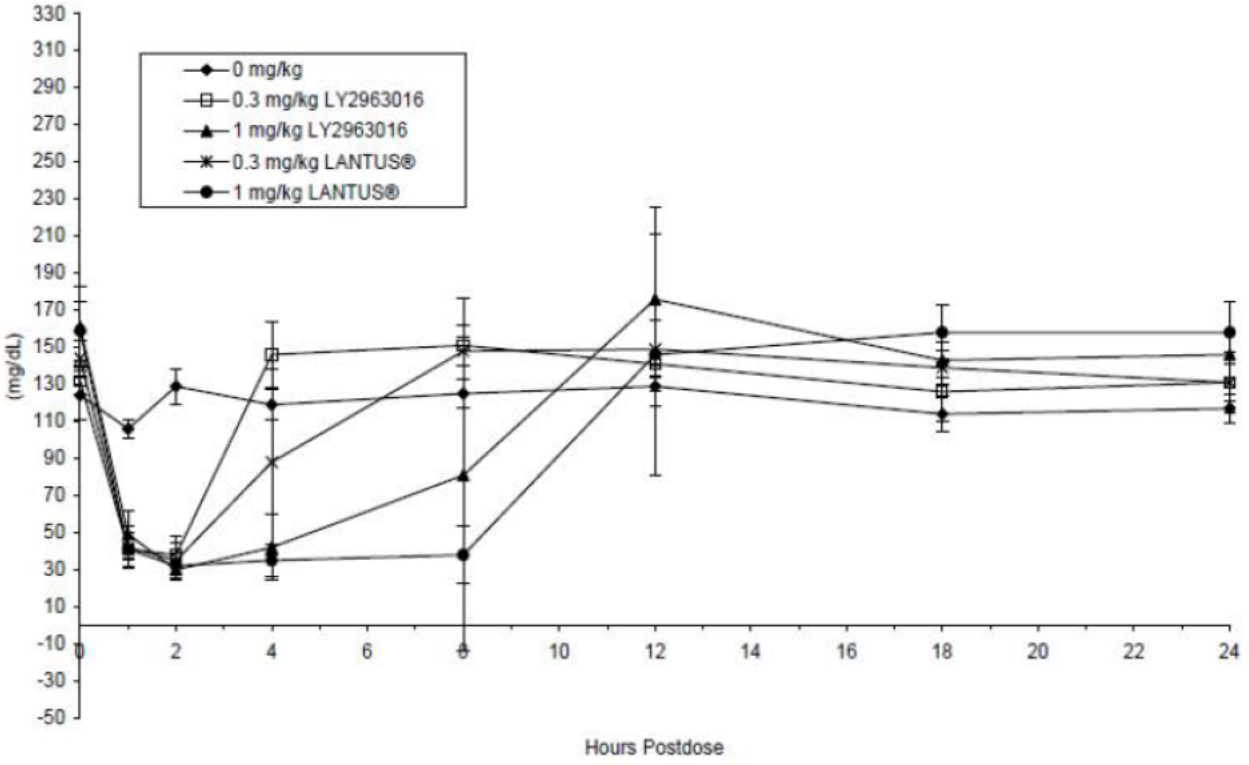

Mean Glucose Data - DSNG 15 - Males

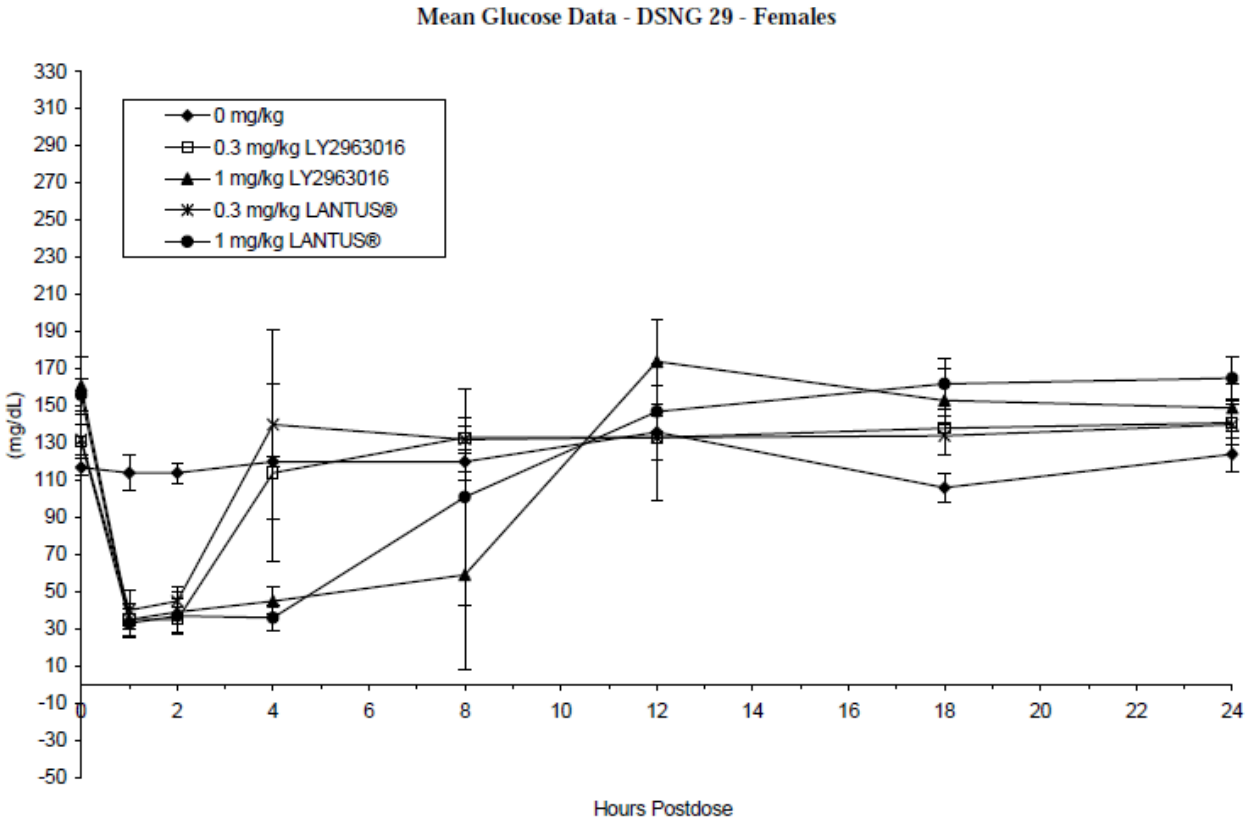


Mean Glucose Data - DSNG 15- Females




Mean Glucose Data - DSNG 29 - Males






- Urinalysis (Blood samples were collected on the day of scheduled sacrifice from Groups 1-4 and Groups 5-7 - Day 30 or 33): No remarkable findings were noted in urinalysis parameters in animals that survived until the end of the dosing phase.

- Gross Pathology (A necropsy was done at scheduled sacrifice): There were no compound related macroscopic observations.

**Table 18 Study No 8229488 - Macroscopic Findings**

**Summary of Macroscopic Observations - Dosing Phase - Final Phase Sacrifice**

| | -- Males -- | | | | | | | -- Females -- | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Number in group: | 10 | 10 | 9 | 10 | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| Examined/No remarkable findings ... | 7 | 7 | 5 | 6 | 7 | 8 | 4 | 8 | 7 | 5 | 6 | 10 | 8 | 3 |
| Liver | | | | | | | | | | | | | | |
| Mottled ..... | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total: ..... | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lung | | | | | | | | | | | | | | |
| Discolored ..... | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total: ..... | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Thymus | | | | | | | | | | | | | | |
| Discolored ..... | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total: ..... | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kidney | | | | | | | | | | | | | | |
| Large ..... | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| Total: ..... | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| Urinary Bladder | | | | | | | | | | | | | | |
| Calculus ..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Total: ..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Stomach, Gl | | | | | | | | | | | | | | |
| Discolored ..... | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total: ..... | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN, Mandibular | | | | | | | | | | | | | | |
| Discolored ..... | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Large ..... | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Total: ..... | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Eye | | | | | | | | | | | | | | |
| Collapsed ..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Discolored ..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Protruding ..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Total: ..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| Testis | | | | | | | | | | | | | | |
| Small ..... | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total: ..... | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Epididymis | | | | | | | | | | | | | | |
| Small ..... | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Summary of Macroscopic Observations
Dosing Phase - Final Phase Sacrifice

| | -- Males -- | | | | | | | -- Females -- | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group: | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Number in group: | 10 | 10 | 9 | 10 | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| Epididymis | | | | | | | | | | | | | | |
| Total: ..... | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN, Axillary | | | | | | | | | | | | | | |
| Discolored ..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Large ..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Total: ..... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Subcutan Site A | | | | | | | | | | | | | | |
| Discolored ..... | 3 | 2 | 3 | 1 | 3 | 1 | 0 | 2 | 0 | 3 | 1 | 0 | 0 | 1 |
| Thickened ..... | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total: ..... | 3 | 2 | 4 | 1 | 3 | 1 | 0 | 2 | 0 | 3 | 1 | 0 | 0 | 1 |
| Subcutan Site B | | | | | | | | | | | | | | |
| Discolored ..... | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| Total: ..... | 1 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 |
| Subcutan Site C | | | | | | | | | | | | | | |
| Discolored ..... | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 |
| Total: ..... | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 |
| Subcutan Site D | | | | | | | | | | | | | | |
| Discolored ..... | 0 | 0 | 1 | 2 | 1 | 0 | 2 | 1 | 0 | 1 | 1 | 0 | 1 | 3 |
| Total: ..... | 0 | 0 | 1 | 2 | 1 | 0 | 2 | 1 | 0 | 1 | 1 | 0 | 1 | 3 |

## Summary of Macroscopic Observations - Unscheduled Sacrifices and Deaths

| | -- Males -- | | -- Females -- | |
|---|---|---|---|---|
| Group: | 3 | 7 | 3 | 7 |
| Number in group: | 1 | 1 | 0 | 1 |
| Examined/No remarkable findings ... | 1 | 0 | 0 | 0 |
| Lung | | | | |
| Discolored ..... | 0 | 0 | 0 | 1 |
| Total: ..... | 0 | 0 | 0 | 1 |
| Stomach, Gl | | | | |
| Discolored ..... | 0 | 1 | 0 | 0 |
| Total: ..... | 0 | 1 | 0 | 0 |
| Subcutan Site B | | | | |
| Discolored ..... | 0 | 1 | 0 | 0 |
| Total: ..... | 0 | 1 | 0 | 0 |

- Organ Weights (Organ weights were recorded at the scheduled sacrifice): Compound-related increases in terminal body weight resulted in changes in organ weight parameters of some organs. For example, there were significant increases in absolute liver weight and liver:brain weight ratios for animals given 3/2 mg/kg LY2963016 or Lantus. These findings were considered secondary to significantly increased terminal body weights.

- Histopathology: 1) Adequate Battery: Yes. Tissues were collected and fixed at the scheduled (or unscheduled) sacrifice. 2) Peer Review: Pathology peer review certification is included in the study report.

Histological Findings: Axonal degeneration of the sciatic nerve occurring at 3/2 mg/kg of LY2963016 or Lantus was observed at 3/2 mg/kg. This finding was consistent with other insulin analog due to prolonged hypoglycemia. Other compound-related findings included increased adipose tissue in the skin/subcutis at ≥ 0.3 mg/kg (due to insulin pharmacology) and decreased vacuolation and cytoplasm of the pancreatic islet cells at ≥ 1 mg/kg (due to feedback inhibition of insulin gene expression by exogenous insulin, expected pharmacological effect of insulin).
Table 19 Key Microscopic Findings of LY2963016 vs. Lantus

**Table 20 Study No 8229488 - Microscopic Findings at Scheduled Sacrifice**

**Summary of Microscopic Observations - Dosing Phase - Final Phase Sacrifice**

Controls from group(s): 1

| Tissues With Diagnoses | Animal sex: | Animals Affected -- Males -- | | | | | | -- Females -- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 2 | 3 | 4 | 5 | 6 | Ctls | 2 | 3 | 4 | 5 | 6 |
| | No. in group: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Brain ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Vacuoles, Neuron | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Spinal Cord ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Adrenal, Cortex ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Adrenal, Medulla ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Pituitary ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Cyst | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nerve, Sciatic ..... | Number examined: | 10 | 9 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Unremarkable: | 9 | 9 | 9 | 6 | 10 | 9 | 10 | 9 | 10 | 6 | 9 | 10 |
| Degeneration, Axon | | 1 | 0 | 0 | 4 | 0 | 1 | 0 | 1 | 0 | 4 | 1 | 0 |
| Trachea ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Esophagus ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Thyroid ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Parathyroid ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 7 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 7 | 0 | 0 |
| Heart ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 9 | 0 | 0 | 8 | 0 | 0 | 10 | 0 | 0 | 9 | 0 | 0 |
| Infiltrate, Lymphocytes | | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Aorta ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10



Reference ID: 3527465

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

Controls from group(s): 1

| Tissues With Diagnoses | Animal sex: | -- Males -- | | | | | | -- Females -- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 2 | 3 | 4 | 5 | 6 | Ctls | 2 | 3 | 4 | 5 | 6 |
| | No. in group: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Tongue ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Muscle, Bi Fem ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Liver ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 7 | 0 | 0 | 6 | 0 | 0 | 5 | 0 | 0 | 8 | 0 | 0 |
| Vacuolation, Hepatocyte | | 3 | 0 | 0 | 4 | 0 | 0 | 4 | 0 | 0 | 2 | 0 | 0 |
| Infiltrate, Mononuclear | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Spleen ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lung ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 9 | 0 | 0 | 9 | 0 | 0 |
| Infiltrate, Mixed | | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | **1** | 0 | 0 |
| Congestion | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Thymus ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Congestion | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Kidney ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 9 | 0 | 0 | 8 | 0 | 0 | 9 | 0 | 0 | 7 | 0 | 0 |
| Basophilic Tubule | | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dilatation, Pelvis | | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mineralization, Tubule | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 |
| Infiltrate, Lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hyaline Droplet, Tubule Cell | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **1** | 0 | 0 |
| Dilatation, Tubule(s) | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **1** | 0 | 0 |
| Urinary Bladder ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Stomach, Gl ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 9 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Infiltrate, Lymphocytes/Macrophages (Mesenteric Fat) | | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration/Necrosis | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

Controls from group(s): 1

| Tissues With Diagnoses | Animal sex: | -- Males -- | | | | | | -- Females -- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 2 | 3 | 4 | 5 | 6 | Ctls | 2 | 3 | 4 | 5 | 6 |
| | No. in group: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Stomach, Nongl ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Hyperkeratosis | | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Duodenum ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Ileum ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Peyer's Patch ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Colon ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Cecum ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Jejunum ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| LN, Mesenteric ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LN, Mandibular ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gl, Mandib Saliv ..... | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Pancreas ..... | Number examined: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Unremarkable: | 10 | 9 | 6 | 3 | 9 | 6 | 9 | 10 | 9 | 3 | 9 | 8 |
| Decreased Zymogen Granules | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Decreased Cytoplasm/Vacuolation, Islet Cells | | 0 | 0 | 2 | 7 | 0 | 2 | 0 | 0 | 1 | 7 | 0 | 2 |
| Infiltrate, Lymphocytes | | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 |
| Apoptosis, Acinar Cells | | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 |
| Atrophy, Acinar Cells | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Atrophy, Lobular | | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

Controls from group(s): 1

| Tissues With Diagnoses | Animal sex: | -- Males -- Animals Affected -- | | | | | | -- Females -- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 2 | 3 | 4 | 5 | 6 | Ctls | 2 | 3 | 4 | 5 | 6 |
| | No. in group: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Nerve, Optic | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Eye | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Skin/Subcutis | Number examined: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Unremarkable: | 10 | 9 | 7 | 4 | 10 | 3 | 10 | 9 | 7 | 0 | 9 | 5 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 1 | 2 | 6 | 0 | 7 | 0 | 1 | 3 | 10 | 1 | 5 |
| Mammary, Male | Number examined: | 9 | 0 | 0 | 10 | 0 | 0 | | | | | | |
| | Unremarkable: | 9 | 0 | 0 | 10 | 0 | 0 | | | | | | |
| Seminal Vesicle | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | | | | | | |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | | | | | | |
| Prostate | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | | | | | | |
| | Unremarkable: | 10 | 0 | 0 | 8 | 0 | 0 | | | | | | |
| Infiltrate, Lymphocytes | | 0 | 0 | 0 | 2 | 0 | 0 | | | | | | |
| Testis | Number examined: | 10 | 0 | 4 | 10 | 0 | 5 | | | | | | |
| | Unremarkable: | 10 | 0 | 4 | 9 | 0 | 5 | | | | | | |
| Atrophy/Degeneration, Seminiferous Tubules (Bilateral) | | 0 | 0 | 0 | 1 | 0 | 0 | | | | | | |
| Epididymis | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | | | | | | |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | | | | | | |
| Debris, Cellular, Lumen (Bilateral) | | 0 | 0 | 0 | 1 | 0 | 0 | | | | | | |
| Decreased Sperm (Bilateral) | | 0 | 0 | 0 | 1 | 0 | 0 | | | | | | |
| Mammary, Female | Number examined: | | | | | | | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | | | | | | | 10 | 0 | 0 | 10 | 0 | 0 |
| Ovary | Number examined: | | | | | | | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | | | | | | | 10 | 0 | 0 | 10 | 0 | 0 |
| Uterus | Number examined: | | | | | | | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | | | | | | | 6 | 0 | 0 | 8 | 0 | 0 |
| Dilatation | | | | | | | | 4 | 0 | 0 | 1 | 0 | 0 |
| Atrophy | | | | | | | | 0 | 0 | 0 | 1 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

Controls from group(s): 1

| Tissues With Diagnoses | Animal sex: | -- Males -- Animals Affected -- | | | | | | -- Females -- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 2 | 3 | 4 | 5 | 6 | Ctls | 2 | 3 | 4 | 5 | 6 |
| | No. in group: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Cervix | Number examined: | | | | | | | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | | | | | | | 10 | 0 | 0 | 9 | 0 | 0 |
| Atrophy | | | | | | | | 0 | 0 | 0 | 1 | 0 | 0 |
| Vagina | Number examined: | | | | | | | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | | | | | | | 10 | 0 | 0 | 9 | 0 | 0 |
| Atrophy, Mucosal | | | | | | | | 0 | 0 | 0 | 1 | 0 | 0 |
| Bone, Femur | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Marrow, Femur | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Bone, Sternum | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Marrow, Sternum | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| LN, Axillary | Number examined: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 | 10 | 0 | 0 |
| Congestion/Hemorrhage | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Death Comment | Number examined: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Scheduled Sacrifice | | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Undetermined | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Subcutan Site A | Number examined: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Unremarkable: | 4 | 2 | 1 | 1 | 2 | 5 | 4 | 2 | 3 | 0 | 2 | 7 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 1 | 7 | 0 | 1 | 0 | 1 | 3 | 10 | 2 | 3 |
| Infiltrate, Lymphocytes/Macrophages | | 5 | 7 | 8 | 2 | 8 | 4 | 6 | 8 | 6 | 3 | 7 | 0 |
| Degeneration, Muscle (Focal) | | 0 | 2 | 4 | 4 | 0 | 0 | 3 | 3 | 3 | 3 | 1 | 0 |
| Congestion/Hemorrhage | | 3 | 4 | 3 | 1 | 5 | 0 | 1 | 1 | 1 | 1 | 1 | 0 |
| Degeneration/Necrosis, Vessels | | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| Granuloma, Hair Shaft | | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | | | | | -- Females -- | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 2 | 3 | 4 | 5 | 6 | Ctls | 2 | 3 | 4 | 5 | 6 |
| Tissues With Diagnoses | No. in group: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Subcutan Site B ..... | Number examined: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Unremarkable: | 6 | 6 | 5 | 2 | 5 | 5 | 6 | 5 | 3 | 0 | 4 | 3 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 7 | 0 | 1 | 0 | 0 | 2 | 10 | 0 | 3 |
| Infiltrate, Lymphocytes/Macrophages | | 4 | 4 | 4 | 3 | 4 | 2 | 2 | 4 | 4 | 2 | 6 | 4 |
| Degeneration, Muscle (Focal) | | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 3 | 1 | 0 | 1 |
| Congestion/Hemorrhage | | 0 | 1 | 0 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| Degeneration/Necrosis, Vessels | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| Granuloma, Hair Shaft | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Subcutan Site C ..... | Number examined: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Unremarkable: | 8 | 8 | 7 | 1 | 7 | 4 | 6 | 5 | 4 | 0 | 5 | 3 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 5 | 0 | 2 | 0 | 1 | 2 | 10 | 2 | 2 |
| Infiltrate, Lymphocytes/Macrophages | | 2 | 2 | 1 | 4 | 2 | 3 | 3 | 4 | 2 | 2 | 2 | 3 |
| Degeneration, Muscle (Focal) | | 2 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 0 | 1 | 1 | 0 |
| Congestion/Hemorrhage | | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 2 |
| Granuloma, Hair Shaft | | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 2 | 0 | 1 | 3 |
| Pigment-Laden Macrophages | | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Subcutan Site D ..... | Number examined: | 10 | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| | Unremarkable: | 7 | 6 | 5 | 1 | 9 | 8 | 9 | 5 | 7 | 1 | 8 | 5 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 5 | 0 | 1 | 0 | 1 | 1 | 9 | 0 | 3 |
| Infiltrate, Lymphocytes/Macrophages | | 2 | 3 | 3 | 6 | 1 | 1 | 1 | 4 | 2 | 4 | 1 | 3 |
| Degeneration, Muscle (Focal) | | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 2 | 1 | 0 |
| Congestion/Hemorrhage | | 0 | 0 | 2 | 1 | 1 | 1 | 1 | 0 | 1 | 2 | 0 | 2 |
| Granuloma, Hair Shaft | | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 | 1 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | -- Females -- | |
|---|---|---|---|---|---|
| | Dosage group: | Ctls | 7 | Ctls | 7 |
| Tissues With Diagnoses | No. in group: | 10 | 9 | 10 | 9 |
| Brain ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Vacuoles, Neuron | | 0 | 0 | 0 | 0 |
| Spinal Cord ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Adrenal, Cortex ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Adrenal, Medulla ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Pituitary ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 8 | 10 | 9 |
| Cyst | | 0 | 1 | 0 | 0 |
| Nerve, Sciatic ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 9 | 5 | 10 | 6 |
| Degeneration, Axon | | 1 | 4 | 0 | 3 |
| Trachea ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Esophagus ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Thyroid ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Parathyroid ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Heart ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 9 | 8 | 10 | 9 |
| Infiltrate, Lymphocytes | | 1 | 1 | 0 | 0 |
| Aorta ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | -- Females -- | |
|---|---|---|---|---|---|
| | Dosage group: | Ctls | 7 | Ctls | 7 |
| Tissues With Diagnoses | No. in group: | 10 | 9 | 10 | 9 |
| Tongue | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Muscle, Bi Fem | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Liver | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 7 | 6 | 5 | 6 |
| Vacuolation, Hepatocyte | | 3 | 3 | 4 | 3 |
| Infiltrate, Mononuclear | | 0 | 1 | 1 | 0 |
| Spleen | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 |
| Lung | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 9 | 8 |
| Infiltrate, Mixed | | 0 | 0 | 1 | 1 |
| Congestion | | 0 | 0 | 0 | 0 |
| Thymus | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 |
| Congestion | | 0 | 0 | 0 | 0 |
| Kidney | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 9 | 8 | 9 | 8 |
| Basophilic Tubule | | 0 | 1 | 0 | 0 |
| Dilatation, Pelvis | | 1 | 0 | 0 | 0 |
| Mineralization, Tubule | | 0 | 0 | 1 | 1 |
| Infiltrate, Lymphocytes | | 0 | 0 | 0 | 0 |
| Hyaline Droplet, Tubule Cell | | 0 | 0 | 0 | 0 |
| Dilatation, Tubule(s) | | 0 | 0 | 0 | 0 |
| Urinary Bladder | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Stomach, Gl | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 9 | 9 | 10 | 9 |
| Infiltrate, Lymphocytes/Macrophages (Mesenteric Fat) | | 1 | 0 | 0 | 0 |
| Degeneration/Necrosis | | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | -- Females -- | |
|---|---|---|---|---|---|
| | Dosage group: | Ctls | 7 | Ctls | 7 |
| Tissues With Diagnoses | No. in group: | 10 | 9 | 10 | 9 |
| Stomach, Nongl | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Hyperkeratosis | | 0 | 0 | 0 | 0 |
| Duodenum | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Ileum | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Peyer's Patch | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Colon | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Cecum | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Jejunum | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| LN, Mesenteric | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 |
| LN, Mandibular | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 |
| Gl, Mandib Saliv | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Pancreas | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 3 | 9 | 1 |
| Decreased Zymogen Granules | | 0 | 0 | 0 | 1 |
| Decreased Cytoplasm/Vacuolation, Islet Cells | | 0 | 6 | 0 | 8 |
| Infiltrate, Lymphocytes | | 0 | 0 | 1 | 0 |
| Apoptosis, Acinar Cells | | 0 | 0 | 0 | 0 |
| Atrophy, Acinar Cells | | 0 | 0 | 0 | 0 |
| Atrophy, Lobular | | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | -- Females -- | |
|---|---|---|---|---|---|
| | Dosage group: | Ctls | 7 | Ctls | 7 |
| Tissues With Diagnoses | No. in group: | 10 | 9 | 10 | 9 |
| Nerve, Optic | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Eye | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Skin/Subcutis | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 1 | 10 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 8 | 0 | 9 |
| Mammary, Male | Number examined: | 9 | 8 | | |
| | Unremarkable: | 9 | 8 | | |
| Seminal Vesicle | Number examined: | 10 | 9 | | |
| | Unremarkable: | 10 | 9 | | |
| Prostate | Number examined: | 10 | 9 | | |
| | Unremarkable: | 10 | 9 | | |
| Infiltrate, Lymphocytes | | 0 | 0 | | |
| Testis | Number examined: | 10 | 9 | | |
| | Unremarkable: | 10 | 9 | | |
| Atrophy/Degeneration, Seminiferous Tubules (Bilateral) | | 0 | 0 | | |
| Epididymis | Number examined: | 10 | 9 | | |
| | Unremarkable: | 10 | 9 | | |
| Debris, Cellular, Lumen (Bilateral) | | 0 | 0 | | |
| Decreased Sperm (Bilateral) | | 0 | 0 | | |
| Mammary, Female | Number examined: | | | 10 | 9 |
| | Unremarkable: | | | 10 | 9 |
| Ovary | Number examined: | | | 10 | 9 |
| | Unremarkable: | | | 10 | 9 |
| Uterus | Number examined: | | | 10 | 9 |
| | Unremarkable: | | | 6 | 8 |
| Dilatation | | | | 4 | 1 |
| Atrophy | | | | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | -- Females -- | |
|---|---|---|---|---|---|
| | Dosage group: | Ctls | 7 | Ctls | 7 |
| Tissues With Diagnoses | No. in group: | 10 | 9 | 10 | 9 |
| Cervix | Number examined: | | | 10 | 9 |
| | Unremarkable: | | | 10 | 9 |
| Atrophy | | | | 0 | 0 |
| Vagina | Number examined: | | | 10 | 8 |
| | Unremarkable: | | | 10 | 8 |
| Atrophy, Mucosal | | | | 0 | 0 |
| Bone, Femur | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Marrow, Femur | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Bone, Sternum | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| Marrow, Sternum | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 9 |
| LN, Axillary | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 10 | 9 | 10 | 8 |
| Congestion/Hemorrhage | | 0 | 0 | 0 | 1 |
| Apoptosis, Lymphocytes | | 0 | 0 | 0 | 0 |
| Death Comment | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 0 | 0 | 0 | 0 |
| Scheduled Sacrifice | | 10 | 9 | 10 | 9 |
| Undetermined | | 0 | 0 | 0 | 0 |
| Subcutan Site A | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 4 | 2 | 4 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 7 | 0 | 9 |
| Infiltrate, Lymphocytes/Macrophages | | 5 | 2 | 6 | 2 |
| Degeneration, Muscle (Focal) | | 0 | 0 | 3 | 1 |
| Congestion/Hemorrhage | | 3 | 0 | 1 | 1 |
| Degeneration/Necrosis, Vessels | | 1 | 0 | 0 | 0 |
| Granuloma, Hair Shaft | | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

Summary of Microscopic Observations
Dosing Phase - Final Phase Sacrifice

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | -- Females -- | |
|---|---|---|---|---|---|
| | Dosage group: | Ctls | 7 | Ctls | 7 |
| Tissues With Diagnoses | No. in group: | 10 | 9 | 10 | 9 |
| Subcutan Site B ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 6 | 1 | 6 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 6 | 0 | 9 |
| Infiltrate, Lymphocytes/Macrophages | | 4 | 4 | 2 | 2 |
| Degeneration, Muscle (Focal) | | 0 | 2 | 1 | 2 |
| Congestion/Hemorrhage | | 0 | 1 | 0 | 1 |
| Degeneration/Necrosis, Vessels | | 0 | 0 | 0 | 0 |
| Granuloma, Hair Shaft | | 0 | 0 | 1 | 1 |
| Subcutan Site C ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 8 | 2 | 6 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 7 | 0 | 9 |
| Infiltrate, Lymphocytes/Macrophages | | 2 | 3 | 3 | 2 |
| Degeneration, Muscle (Focal) | | 2 | 1 | 2 | 1 |
| Congestion/Hemorrhage | | 0 | 1 | 0 | 2 |
| Granuloma, Hair Shaft | | 0 | 0 | 1 | 1 |
| Pigment-Laden Macrophages | | 0 | 0 | 0 | 0 |
| Subcutan Site D ..... | Number examined: | 10 | 9 | 10 | 9 |
| | Unremarkable: | 7 | 1 | 9 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 7 | 0 | 9 |
| Infiltrate, Lymphocytes/Macrophages | | 2 | 3 | 1 | 4 |
| Degeneration, Muscle (Focal) | | 0 | 1 | 1 | 1 |
| Congestion/Hemorrhage | | 0 | 1 | 1 | 2 |
| Granuloma, Hair Shaft | | 1 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All scheduled; Date of death range: 20.Aug.10 To 23.Aug.10

**Table 21 Study No 8229488 - Microscopic Findings at Uncheduled Sacrifice**

## Summary of Microscopic Observations - Unscheduled Sacrifices and Deaths

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | | -- Females -- | | |
|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 3 | 7 | Ctls | 3 | 7 |
| Tissues With Diagnoses | No. in group: | 0 | 1 | 1 | 0 | 0 | 1 |
| Brain ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 0 | 1 | 0 | 0 | 1 |
| Vacuoles, Neuron | | 0 | 1 | 0 | 0 | 0 | 0 |
| Spinal Cord ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Adrenal, Cortex ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Adrenal, Medulla ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Pituitary ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Cyst | | 0 | 0 | 0 | 0 | 0 | 0 |
| Nerve, Sciatic ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 0 | 0 | 0 | 1 |
| Degeneration, Axon | | 0 | 0 | 1 | 0 | 0 | 0 |
| Trachea ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Esophagus ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Thyroid ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Parathyroid ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Heart ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Infiltrate, Lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 |
| Aorta ..... | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |

All Diagnoses; Phases: All; Death types: All unscheduled; Date of death range: 25.Jul.10 To 02.Aug.10

Summary of Microscopic Observations
Unscheduled Sacrifices and Deaths

| Controls from group(s): 1 | Animal sex: | -- Males -- | | | -- Females -- | | |
|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 3 | 7 | Ctls | 3 | 7 |
| Tissues With Diagnoses | No. in group: | 0 | 1 | 1 | 0 | 0 | 1 |
| Tongue | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Muscle, Bi Fem | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Liver | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 0 | 0 | 0 | 1 |
| Vacuolation, Hepatocyte | | 0 | 0 | 1 | 0 | 0 | 0 |
| Infiltrate, Mononuclear | | 0 | 0 | 0 | 0 | 0 | 0 |
| Spleen | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 0 | 0 | 0 | 1 |
| Apoptosis, Lymphocytes | | 0 | 0 | 1 | 0 | 0 | 0 |
| Lung | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 0 | 1 | 0 | 0 | 1 |
| Infiltrate, Mixed | | 0 | 0 | 0 | 0 | 0 | 0 |
| Congestion | | 0 | 1 | 0 | 0 | 0 | 0 |
| Thymus | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 |
| Apoptosis, Lymphocytes | | 0 | 0 | 1 | 0 | 0 | 1 |
| Congestion | | 0 | 1 | 0 | 0 | 0 | 0 |
| Kidney | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 0 |
| Basophilic Tubule | | 0 | 0 | 0 | 0 | 0 | 0 |
| Dilatation, Pelvis | | 0 | 0 | 0 | 0 | 0 | 0 |
| Mineralization, Tubule | | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, Lymphocytes | | 0 | 0 | 0 | 0 | 0 | 1 |
| Hyaline Droplet, Tubule Cell | | 0 | 0 | 0 | 0 | 0 | 0 |
| Dilatation, Tubule(s) | | 0 | 0 | 0 | 0 | 0 | 0 |
| Urinary Bladder | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Stomach, Gl | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 0 | 1 | 0 | 0 | 1 |
| Infiltrate, Lymphocytes/Macrophages (Mesenteric Fat) | | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration/Necrosis | | 0 | 1 | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All unscheduled; Date of death range: 25.Jul.10 To 02.Aug.10

Summary of Microscopic Observations
Unscheduled Sacrifices and Deaths

| Controls from group(s): 1 | Animal sex: | -- Males -- | | | -- Females -- | | |
|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 3 | 7 | Ctls | 3 | 7 |
| Tissues With Diagnoses | No. in group: | 0 | 1 | 1 | 0 | 0 | 1 |
| Stomach, Nongl | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Hyperkeratosis | | 0 | 0 | 0 | 0 | 0 | 0 |
| Duodenum | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Ileum | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Peyer's Patch | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Colon | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Cecum | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Jejunum | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| LN, Mesenteric | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 0 | 0 | 0 | 1 |
| Apoptosis, Lymphocytes | | 0 | 0 | 1 | 0 | 0 | 0 |
| LN, Mandibular | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 0 | 0 | 0 | 0 |
| Apoptosis, Lymphocytes | | 0 | 0 | 1 | 0 | 0 | 1 |
| Gl, Mandib Saliv | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Pancreas | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 |
| Decreased Zymogen Granules | | 0 | 1 | 1 | 0 | 0 | 0 |
| Decreased Cytoplasm/Vacuolation, Islet Cells | | 0 | 0 | 1 | 0 | 0 | 1 |
| Infiltrate, Lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 |
| Apoptosis, Acinar Cells | | 0 | 0 | 0 | 0 | 0 | 0 |
| Atrophy, Acinar Cells | | 0 | 0 | 0 | 0 | 0 | 0 |
| Atrophy, Lobular | | 0 | 0 | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All unscheduled; Date of death range: 25.Jul.10 To 02.Aug.10

Summary of Microscopic Observations
Unscheduled Sacrifices and Deaths

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | | -- Females -- | | |
|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 3 | 7 | Ctls | 3 | 7 |
| Tissues With Diagnoses | No. in group: | 0 | 1 | 1 | 0 | 0 | 1 |
| Nerve, Optic | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Eye | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Skin/Subcutis | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 0 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 1 | 0 | 0 | 1 |
| Mammary, Male | Number examined: | 0 | 1 | 1 | | | |
| | Unremarkable: | 0 | 1 | 1 | | | |
| Seminal Vesicle | Number examined: | 0 | 1 | 1 | | | |
| | Unremarkable: | 0 | 1 | 1 | | | |
| Prostate | Number examined: | 0 | 1 | 1 | | | |
| | Unremarkable: | 0 | 1 | 0 | | | |
| Infiltrate, Lymphocytes | | 0 | 0 | 1 | | | |
| Testis | Number examined: | 0 | 1 | 1 | | | |
| | Unremarkable: | 0 | 1 | 1 | | | |
| Atrophy/Degeneration, Seminiferous Tubules (Bilateral) | | 0 | 0 | 0 | | | |
| Epididymis | Number examined: | 0 | 1 | 1 | | | |
| | Unremarkable: | 0 | 1 | 1 | | | |
| Debris, Cellular, Lumen (Bilateral) | | 0 | 0 | 0 | | | |
| Decreased Sperm (Bilateral) | | 0 | 0 | 0 | | | |
| Mammary, Female | Number examined: | | | | 0 | 0 | 1 |
| | Unremarkable: | | | | 0 | 0 | 1 |
| Ovary | Number examined: | | | | 0 | 0 | 1 |
| | Unremarkable: | | | | 0 | 0 | 1 |
| Uterus | Number examined: | | | | 0 | 0 | 1 |
| | Unremarkable: | | | | 0 | 0 | 0 |
| Dilatation | | | | | 0 | 0 | 1 |
| Atrophy | | | | | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All unscheduled; Date of death range: 25.Jul.10 To 02.Aug.10

Summary of Microscopic Observations
Unscheduled Sacrifices and Deaths

| Controls from group(s): 1 | Animal sex: | -- Animals Affected -- Males -- | | | -- Females -- | | |
|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 3 | 7 | Ctls | 3 | 7 |
| Tissues With Diagnoses | No. in group: | 0 | 1 | 1 | 0 | 0 | 1 |
| Cervix | Number examined: | | | | 0 | 0 | 1 |
| | Unremarkable: | | | | 0 | 0 | 1 |
| Atrophy | | | | | 0 | 0 | 0 |
| Vagina | Number examined: | | | | 0 | 0 | 1 |
| | Unremarkable: | | | | 0 | 0 | 1 |
| Atrophy, Mucosal | | | | | 0 | 0 | 0 |
| Bone, Femur | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Marrow, Femur | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Bone, Sternum | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| Marrow, Sternum | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 1 |
| LN, Axillary | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 0 | 1 | 0 | 0 | 1 |
| Congestion/Hemorrhage | | 0 | 0 | 0 | 0 | 0 | 0 |
| Apoptosis, Lymphocytes | | 0 | 1 | 0 | 0 | 0 | 0 |
| Death Comment | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 |
| Scheduled Sacrifice | | 0 | 0 | 0 | 0 | 0 | 0 |
| Undetermined | | 0 | 1 | 1 | 0 | 0 | 1 |
| Subcutan Site A | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 0 | 0 | 1 |
| Infiltrate, Lymphocytes/Macrophages | | 0 | 0 | 0 | 0 | 0 | 1 |
| Degeneration, Muscle (Focal) | | 0 | 0 | 0 | 0 | 0 | 0 |
| Congestion/Hemorrhage | | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration/Necrosis, Vessels | | 0 | 0 | 0 | 0 | 0 | 0 |
| Granuloma, Hair Shaft | | 0 | 0 | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All unscheduled; Date of death range: 25.Jul.10 To 02.Aug.10

Summary of Microscopic Observations
Unscheduled Sacrifices and Deaths

| Controls from group(s): 1 | Animal sex: | -- Males -- | | | -- Females -- | | |
|---|---|---|---|---|---|---|---|
| | Dosage group: | Ctls | 3 | 7 | Ctls | 3 | 7 |
| Tissues With Diagnoses | No. in group: | 0 | 1 | 1 | 0 | 0 | 1 |
| Subcutan Site B | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 0 | 0 | 1 |
| Infiltrate, Lymphocytes/Macrophages | | 0 | 0 | 1 | 0 | 0 | 0 |
| Degeneration, Muscle (Focal) | | 0 | 0 | 0 | 0 | 0 | 0 |
| Congestion/Hemorrhage | | 0 | 1 | 0 | 0 | 0 | 0 |
| Degeneration/Necrosis, Vessels | | 0 | 1 | 0 | 0 | 0 | 0 |
| Granuloma, Hair Shaft | | 0 | 0 | 0 | 0 | 0 | 0 |
| Subcutan Site C | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 0 | 1 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 0 | 0 | 1 |
| Infiltrate, Lymphocytes/Macrophages | | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration, Muscle (Focal) | | 0 | 0 | 0 | 0 | 0 | 0 |
| Congestion/Hemorrhage | | 0 | 1 | 0 | 0 | 0 | 0 |
| Granuloma, Hair Shaft | | 0 | 0 | 0 | 0 | 0 | 0 |
| Pigment-Laden Macrophages | | 0 | 0 | 0 | 0 | 0 | 0 |
| Subcutan Site D | Number examined: | 0 | 1 | 1 | 0 | 0 | 1 |
| | Unremarkable: | 0 | 1 | 1 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 0 | 0 | 1 |
| Infiltrate, Lymphocytes/Macrophages | | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration, Muscle (Focal) | | 0 | 0 | 0 | 0 | 0 | 0 |
| Congestion/Hemorrhage | | 0 | 0 | 0 | 0 | 0 | 0 |
| Granuloma, Hair Shaft | | 0 | 0 | 0 | 0 | 0 | 0 |

All Diagnoses; Phases: All; Death types: All unscheduled; Date of death range: 25.Jul.10 To 02.Aug.10

**Table 22 Study No 8229488 - Microscopic Findings re: Severity**

**Incidence and Average Severity[a] of LY2963016-Related Microscopic Findings**

| | LY2963016 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sex | Males | | | | Females | | | |
| Dose Level (mg/kg) | 0 | 0.3 | 1[b] | 3/2 | 0 | 0.3 | 1 | 3/2 |
| Sciatic Nerve | | | | | | | | |
| Number Examined | 10 | 9 | 10 | 10 | 10 | 10 | 10 | 10 |
| Degeneration, Axons | 1 (1.0) | 0 (0.0) | 0 (0.0) | 4 (1.8) | 0 (0.0) | 1 (1.0) | 0 (0.0) | 4 (1.5) |
| Skin/Subcutis | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 1 (1.0) | 2 (1.5) | 6 (1.7) | 0 (0.0) | 1 (1.0) | 3 (1.0) | 10 (2.2) |
| Subcutaneous Injection Site A | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 0 (0.0) | 1 (1.0) | 7 (1.4) | 0 (0.0) | 1 (1.0) | 3 (1.0) | 10 (2.0) |
| Subcutaneous Injection Site B | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 0 (0.0) | 0 (0.0) | 7 (1.3) | 0 (0.0) | 0 (0.0) | 2 (1.0) | 10 (2.3) |
| Subcutaneous Injection Site C | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 0 (0.0) | 0 (0.0) | 5 (1.2) | 0 (0.0) | 1 (1.0) | 2 (1.5) | 10 (2.2) |
| Subcutaneous Injection Site D | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 0 (0.0) | 0 (0.0) | 5 (1.8) | 0 (0.0) | 1 (1.0) | 1 (1.0) | 9 (2.2) |
| Pancreas | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Decreased Cytoplasm/Vacuolation, Islet Cells | 0 (0.0) | 0 (0.0) | 2 (1.0) | 7 (1.3) | 0 (0.0) | 0 (0.0) | 1 (1.0) | 7 (1.4) |

a Incidence and average severity ( ) based on number of animals with finding.
b Includes unscheduled sacrifice animal.

**Incidence and Average Severity[a] of LANTUS®-Related Microscopic Findings**

| | LANTUS® | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sex | Males | | | | Females | | | |
| Dose Level (mg/kg) | 0 | 0.3 | 1 | 3/2[b] | 0 | 0.3 | 1 | 3/2[b] |
| Sciatic Nerve | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Degeneration, Axons | 1 (1.0) | 0 (0.0) | 1 (1.0) | 5 (1.8) | 0 (0.0) | 1 (1.0) | 0 (0.0) | 3 (2.0) |
| Skin/Subcutis | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 0 (0.0) | 7 (1.3) | 9 (2.0) | 0 (0.0) | 1 (1.0) | 5 (1.0) | 10 (2.3) |
| Subcutaneous Injection Site A | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 0 (0.0) | 1 (1.0) | 7 (1.6) | 0 (0.0) | 2 (1.0) | 3 (1.0) | 10 (1.8) |
| Subcutaneous Injection Site B | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 0 (0.0) | 1(1.0) | 6 (1.7) | 0 (0.0) | 0 (0.0) | 3 (1.3) | 10 (2.0) |
| Subcutaneous Injection Site C | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 0 (0.0) | 2 (1.0) | 7 (1.6) | 0 (0.0) | 2 (1.0) | 2 (1.0) | 10 (2.0) |
| Subcutaneous Injection Site D | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Increased Adipose Tissue, Deep Dermis | 0 (0.0) | 0 (0.0) | 1 (1.0) | 7 (1.3) | 0 (0.0) | 0 (0.0) | 3 (1.0) | 10 (2.2) |
| Pancreas | | | | | | | | |
| Number Examined | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Decreased Cytoplasm/Vacuolation, Islet Cells | 0 (0.0) | 0 (0.0) | 2 (1.0) | 7 (1.6) | 0 (0.0) | 0 (0.0) | 2 (1.0) | 9 (1.4) |

a Incidence and average severity ( ) based on number of animals with finding.
b Includes unscheduled sacrifice animal.

- Special Evaluation: Not applicable.

- Toxicokinetics (Blood samples were collected on 1) Days 1 and 29 with Phase I TK animals for Groups 8, 9, 10, 12, and 13 and 2) Days 1, 15, and 32 with Phase I TK animals for Groups 11 and 14. Blood sample were also collected on Day 1 with Phase II TK animals for Groups 8-14. Time points: predose, 1, 2, 4, 8, 12, 18, and 24 hrs postdose).

Serum concentration levels from the control groups and predose were generally below of quantification (< 60 pM) or close to the lower limit of quantification (<120 pM).

Many of the Phase I TK samples were severely hemolyzed or had questionable stability or had insufficient volume to achieve a valid result. A number of the Phase II TK samples were also severely hemolyzed. Based on samples of Phase II TK (also called Repeat Day 1), exposures to LY2963016 and Lantus (AUC and $C_{max}$) generally increased with increasing dose, but were not consistently dose-proportional. Mean concentration-time profiles were generally similar on repeat Day and Day 29/32, indicating no accumulation of LY2964016 or Lantus. However, such finding might be confounded by potentially underreported data on Day 29/32. Exposures to LY2963016 and Lantus appeared to be generally similar to a given dose level.

**Table 23 Study No 8229488 TK Parameters for LY2963016 and Lantus on Repeat Day 1 (from Phase II TK animals)**



Reference ID: 3527465

**Exposure Parameters for Repeat Day 1**

| Parameter | Administered Dose (mg/kg) | | |
|---|---|---|---|
| | 0.3 | 1 | 2 |
| Sex | M/F Combined | M/F Combined | M/F Combined |
| LY2963016 | | | |
| Repeat Day 1 | | | |
| $C_{max}$ (pM) | 7903 | 14600 | 23300 |
| $AUC_{0-24hr}$ (pM·hr) | 24509 | 71986 | 236377 |
| SEM $AUC_{0-24hr}$ (pM·hr) | 4543 | 11900 | 20122 |
| LANTUS® | | | |
| Repeat Day 1 | | | |
| $C_{max}$ (pM) | 11017 | 19200 | 28060 |
| $AUC_{0-24hr}$ (pM·hr) | 31679 | 107598 | 241432 |
| SEM $AUC_{0-24hr}$ (pM·hr) | 4440 | 8583 | 19675 |

M/F Combined = Male and Female Combined.
$C_{max}$ = Maximum serum concentration.
pM = Pico molar.
SEM = Standard error of the mean.
$AUC_{0-24hr}$ = Area under the serum concentration versus time curve from 0 to 24 hours.

- Dosing Solution Analysis: This was done by Lancaster Laboratories, Inc. Mean concentrations of LY2963016 and Lantus for dose concentrations of 1, 2, 3 mg/ml ranged from 94.2-104% of theoretical values and were acceptable for use. However, for 0.3 mg/ml, the ranges of theoretical values were outside of acceptable range (lower concentration verification results ranged from 34.7-87% due to a possible cloudy precipitate formation). The sponsor argued that such result would not impact study integrity due to 1) compounds were clear in solution when animals were dosed, 2) 0.3 mg/kg dose groups showed a marked PD effect as expected by reducing glucose levels 1 and 2 hrs postdose, and 3) the glucose curves at each concurrent timepoints were similar at 0.3 mg/kg LY2963016 or Lantus.

The potency assay tested at the end of the study showed that LY2963016 had a potency of 3.675 mg/ml at initial dosing phase and 3.727 mg/ml after Phase II. The RP-HPLC assay after Phase II showed LY2963016 had purity of 98.4%. These results indicated that lY2963016 was stable through the period of study.

**Table 24 Study No 8229488 – Dosing Solution Analysis**

**Results of Concentration Verification**

| Theoretical Concentrations | LY2963016 mg/mL | | | | |
|---|---|---|---|---|---|
| | 0 | 0.3 | 1 | 2 | 3 |
| Phase 1 | | | | | |
| Day 1 | | | | | |
| Replicate 1 | 0 | 0.246 | 0.931 | - | 2.942 |
| Replicate 2 | - | 0.249 | 0.952 | - | 2.946 |
| Overall Mean (% of theoretical) | - | 0.248 (82.6) | 0.942 (94.2) | - | 2.944 (98.1) |
| % Relative Standard Deviation | | 0.9 | 1.6 | - | 0.1 |
| Week 3 | | | | | |
| Replicate 1 | - | 0.114 | - | 1.946 | - |
| Replicate 2 | - | 0.129 | - | 1.947 | - |
| Overall Mean (% of theoretical) | - | 0.121 (40.3) | - | 1.947 (97.4) | - |
| % Relative Standard Deviation | - | 8.7 | - | <0.1 | |
| Phase 2 | | | | | |
| Day 1 | | | | | |
| Replicate 1 | 0 | 0.278 | 0.992 | 1.982 | - |
| Replicate 2 | - | 0.243 | 0.993 | 1.972 | - |
| Overall Mean (% of theoretical) | - | 0.261 (87) | 0.993 (99.3) | 1.977 (98.9) | - |
| % Relative Standard Deviation | | 9.5 | 0.1 | 0.4 | |

- = Not applicable.

| Theoretical Concentrations | LANTUS® mg/mL | | | |
|---|---|---|---|---|
| | 0.3 | 1 | 2 | 3 |
| Phase 1 | | | | |
| Day 1 | | | | |
| Replicate 1 | 0.243 | 0.989 | - | 3.033 |
| Replicate 2 | 0.240 | 0.980 | - | 3.035 |
| Overall Mean (% of theoretical) | 0.242 (80.7) | 0.985 (98.5) | - | 3.034 (101) |
| % Relative Standard Deviation | 0.9 | 0.6 | - | <0.1 |
| Week 3 | | | | |
| Replicate 1 | 0.117 | - | 2.047 | - |
| Replicate 2 | 0.113 | - | 2.050 | - |
| Overall Mean (% of theoretical) | 0.115 (38.3) | - | 2.049 (102) | - |
| % Relative Standard Deviation | 2.5 | - | 0.1 | - |
| Phase 2 | | | | |
| Day 1 | | | | |
| Replicate 1 | 0.106 | 1.038 | 2.065 | - |
| Replicate 2 | 0.101 | 1.037 | 2.060 | - |
| Overall Mean (% of theoretical) | 0.104 (34.7) | 1.038 (104) | 2.063 (103) | - |
| % Relative Standard Deviation | 3.4 | 0.1 | 0.2 | - |

- = Not applicable.

TX 37440 - Attachment
Study: 8229488
Compound: LY2963016 (BIV/LANTUS®)

| | | |
|---|---|---|
| Potency by RP-HPLC: | Analyst: | Amy Palmeri |
| | Analysis Date: | 12/31/2010 |
| | Method/Revision: | LL-1283.04 |
| | Notebook number: | 124803 |
| | Notebook pages: | 1-6 |
| | Run Number: | 2010365062247 |
| | Instrument Number: | P_LC44 |
| Purity by RP-HPLC: | Analyst: | Justin Hayes |
| | Analysis Date: | 1/4/2011 |
| | Method/Revision: | LL-1288.02 |
| | Notebook number: | 124804 |
| | Notebook pages: | 1-2 |
| | Run Number: | 2011004161416 |
| | Instrument Number: | P_LC44 |
| pH: | Analyst: | Amy Palmeri |
| | Analysis Date: | 12/31/2010 |
| | Method/Revision: | USP <791> |
| | Notebook number: | 124805 |
| | Notebook pages: | 1-2 |
| Appearance: | Analyst: | Amy Palmeri |
| | Analysis Date: | 12/31/2010 |
| | Method/Revision: | Visual |
| | Notebook number: | 124805 |
| | Notebook pages: | 1 |

| Sample ID | Potency (mg/mL) | Purity (Area %) | Appearance | pH |
|---|---|---|---|---|
| NS-06175864 Prep 1 | 3.707 | 98.4 | A clear, colorless solution. | 4.3 |
| NS-06175864 Prep 2 | 3.746 | NA | | |
| NS-06175864 (Mean Result) | 3.727 | NA | | |
| %RSD | 0.7 | NA | | |

NA = Not applicable

## Study 8259267 – 4 wk rat study with EU Lantus

**Study title: A repeat-dose toxicity and toxicokinetic study in rats given LY2963016 or Lantus® (European-approved) by subcutaneous injection for 4 weeks**

| | |
|---|---|
| Study no.: | 8259267 |
| Study report location: | Eli Lilly and Company (Indianapolis, Indiana) |
| Conducting laboratory and location: | (b) (4) |
| Date of study initiation: | April 16, 2012 |
| GLP compliance: | Yes |
| QA statement: | Yes |
| Drug, lot #, and % purity: | LY2963016 (Lot # A889460) and 101% |

**Key Study Findings**

- LY2963016 or Lantus given to rats at 0, 0.3, 1, and 2 mg/kg for 4 weeks via sc injection resulted in markedly reduced glucose levels with dose-dependent duration at all dose levels.
- Compound-related early deaths or sacrificed occurred in 5 animals (2 M: at 2 mg/kg Lantus, 1 found dead on Day 21 and 1 sacrificed in moribund condition on Day 25, 1 F at 2 mg/kg LY2963016 found dead on Day 26, 1F at 1mg/kg Lantus found dead on Day 1, 1F at 1mg/kg

LY2963016 sacrificed in moribund condition on Day 29. There were also 25 TK animals found dead or sacrificed in moribund condition (1 F at 0.3 mg/kg LY2963016, 1 M/2F at 1 mg/kg/ LY2963016, 2M/5F at 2 mg/kg LY2963016, 1M/3F at 0.3 mg/kg Lantus, 4M/5F at 2 mg/kg Lantus. Based on findings (moderated to marked hypoglycemia), the 0.3 mg/kg was the MTD for both LY2963016 and Lantus.

- Clinical observations that occurred in one or more animals that died or were sacrificed included hunched posture, hypoactivity, recumbency, irregular or labored respiration, cold to touch, or ataxia, which were consistent with signs of hypoglycemia.
- There were LY2963016 or Lantus related increases I body weight gain and food consumption at 2 mg/kg, likely compensatory to reduced glucose levels.
- Decreased glucose concentrations occurred in animals given ≥ 0.3 mg/kg LY2963016 or Lantus. The decrease in glucose concentration as marked 1-4 hrs postdose at all doses (≥ 0.3 mg/kg) of LY2963016 and Lantus. The effect on glucose concentration was dose-dependent at later time points and persisted for longer durations at higher doses (through 8 hr postdose at 1 mg/kg and through 12-18 hrs postdose at 2 mg/kg). Decreased glucose concentration was generally reversed by 24 hrs postdose for all dose levels of LY2963013 and Lantus.
- There were no compound-related organ weight changes or macroscopic findings.
- Target organs were identified (occurred in low and mid dose groups): skin/subcutis, injection sites, sciatic nerve, and pancreas.
- Increased fat in the skin, subcutis, and at the injection sites (lipogenic pharmacological activity for insulins) were seen with LY2963016 and Lantus in males at 2 mg/kg LY2963016 , females at ≥ 1 mg/kg LY2963016, and males/females at 2 mg/kg Lantus.
- Minimal degeneration of axons in the sciatic nerve (secondary to hypoglycemia) was seen in females at 2 mg/kg LY2963016.
- Islet cell degranulation or atrophy (i.e. decreased islet cell cytoplasm/vacuolation due to down-regulation of endogenous insulin synthesis, secondary to hypoglycemia) was seen in males/females at ≥ 1 mg/kg LY2963016 or Lanus.
- Exposure to LY2963016 and Lantus on Days 1 and 29 of dosing phase, generally increased with increasing dose but was not consistently dose-proportional. Mean concentration-time profiles were generally higher on Day 29 compared with Day 1, suggesting a slight accumulation of LY2963016 or Lantus in rat serum after multiple dosing. Exposure to LY2963016 and Lantus appear generally similar across all dose levels.
- The NOAEL was 0.3 mg/kg/ for both LY2963016 and Lantus.



Reference ID: 3527465

Methods

| | |
|---|---|
| Doses: | 0.3, 1, 2 mg/kg for LY2963016 and Lantus |
| Frequency of dosing: | Daily |
| Route of administration: | Subcutaneous injection (rotating through 4 quadrants of the dorsal region – UL, UR, LL, LR) |
| Dose volume: | 3.64 mg/ml (100 IU/ml) |
| Formulation/Vehicle: | Vehicle control and diluent: 17 mg/ml glycerin, 2.7 mg/ml m-cresol,0.0373 mg/ml zinc oxide |
| Species/Strain: | Crl:CD(SD) rats |
| Number/Sex/Group: | Main: 10/sex/group, TK: 25/sex/group |
| Age: | 10-11 weeks old |
| Weight: | 302-479 g for males and 203-294 g for females |
| Satellite groups: | TK group |
| Unique study design: | -- |
| Deviation from study protocol: | Yes but not affected the overall interpretation of study findings. |

**Table 25 Study No 8259267: Study Design**

| Group | Subgroup | No. of Animals Male | No. of Animals Female | Dose Level (mg LY2963016 or LANTUS®/kg) | Dose Concentration (mg LY2963016 or LANTUS®/mL) |
|---|---|---|---|---|---|
| LY2963016 | | | | | |
| 1 (Control) | 1 (Toxicity) | 10 | 10 | 0 | 0 |
| | 2 (Toxicokinetic) | 25 | 25 | 0 | 0 |
| 2 (Low) | 1 (Toxicity) | 10 | 10 | 0.3 | 0.6 |
| | 2 (Toxicokinetic) | 25 | 25 | 0.3 | 0.6 |
| 3 (Mid) | 1 (Toxicity) | 10 | 10 | 1 | 1 |
| | 2 (Toxicokinetic) | 25 | 25 | 1 | 1 |
| 4 (High) | 1 (Toxicity) | 10 | 10 | 2 | 2 |
| | 2 (Toxicokinetic) | 25 | 25 | 2 | 2 |
| LANTUS® | | | | | |
| 5 (Low) | 1 (Toxicity) | 10 | 10 | 0.3 | 0.6 |
| | 2 (Toxicokinetic) | 25 | 25 | 0.3 | 0.6 |
| 6 (Mid) | 1 (Toxicity) | 10 | 10 | 1 | 1 |
| | 2 (Toxicokinetic) | 25 | 25 | 1 | 1 |
| 7 (High) | 1 (Toxicity) | 10 | 10 | 2 | 2 |
| | 2 (Toxicokinetic) | 25 | 25 | 2 | 2 |

Observations and Results

- Mortality (twice daily): a total of 30 animals were sacrificed in moribund condition or found dead. Of those, 8 TK animals were sacrificed in moribund condition or found dead shortly after blood collection on Days 1 and 2, which was likely related to the volume of blood collected and stress of the collection procedures in combination with the pharmacologic effects of the test article.

All deaths or sacrifices at ≥ 1 mg/kg LY2963016 or Lantus were considered compound-related hypoglycemia. Although administration of Lantus may have contributed to deaths at 0.3 mg/kg, TK animals were potentially compromised and no other deaths occurred at 0.3 mg/kg LY2963016 or Lantus. Therefore, 0.3 mg/kg was the MTD for both compounds.

**Table 26 Study No 8259267: Mortality**



Reference ID: 3527465

**Unscheduled Sacrifices and Deaths**

| Number of Animals Sacrificed or Found Dead | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sex | Male | | | | Female | | | |
| Dose (mg LY2963016 or LANTUS®/kg) | 0 | 0.3 | 1 | 2 | 0 | 0.3 | 1 | 2 |
| Toxicity Animals | | | | | | | | |
| LY2963016 | - | - | - | - | - | - | 1 | 1 |
| LANTUS® | - | - | - | 2 | - | - | 1 | - |
| Toxicokinetic Animals | | | | | | | | |
| LY2963016 | - | 1[a] | 1 | 2 | - | 1[b] | 2[b] | 5[b] |
| LANTUS® | - | 1[b] | - | 4 | - | 3[b] | - | 5[b] |

- = No deaths occurred.

a Animal was sacrificed due to a swollen nose and malocclusion that could not be palliated.

b Includes toxicokinetic animals replaced after blood collections on Day 1 or 2 of the dosing phase.

- Clinical Signs (twice daily): All compound related clinical observations occurred in those sacrificed or that died and were consistent with signs of hypoglycemia.

- Body Weights (at least once during predose, before dosing on Day 1, weekly thereafter, day of scheduled sacrifices): Males at 2 mg/kg LY2963016 or Lantus had slightly increased mean body weights and body weight gains relative to control animals (10 and 14% respectively by Day 29). Body weight difference increased gradually during the dosing phase. Body weight gain was 60 and 77% higher for males at 2 mg/kg LY2963016 or Lantus respectively over the course of the dosing phase relative to control males. Also, females at 2 mg/kg LY2963016 or Lantus had slightly increased mean body weights and body weight gains relative to control animals (14 and 16% respectively by Day 29). Overall body weight gain was 113 and 131% higher in females at 2 mg/kg LY2963016 or Lantus, respectively, relative to control females.

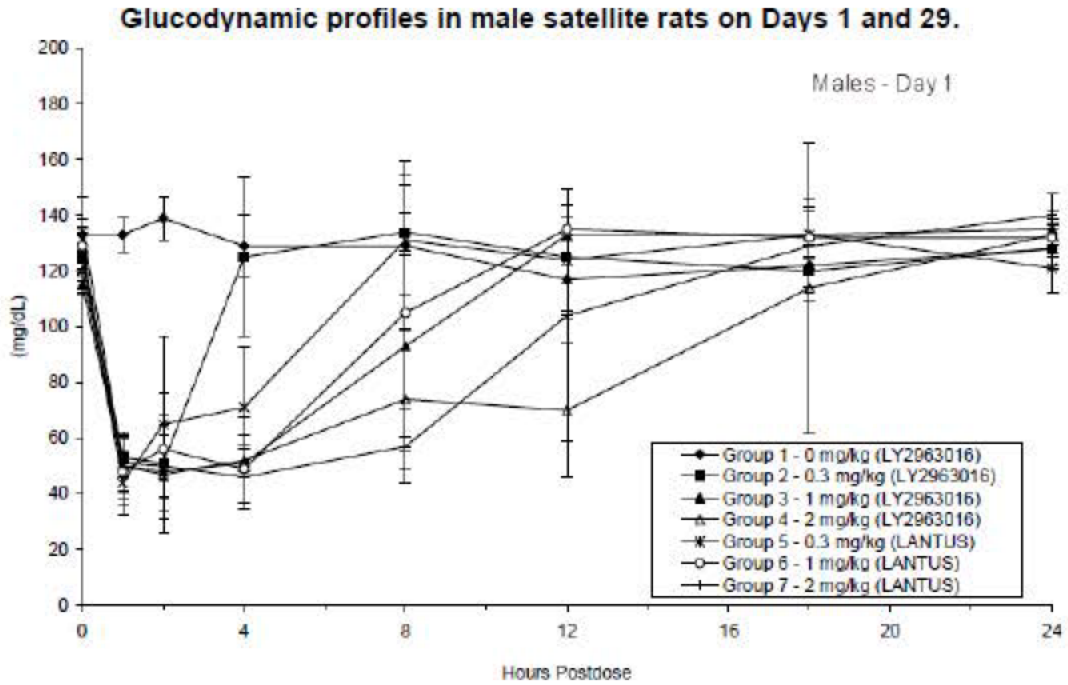
- Feed Consumption (twice weekly): It was significantly increased (11 to 45% relative to control animals) at 2 mg/kg LY2963016 or Lantus, correlating with effects on body weight. Such increase was likely compensatory to reduced glucose levels.

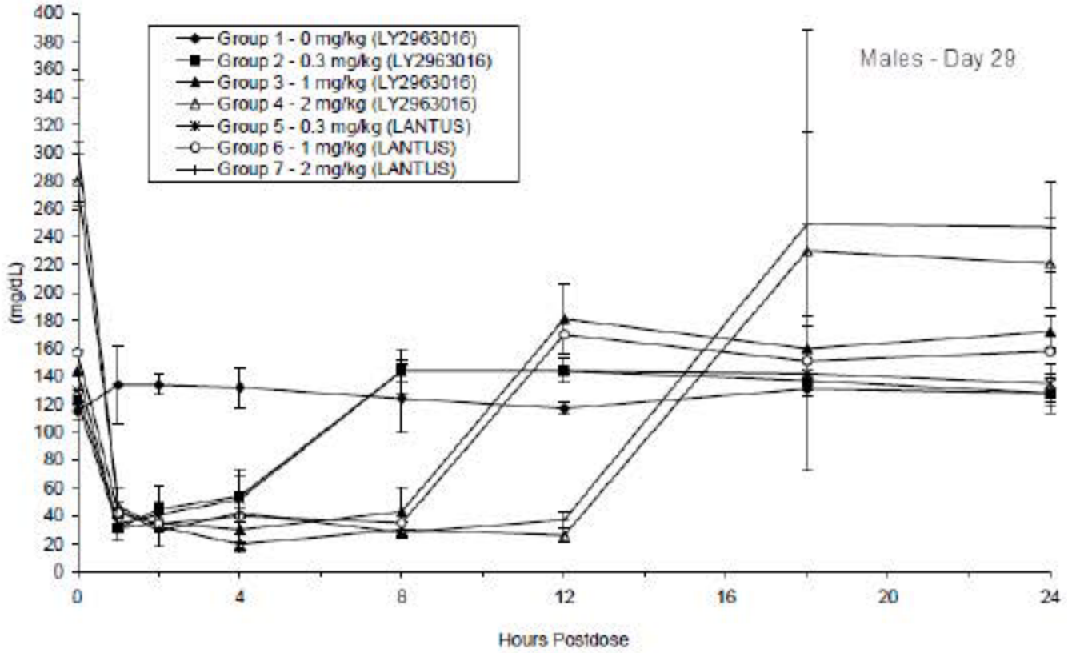
- Ophthalmoscopy (predose and on Day 26 of the dosing phase): No ophthalmic findings were observed.

- ECG: n/a

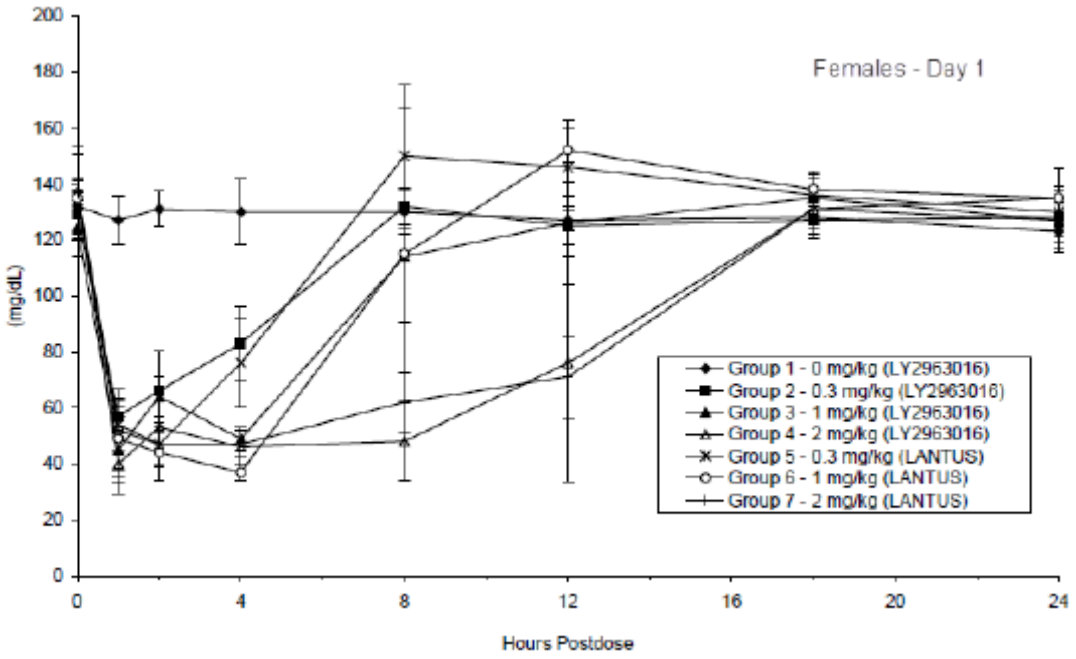
- Clinical Pathology – Hematology/clinical chemistry/urinalysis (blood sample were collected on Days 1 and 29 and approx. 1, 2, 4, 8, 12, 18, and 24 hrs postdose): There was mild to moderate increases in serum glucose concentrations at ≥ 1 mg/kg LY2963016 and males at ≥ 0.3 mg/kg Lantus and females at ≥ 1 mg/kg Lantus. This was likely reflected a rebound response to decreases in glucose concentration that may have occurred at earlier time points and were consistent with pancreatic islet cell atrophy observed microscopically. In general, these decreases were of similar magnitude in animals given LY2963016 or Lantus.

**Figure 2 Study No 8259267 – Glucose Data on Days 1 and 29**




**Glucodynamic profiles in female satellite rats on Days 1 and 29.**




- Gross Pathology (at the scheduled sacrifices): No compound-related macroscopic findings were noted at scheduled or unscheduled sacrifices.

- Organ Weights (at the scheduled sacrifices): There were increases in organ weight considered secondary to increased terminal body weight.

- Histopathology (at the scheduled sacrifices): 1) Adequate Battery: Yes and 2) Peer Review: Yes. The pancrease, sciatic nerve, and skin/subcutis, including injection sites, were identified as target organs. These tissues from all animals in low and mid dose groups were examined. All other were held for possible further examination.

Histological Findings: There were microscopic findings in the sciatic nerve (minimal axonal degeneration in females at 2 mg/kg LY2963016), skin/subcutis (increased adipose tissue across all skin section and sc injection sites; expected insulin pharmacology), and pancreas (minimally decreased cytoplasmic vacuolation and/or decreases cytoplasmic volume of islet cells in malesand females at ≥ 1 mg/kg LY2963016 or Lantus.

**Table 27 Study No 8259267 Summary of Microscopic Findings – Scheduled Sacrifice**

**Incidence of Microscopic Observations - Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)**

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/M 10 | 2/1/M 10 | 3/1/M 10 | 4/1/M 10 | 5/1/M 10 | 6/1/M 10 | 7/1/M 8 |
|---|---|---|---|---|---|---|---|---|
| Adrenal, Cortex | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 9 | 0 | 0 | 9 | 0 | 0 | 8 |
| Degeneration, cystic | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vacuolation | | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Brain | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 8 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Cecum | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 8 | 0 | 0 | 10 | 0 | 0 | 8 |
| Congestion | | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Epididymis | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 7 | 0 | 0 | 6 | 0 | 0 | 8 |
| Infiltrate, lymphocytes/macrophages | | 3 | 0 | 0 | 4 | 0 | 0 | 0 |
| Esophagus | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 8 |
| Yeast, epithelium | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/M 10 | 2/1/M 10 | 3/1/M 10 | 4/1/M 10 | 5/1/M 10 | 6/1/M 10 | 7/1/M 8 |
|---|---|---|---|---|---|---|---|---|
| Eye | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 7 |
| Rosette, retina | | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Heart | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 7 | 0 | 0 | 3 | 0 | 0 | 4 |
| Cyst, valve | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration/necrosis, myocardium, focal/multifocal | | 1 | 0 | 0 | 4 | 0 | 0 | 3 |
| Hyperplasia, endothelial | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 3 | 0 | 0 | 1 |
| Inflammation, chronic | | 1 | 0 | 0 | 1 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/M 10 | 2/1/M 10 | 3/1/M 10 | 4/1/M 10 | 5/1/M 10 | 6/1/M 10 | 7/1/M 8 |
|---|---|---|---|---|---|---|---|---|
| Kidney | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 3 | 0 | 0 | 2 | 0 | 0 | 2 |
| Basophilic tubule | | 3 | 0 | 0 | 5 | 0 | 0 | 2 |
| Cast, proteinaceous | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cyst | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration/necrosis, single cell, increased | | 3 | 0 | 0 | 5 | 0 | 0 | 2 |
| Dilatation, pelvis | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hyaline droplet, increased, tubule cell | | 0 | 0 | 0 | 2 | 0 | 0 | 1 |
| Hyperplasia/hypertrophy, tubule cell | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 3 | 0 | 0 | 4 | 0 | 0 | 1 |
| Mineralization | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Liver | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 2 | 0 | 0 | 2 | 0 | 0 | 1 |
| Degeneration/necrosis hepatocytes | | 1 | 0 | 0 | 3 | 0 | 0 | 0 |
| Hematopoiesis, extramedullary | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 8 | 0 | 0 | 8 | 0 | 0 | 7 |



Reference ID: 3527465

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/M 10 | 2/1/M 10 | 3/1/M 10 | 4/1/M 10 | 5/1/M 10 | 6/1/M 10 | 7/1/M 8 |
|---|---|---|---|---|---|---|---|---|
| Lung | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 3 | 0 | 0 | 8 | 0 | 0 | 8 |
| Crystals, hemoglobin | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fibrosis, pleural/subpleural | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hemorrhage | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, macrophages, alveolus | | 5 | 0 | 0 | 2 | 0 | 0 | 0 |
| Mineralization, vessel | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Lymph Node, Axillary | Number Examined: | 9 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 6 | 0 | 0 | 10 | 0 | 0 | 6 |
| Dilatation, sinus | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Erythrocytes, sinus | | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| Lymph Node, Mandibular | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 4 | 0 | 0 | 4 | 0 | 0 | 5 |
| Erythrocytes, sinus | | 4 | 0 | 0 | 6 | 0 | 0 | 3 |
| Hemorrhage | | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lymph Node, Mesenteric | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 8 |
| Infiltrate, eosinophils | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/M 10 | 2/1/M 10 | 3/1/M 10 | 4/1/M 10 | 5/1/M 10 | 6/1/M 10 | 7/1/M 8 |
|---|---|---|---|---|---|---|---|---|
| Mammary Gland | Number Examined: | 10 | 0 | 0 | 8 | 0 | 0 | 7 |
| | Unremarkable: | 10 | 0 | 0 | 8 | 0 | 0 | 7 |
| M-Carcinoma | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mandibular Salivary Gland | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 8 |
| Vacuolation | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Muscle, Biceps Femoris | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 8 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Nerve, Sciatic | Number Examined: | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| | Unremarkable: | 9 | 9 | 9 | 8 | 10 | 10 | 8 |
| Degeneration, axon | | 0 | 1 | 0 | 2 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| Ovary | Number Examined: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mineralization | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: | 1/1/M | 2/1/M | 3/1/M | 4/1/M | 5/1/M | 6/1/M | 7/1/M |
|---|---|---|---|---|---|---|---|---|
| | Number of Animals: | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| Pancreas | Number Examined: | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| | Unremarkable: | 6 | 9 | 3 | 2 | 8 | 5 | 1 |
| Alteration, basophilic | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Atrophy | | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| Decreased cytoplasm/vacuolation, islet cells | | 0 | 0 | 3 | 7 | 0 | 3 | 7 |
| Infiltrate, lymphocytes/macrophages | | 4 | 0 | 4 | 3 | 2 | 4 | 1 |
| Pituitary | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 9 | 0 | 0 | 10 | 0 | 0 | 8 |
| Cyst | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Prostate | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 9 | 0 | 0 | 8 | 0 | 0 | 8 |
| Infiltrate, lymphocytes/macrophages | | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| Inflammation, acute | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Skin/Subcutis | Number Examined: | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| | Unremarkable: | 10 | 10 | 10 | 4 | 10 | 9 | 4 |
| Erosion/ulcer | | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 6 | 0 | 0 | 4 |
| Inflammation, chronic-active | | 0 | 0 | 0 | 0 | 0 | 1 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: | 1/1/M | 2/1/M | 3/1/M | 4/1/M | 5/1/M | 6/1/M | 7/1/M |
|---|---|---|---|---|---|---|---|---|
| | Number of Animals: | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| Spleen | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 9 | 0 | 0 | 10 | 0 | 0 | 8 |
| Pigment, increased | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Subcutaneous | Number Examined: | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| Injection Site, A | Unremarkable: | 3 | 5 | 3 | 3 | 4 | 5 | 3 |
| Congestion/hemorrhage | | 2 | 2 | 2 | 0 | 2 | 3 | 0 |
| Degeneration muscle | | 2 | 2 | 3 | 1 | 3 | 1 | 0 |
| Erosion/ulcer | | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 6 | 0 | 1 | 4 |
| Infiltrate, lymphocytes/macrophages | | 3 | 1 | 2 | 0 | 2 | 1 | 1 |
| Inflammation, chronic/chronic-active | | 4 | 3 | 5 | 4 | 2 | 3 | 1 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/M 10 | 2/1/M 10 | 3/1/M 10 | 4/1/M 10 | 5/1/M 10 | 6/1/M 10 | 7/1/M 8 |
|---|---|---|---|---|---|---|---|---|
| Subcutaneous Injection Site, B | Number Examined: | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| | Unremarkable: | 7 | 9 | 5 | 3 | 9 | 5 | 2 |
| Congestion/hemorrhage | | 2 | 0 | 2 | 0 | 0 | 2 | 1 |
| Degeneration, muscle | | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 4 | 0 | 0 | 6 |
| Infiltrate, lymphocytes/macrophages | | 1 | 1 | 0 | 3 | 1 | 2 | 1 |
| Inflammation, chronic/chronic-active | | 2 | 0 | 4 | 0 | 0 | 2 | 1 |
| Subcutaneous Injection Site, C | Number Examined: | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| | Unremarkable: | 9 | 10 | 8 | 3 | 8 | 4 | 3 |
| Congestion/hemorrhage | | 0 | 0 | 0 | 0 | 1 | 2 | 0 |
| Degeneration, muscle | | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Granuloma, hair shaft | | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 6 | 0 | 0 | 4 |
| Infiltrate, lymphocytes/macrophages | | 1 | 0 | 0 | 1 | 0 | 3 | 1 |
| Inflammation, chronic/chronic-active | | 0 | 0 | 1 | 0 | 1 | 3 | 0 |
| Pigment | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/M 10 | 2/1/M 10 | 3/1/M 10 | 4/1/M 10 | 5/1/M 10 | 6/1/M 10 | 7/1/M 8 |
|---|---|---|---|---|---|---|---|---|
| Subcutaneous Injection Site, D | Number Examined: | 10 | 10 | 10 | 10 | 10 | 10 | 8 |
| | Unremarkable: | 7 | 7 | 5 | 3 | 5 | 1 | 2 |
| Congestion/hemorrhage | | 0 | 2 | 2 | 0 | 1 | 4 | 1 |
| Degeneration muscle | | 1 | 0 | 1 | 0 | 0 | 1 | 1 |
| Erosion/ulcer | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Granuloma, hair shaft | | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 6 | 0 | 0 | 3 |
| Infiltrate, lymphocytes/macrophages | | 2 | 2 | 2 | 0 | 3 | 2 | 3 |
| Inflammation, chronic/chronic-active | | 1 | 1 | 2 | 2 | 2 | 5 | 1 |
| Inflammation, vessel | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pigment | | 0 | 0 | 0 | 0 | 1 | 1 | 0 |
| Testis | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 10 | 0 | 0 | 8 | 0 | 0 | 8 |
| Hypoplasia/atrophy, focal/multifocal | | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| Thymus | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 9 | 0 | 0 | 8 | 0 | 0 | 7 |
| Hemorrhage | | 1 | 0 | 0 | 2 | 0 | 0 | 1 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/M 10 | 2/1/M 10 | 3/1/M 10 | 4/1/M 10 | 5/1/M 10 | 6/1/M 10 | 7/1/M 8 |
|---|---|---|---|---|---|---|---|---|
| Thyroid | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Urinary Bladder | Number Examined: | 10 | 0 | 0 | 10 | 0 | 0 | 8 |
| | Unremarkable: | 10 | 0 | 0 | 10 | 0 | 0 | 6 |
| Congestion | | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Infiltrate, lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Vagina | Number Examined: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Diestrus | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Estrus | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Metestrus | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Proestrus | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: | 1/1/F | 2/1/F | 3/1/F | 4/1/F | 5/1/F | 6/1/F | 7/1/F |
|---|---|---|---|---|---|---|---|---|
| | Number of Animals: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| Adrenal, Cortex | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 9 |
| Degeneration, cystic | | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Vacuolation | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Brain | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cecum | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Congestion | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Epididymis | Number Examined: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Esophagus | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Yeast, epithelium | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: | 1/1/F | 2/1/F | 3/1/F | 4/1/F | 5/1/F | 6/1/F | 7/1/F |
|---|---|---|---|---|---|---|---|---|
| | Number of Animals: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| Eye | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Rosette, retina | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Heart | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 8 | 0 | 0 | 5 | 0 | 0 | 7 |
| Cyst, valve | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration/necrosis, myocardium, focal/multifocal | | 2 | 0 | 0 | 3 | 0 | 0 | 1 |
| Hyperplasia, endothelial | | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Infiltrate, lymphocytes/macrophages | | 1 | 0 | 0 | 1 | 0 | 0 | 1 |
| Inflammation, chronic | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |



Reference ID: 3527465

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: | 1/1/F | 2/1/F | 3/1/F | 4/1/F | 5/1/F | 6/1/F | 7/1/F |
|---|---|---|---|---|---|---|---|---|
| | Number of Animals: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| Kidney | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 4 | 0 | 0 | 6 | 0 | 0 | 4 |
| Basophilic tubule | | 2 | 0 | 0 | 2 | 0 | 0 | 2 |
| Cast, proteinaceous | | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Cyst | | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Degeneration/necrosis, single cell, increased | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dilatation, pelvis | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hyaline droplet, increased, tubule cell | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hyperplasia/hypertrophy, tubule cell | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 2 | 0 | 0 | 3 | 0 | 0 | 3 |
| Mineralization | | 3 | 0 | 0 | 0 | 0 | 0 | 1 |
| Liver | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 0 | 0 | 0 | 3 | 0 | 0 | 6 |
| Degeneration/necrosis hepatocytes | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hematopoiesis, extramedullary | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 10 | 0 | 0 | 6 | 0 | 0 | 4 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/F 10 | 2/1/F 10 | 3/1/F 9 | 4/1/F 9 | 5/1/F 10 | 6/1/F 9 | 7/1/F 10 |
|---|---|---|---|---|---|---|---|---|
| Lung | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 7 | 0 | 0 | 7 | 0 | 0 | 10 |
| Crystals, hemoglobin | | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| Fibrosis, pleural/subpleural | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Hemorrhage | | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, macrophages, alveolus | | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mineralization, vessel | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lymph Node, Axillary | Number Examined: | 9 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 7 | 0 | 0 | 8 | 0 | 0 | 9 |
| Dilatation, sinus | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Erythrocytes, sinus | | 2 | 0 | 0 | 1 | 0 | 0 | 1 |
| Lymph Node, Mandibular | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 6 | 0 | 0 | 6 | 0 | 0 | 5 |
| Erythrocytes, sinus | | 4 | 0 | 0 | 3 | 0 | 0 | 5 |
| Hemorrhage | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Lymph Node, Mesenteric | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Infiltrate, eosinophils | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/F 10 | 2/1/F 10 | 3/1/F 9 | 4/1/F 9 | 5/1/F 10 | 6/1/F 9 | 7/1/F 10 |
|---|---|---|---|---|---|---|---|---|
| Mammary Gland | Number Examined: | 10 | 0 | 0 | 9 | 0 | 1 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| M-Carcinoma | | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Mandibular Salivary Gland | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Vacuolation | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Muscle, Biceps Femoris | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Nerve, Sciatic | Number Examined: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| | Unremarkable: | 8 | 9 | 9 | 3 | 9 | 9 | 8 |
| Degeneration, axon | | 2 | 1 | 0 | 6 | 1 | 0 | 2 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ovary | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 8 | 0 | 0 | 9 | 0 | 0 | 10 |
| Mineralization | | 2 | 0 | 0 | 0 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/F 10 | 2/1/F 10 | 3/1/F 9 | 4/1/F 9 | 5/1/F 10 | 6/1/F 9 | 7/1/F 10 |
|---|---|---|---|---|---|---|---|---|
| Pancreas | Number Examined: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| | Unremarkable: | 5 | 7 | 4 | 1 | 9 | 6 | 1 |
| Alteration, basophilic | | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Atrophy | | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| Decreased cytoplasm/vacuolation, islet cells | | 0 | 0 | 1 | 8 | 0 | 2 | 7 |
| Infiltrate, lymphocytes/macrophages | | 4 | 3 | 4 | 1 | 1 | 1 | 2 |
| Pituitary | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Cyst | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Prostate | Number Examined: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Inflammation, acute | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Skin/Subcutis | Number Examined: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| | Unremarkable: | 10 | 10 | 8 | 4 | 10 | 9 | 4 |
| Erosion/ulcer | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 1 | 5 | 0 | 0 | 6 |
| Inflammation, chronic-active | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/F 10 | 2/1/F 10 | 3/1/F 9 | 4/1/F 9 | 5/1/F 10 | 6/1/F 9 | 7/1/F 10 |
|---|---|---|---|---|---|---|---|---|
| Spleen | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Pigment, increased | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Subcutaneous Injection Site, A | Number Examined: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| | Unremarkable: | 7 | 8 | 7 | 0 | 8 | 8 | 2 |
| Congestion/hemorrhage | | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| Degeneration muscle | | 3 | 0 | 0 | 2 | 0 | 1 | 3 |
| Erosion/ulcer | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 1 | 1 | 7 | 0 | 0 | 7 |
| Infiltrate, lymphocytes/macrophages | | 0 | 1 | 1 | 1 | 1 | 0 | 1 |
| Inflammation, chronic/chronic-active | | 1 | 0 | 0 | 1 | 1 | 1 | 1 |



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Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: | 1/1/F | 2/1/F | 3/1/F | 4/1/F | 5/1/F | 6/1/F | 7/1/F |
|---|---|---|---|---|---|---|---|---|
| | Number of Animals: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| Subcutaneous | Number Examined: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| Injection Site, B | Unremarkable: | 8 | 7 | 8 | 0 | 8 | 7 | 5 |
| Congestion/hemorrhage | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Degeneration, muscle | | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 1 | 8 | 0 | 1 | 5 |
| Infiltrate, lymphocytes/macrophages | | 1 | 3 | 0 | 1 | 1 | 1 | 0 |
| Inflammation, chronic/chronic-active | | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Subcutaneous | Number Examined: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| Injection Site, C | Unremarkable: | 9 | 8 | 6 | 4 | 8 | 5 | 4 |
| Congestion/hemorrhage | | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Degeneration, muscle | | 0 | 1 | 0 | 0 | 1 | 1 | 1 |
| Granuloma, hair shaft | | 1 | 0 | 0 | 0 | 1 | 3 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 0 | 5 | 0 | 0 | 6 |
| Infiltrate, lymphocytes/macrophages | | 0 | 2 | 1 | 1 | 1 | 0 | 1 |
| Inflammation, chronic/chronic-active | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pigment | | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/F 10 | 2/1/F 10 | 3/1/F 9 | 4/1/F 9 | 5/1/F 10 | 6/1/F 9 | 7/1/F 10 |
|---|---|---|---|---|---|---|---|---|
| Subcutaneous Injection Site, D | Number Examined: | 10 | 10 | 9 | 9 | 10 | 9 | 10 |
| | Unremarkable: | 6 | 6 | 2 | 2 | 7 | 4 | 3 |
| Congestion/hemorrhage | | 0 | 0 | 2 | 1 | 0 | 2 | 0 |
| Degeneration muscle | | 1 | 1 | 0 | 1 | 1 | 1 | 2 |
| Erosion/ulcer | | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Granuloma, hair shaft | | 3 | 1 | 1 | 0 | 0 | 1 | 0 |
| Increased Adipose Tissue, Deep Dermis | | 0 | 0 | 1 | 5 | 0 | 1 | 5 |
| Infiltrate, lymphocytes/macrophages | | 0 | 0 | 4 | 4 | 1 | 0 | 0 |
| Inflammation, chronic/chronic-active | | 0 | 0 | 1 | 0 | 1 | 1 | 1 |
| Inflammation, vessel | | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| Pigment | | 0 | 1 | 0 | 0 | 2 | 0 | 0 |
| Testis | Number Examined: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hypoplasia/atrophy, focal/multifocal | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Thymus | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 9 | 0 | 0 | 8 | 0 | 0 | 9 |
| Hemorrhage | | 1 | 0 | 0 | 1 | 0 | 0 | 1 |

Incidence of Microscopic Observations
Terminal Sacrifice (Dosing Phase - Final Phase Sacrifice)

| Test Article | dosage) | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| LY2963016 | mL/kg | 0 | 0.3 | 1 | 2 | 0 | 0 | 0 |
| LANTUS® | mL/kg | 0 | 0 | 0 | 0 | 0.3 | 1 | 2 |

| Tissue/ Observation | Group/Subgroup/Sex: Number of Animals: | 1/1/F 10 | 2/1/F 10 | 3/1/F 9 | 4/1/F 9 | 5/1/F 10 | 6/1/F 9 | 7/1/F 10 |
|---|---|---|---|---|---|---|---|---|
| Thyroid | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 9 | 0 | 0 | 9 | 0 | 0 | 10 |
| Infiltrate, lymphocytes/macrophages | | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Urinary Bladder | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| Congestion | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Infiltrate, lymphocytes | | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Vagina | Number Examined: | 10 | 0 | 0 | 9 | 0 | 0 | 10 |
| | Unremarkable: | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Diestrus | | 4 | 0 | 0 | 1 | 0 | 0 | 2 |
| Estrus | | 1 | 0 | 0 | 0 | 0 | 0 | 5 |
| Metestrus | | 2 | 0 | 0 | 2 | 0 | 0 | 2 |
| Proestrus | | 3 | 0 | 0 | 6 | 0 | 0 | 1 |

- Special Evaluation – Antibody analysis (day of terminal sacrifice)



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- Toxicokinetics (Blood samples were collected on Days 1 and 29): Exposure to LY2963016 and Lantus generally increased with increasing dose but was not consistently dose-proportional. Males and females generally had similar mean serum concentration-time profiles. There was slight accumulation of LY2963013 or Lantus. Exposure to LY2963016 and Lantus appeared to be generally similar across all dose levels.

On Day 29, a dose of 0.3 mg/kg LY2963016 corresponded to mean Cmax values of 11,227 and 14,033 pM and AUC0-24hr values of 58,558 and 41,784 pM•hr for males and females, respectively, and a dose of 0.3 mg/kg LANTUS® corresponded to mean Cmax values of 12,230 and 9537 pM and AUC0-24hr values of 44,819 and 31,585 pM•hr for males and females, respectively.

**Table 28 Study No 8259267 - TK data**

**Mean Plasma Toxicokinetic Data Summary**

| Parameter | Administered Dose (mg/kg) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.3 | | | 1 | | | 2 | | |
| Sex | M | F | M/F Combined | M | F | M/F Combined | M | F | M/F Combined |
| LY2963016 | | | | | | | | | |
| Day 1 | | | | | | | | | |
| $C_{max}$ (pM) | 4440 | 7813 | 5917 | 16390 | 20133 | 18262 | 26800 | 23200 | 25360 |
| SD $C_{max}$ (pM) | 286 | 2075 | 2523 | 9514 | 6507 | 7573 | 3300 | NA | 4835 |
| $AUC_{0-24hr}$ (pM·hr) | 15961 | 26376 | 21016 | 95241 | 98567 | 96904 | 223386 | 222888 | 224217 |
| SEM $AUC_{0-24hr}$ (pM·hr) | 2526 | 4489 | 2806 | 18560 | 11584 | 10812 | 30179 | 33856 | 20045 |
| Day 29 | | | | | | | | | |
| $C_{max}$ (pM) | 11227 | 14033 | 11012 | 25700 | 17967 | 20850 | 106733 | 38600 | 69137 |
| SD $C_{max}$ (pM) | 5198 | 4934 | 4879 | 4877 | 907 | 7837 | 98063 | NA | 75440 |
| $AUC_{0-24hr}$ (pM·hr) | 58558 | 41784 | 50182 | 142077 | 116315 | 128699 | 688670 | 391906 | 551466 |
| SEM $AUC_{0-24hr}$ (pM·hr) | 9811 | 8353 | 8236 | 14424 | 14743 | 11636 | 179199 | 54833 | 103131 |
| LANTUS® | | | | | | | | | |
| Day 1 | | | | | | | | | |
| $C_{max}$ (pM) | 6947 | 6310 | 6628 | 14333 | 23000 | 18300 | 17567 | 41500 | 28497 |
| SD $C_{max}$ (pM) | 1410 | 2793 | 2009 | 8009 | 4513 | 6084 | 929 | 19419 | 19742 |
| $AUC_{0-24hr}$ (pM·hr) | 19216 | 17095 | 18122 | 82629 | 100983 | 92089 | 174405 | 239692 | 207049 |
| SEM $AUC_{0-24hr}$ (pM·hr) | 1376 | 3197 | 1665 | 14735 | 19615 | 11923 | 24420 | 36968 | 26523 |
| Day 29 | | | | | | | | | |
| $C_{max}$ (pM) | 12230 | 9537 | 10883 | 30933 | 33900 | 32417 | 38800 | 47433 | 35720 |
| SD $C_{max}$ (pM) | 2484 | 2658 | 2733 | 3623 | 18489 | 12026 | NA | 10872 | 17805 |
| $AUC_{0-24hr}$ (pM·hr) | 44819 | 31585 | 37545 | 167112 | 136217 | 152919 | 313244 | 478785 | 406818 |
| SEM $AUC_{0-24hr}$ (pM·hr) | 5519 | 3064 | 3595 | 22435 | 33638 | 22282 | 38204 | 98168 | 65041 |

F = Female; M = Male; SD = Standard deviation; SEM = Standard error of the mean.

- Dosing Solution Analysis: Concentrations of LY2963016 and Lantus for dose concentrations of 0.6, 1, and 2 mg/ml ranged from 95.8 – 102% of theoretical values and were acceptable for use on the study. At the end of the dosing phase, Lot No A889460 was stable over the course of use on study. The lot, as supplied, had a potency of 3.67 mg/ml and a purity of 99.21% with pH of 4.00. At the end of dosing phase, the lot had a potency of 3.684 mg/ml and a purity of 99.6% with pH of 4.5.

**Table 29 Study No 8259267 - Dosing Solution Analysis**



Reference ID: 3527465

**LY2963016 (Lot A889460) Analytical Results**

| | Potency (%) | Purity (%) | pH | Appearance |
|---|---|---|---|---|
| Lot as supplied | 3.67 | 99.21 | 4.00 | Clear, colorless solution |
| End of study | 3.684 | 99.6 | 4.5 | Clear, colorless solution |

# 7 Genetic Toxicology

- LY2963016

No genotoxicity studies were conducted, in agreement with ICHS6 guidance.

- Lantus

Lantus was not mutagenic determined by 1) Ames and HGPRT tests for gene mutations and 2) cytogenetics in vitro (V79 cells) and in vivo (Chinese hamsters) for chromosomal aberrations.

# 8 Carcinogenicity

- LY2963016

No carcinogenicity studies have been conducted with LY2963016. There was no evidence of increased cell proliferative activity in a 1 month toxicity study in rats (Study No 8229488).

In addition, there were no significant differences in potency or efficacy in vitro assays (i.e. binding affinity, auto-phosphorylation, lipogenesis, or mitogenicity under Study No DBT93), indicating that LY2963016 showed similar cell proliferative activity to Lantus.

- Lantus

There were standard 2-year carcinogenicity studies done in mice and rats given up to 0.455 mg/kg (10x for rats and 5x for mice over human starting dose 0.008 mg/kg/day – 10 IU day). The female mouse findings were inconclusive due to excessive mortality in all dose groups during the study. Histiocytomas were seen at injection sites in males rats (statistically significant) and male mice (not statistically significant) in vehicle groups. These tumors were not seen in female rats and mice in saline control, or insulin comparator groups with a different vehicle. Therefore, the relevance of these findings to humans was unknown.

# 9 Reproductive and Developmental Toxicology

- LY2963016

No reproductive and developmental toxicity studies have been conducted with LY2963016.

- Lantus

Pregnancy Category C

A combined fertility and prenatal/postnatal study of Lantus was conducted in rats up to 0.36 mg/kg/day (7X higher than starting human dose 0.008 mg/kg/day). Material toxicity (i.e. hypoglycemia) was observed. A decrease in rearing rate occurred in the high-dose group only.



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Reproduction and teratology studies were done with Lantus and regular human insulin in rats (up to 0.36 mg/kg/day) and rabbits (0.072 mg/kg/day). The effects of Lantus did not generally differ from those observed with regular human insulin in rats or rabbits. However, 5 fetuses from 2 litters of the high dose group did have dilation of the cerebral ventricles. Nonetheless, fertility and early embryonic development appeared normal.

# 10 Special Toxicology Studies

Local tolerance was assessed in the 28 day repeat dose toxicity study (Study No 8229488). No clinical or histological signs were noted at the injection sites with LY2963016 (same with Lantus).

# 11 Integrated Summary and Safety Evaluation

Eli Lilly and Company submitted NDA 205692 under 505b2 is for LY2963016 (insulin glargine) to treat T1DM and T2DM patients. It is designed to be similar to Lantus (NDA 021081). The drug product is composed of 100 IU/ml (3.64 mg/ml) LY2963016 and inactive ingredients such as m-cresol, glycerin, zinc oxide, water, hydrochloric acid and sodium hydroxide. These inactive ingredients are commonly used in other approved products.

With regards to clinical development of LY2963016, the sponsor has used both US-approved Lantus as well as EU-approved insulin glargine (manufactured by Sanofi-Aventis) as the comparator product. In supporting their 505b2 application, the sponsor included bridging studies such as clinical Phase I PK and PD and Phase III safety and efficacy of LY2963016 and Lantus.

With regards to nonclinical development of LY2963016, Lilly conducted two in vivo pharmacology studies (in vitro assays: receptor binding, functional activity, lipogenesis, and Mitogenic activity) and two 28 day repeat dose toxicity rat studies (with TK data).

The in vitro pharmacology studies **(Study No DBT93 and Study No DBT149 by Lilly)** showed that the characteristics of LY2963016, including insulin and IGF-1 receptor pharmacology (i.e. binding potency and functional activity in 293HEK-EBNA cells overexpressing insulin or insulin-like receptors), metabolic potency (in 3T3L1 adipocyte cells), and mitogenicity (in IGF-1 deficient rat H4IIe hepatoma cell line and IGF-1 expressed SAOS osteosarcoma cell line) were comparable to commercially available insulin glargine (both US and EU approved Lantus).

The 4-week toxicity rat studies **(Study No 8229488 and Study No 8259267 by (b) (4))** showed that the characteristics of LY2963016 including in vivo pharmacodynamics (i.e. glucose lowering) and pharmacokinetics/toxicokinetics (i.e. AUC and $C_{max}$) were comparable to US- or EU-approved Lantus.

In Study No 8229488, rats were given LY2963015 or US-Lantus (0.3, 1, 3/2 mg/kg) via sc injection for 28 days. Most findings were associated with hypoglucemia in animals given LY2963016 or US-approved Lantus. Due to serum shortfall from the original Phase I TK animals (i.e. hemolyzed samples or insufficient sample volumes), another set of TK animals (Phase II) were added to repeat all Day 1 timepoints. Since there was no apparent drug accumulation with repeat dosing, no additional samples from Phase II TK animals were collected beyond Day 1. The analysis showed that the TK profiles for LY2963016 and insulin glargine were similar at all doses. The lowest glucose levels were seen 1-2 hrs postdose at in



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all dose groups of LY2963016 and Lantus. The durations of glucose lowering effects were dose related and lasted approx. 2 hrs postdose for 0.3 mg/kg, 8 hrs postdose for 1 mg/kg, and 12-18 hrs postdose for 3/2 mg/kg groups (for LY2963016 and Lantus). However, the glucose concentrations returned to normal within 24 hrs postdosing. In addition, there was no apparent drug accumulation with repeat dosing. Microscopic findings were observed in sciatic nerve, skin/subcutis, and pancreas. Overall, findings with LY2963016 and US-approved Lantus were similar. The NOAEL was 0.3 mg/kg given LY2963016 or US-approved Lantus.

In Study No 8259267, rats were given LY2963015 or EU-Lantus (0.3, 1, 2 mg/kg) via sc injection for 28 days. Most findings again were associated mostly with hypoglucemia in animals given LY2963016 or EU-approved Lantus. Decreased glucose was seen in rats given ≥ 0.3 mg/kg LY2963016 or EU-approved Lantus. The decreased glucose was marked 1-4 hrs after injection in all dose groups. Microscopic findings were observed in sciatic nerve, skin/subcutis, and pancreas. Overall, findings with LY2963016 and EU-approved Lantus were similar. The NOAEL was 0.3 mg/kg given LY2963016 or EU-approved Lantus.

All in all, for LY2963016 and EU- and US-approved Lantus, in vitro pharmacology studies showed that LY2963016 was a potent and functionally active insulin molecule by using a panel of 8 different in vitro assays. Whether formulated at (b) (4) or Lilly France, LY2963016 showed similar in vitro pharmacology profile to either US- or EU-approved Lantus. Also, two toxicity studies demonstrated a similar spectrum of effects that were typically associated with hypoglycemia, including clinical signs and microscopic findings in sciatic nerve, skin/subcutis, and pancreas.

**Table 30 Key Study Findings on Study No 8259267 (LY2963016 vs. EU approved Lantus) and Study No 8229488 (LY2963016 vs. US approved Lantus)**

**Comparison of Important Attributes from 1-Month Subchronic Toxicity Studies of LY2963016**



Reference ID: 3527465

| Study 8259267 | Study 8229488 |
|---|---|
| | |
| **Inlife Start**: 26 Apr 2012 | **Inlife Start**: 22 Jul 2010 |
| **Inlife End**: 26 May 2012 | **Inlife End**: 23 Aug 2010 |
| **Species**: Rat, Crl:CD (Sprague Dawley) | **Species**: Rat, Crl:CD (Sprague Dawley) |
| **Age**: 10 – 11 weeks | **Age**: 10 – 11 weeks |
| **LY2963016 Lot**: A889460 | **LY2963016 Lot**: MST06628-H |
| **EU-approved LANTUS® Lots**: A1306, 1A1334 | **US-approved LANTUS® Lot**: 0F015A |
| **Doses**: 0, 0.3, 1.0, 2.0 mg/kg | **Doses**: 0, 0.3, 1.0, 3.0/2.0 mg/kg |
| **PK/PD**: Days 1 and 29 | **PK/PD**: Days 1, 15, and 29/32 |
| **Results**<br>• MTD exceeded for LY2963016 and LANTUS® at ≥1.0 mg/kg due to multiple hypoglycemia deaths and clinical signs<br>• Hypoglycemia occurred in all LY2963016 and LANTUS® groups<br>  ○ Dose-dependent duration<br>  ○ Compensatory ↑FC/BWG at 2.0 mg/kg<br>• Sciatic nerve degeneration for LY2963016 and LANTUS® at 2.0 mg/kg<br>• Islet cell atrophy for LY2963016 and LANTUS® at ≥1.0 mg/kg<br>• ↑Fat in skin subcutis for LY2963016 and LANTUS® at ≥1.0 mg/kg | **Results**<br>• MTD exceeded for LY2963016 and LANTUS® at ≥1.0 mg/kg due to multiple hypoglycemia deaths and clinical signs<br>• Hypoglycemia occurred in all LY2963016 and LANTUS® groups<br>  ○ Dose-dependent duration<br>  ○ Compensatory ↑FC/BWG at 3.0/2.0 mg/kg<br>• Sciatic nerve degeneration for LY2963016 and LANTUS® at 3.0/2.0 mg/kg<br>• Islet cell atrophy for LY2963016 and LANTUS® at ≥1.0 mg/kg<br>• ↑Fat in skin/subcutis for LY2963016 and LANTUS® at ≥0.3 mg/kg |
| **MTD: 0.3 mg/kg for LY2963016 and EU-approved LANTUS®** | **MTD: 0.3 mg/kg for LY2963016 and US-approved LANTUS®** |

Abbreviations: BWG = body weight gain, FC = food consumption, MTD = maximum tolerated dose.

The toxicity profile of LY2963016, EU-approved Lantus, and US-approved Lantus were similar. Since the MTD for LY2963016, EU- approved Lantus was 0.3 mg/kg. The margins of safety were approx. 15.9-fold.

**Table 31 Exposure Multiple**

**Margin of Safety in Humans for Target Organ Toxicity and MTD for Hypoglycemia in Rats Following Subchronic Daily Subcutaneous Injection of LY2963016**

| Species | AUC (pmole•h/L) | AUC Exposure Multiple[a] |
|---|---|---|
| Human: | | |
| Study ABEM: 0.6 U/kg[b] | 3160 | – |
| Rat: | | |
| 1-Month Rat Study 8259267 (Reference: EU-approved LANTUS®) | | |
| 2.0 mg/kg | 551466[c] | 175-fold |
| 1.0 mg/kg | 128699[c] | 40.7-fold |
| 0.3 mg/kg[d] | 50182[c] | 15.9-fold |

Abbreviations: AUC = area under plasma concentration-time curve, MTD = maximum-tolerated dose, U = units.

[a] Exposure multiple is the [(AUC in animals) ÷ (AUC in humans)].

[b] Geometric mean for $AUC_{0\text{-}24\ hr}$ in healthy subjects from Study 14L-MC-ABEM.

[c] $AUC_{0\text{-}24\ hr}$ from Study 8259267 measured on Day 29.

[d] MTD.

# 12 Appendix/Attachments

## Nonclinical Program Overview

### Pharmacology: Overview

| Type of Study | Test System | Route | Testing Facility | Document ID | Section # |
|---|---|---|---|---|---|
| **Primary Pharmacodynamics** | | | | | |
| In vitro pharmacology | | | | | |
| • Binding affinity to hIGF-1R, hIR-A, and hIR-B | 293HEK cells | In vitro | Eli Lilly and Company<br>Lilly Corporate Center<br>Indianapolis, IN 46285 USA | DBT149<br>DBT93 | 2.6.2.2.1.1.1<br>2.6.2.2.1.1.2 |
| • Activation of hIR-A and hIR-B | 293HEK cells<br>SAOS2 cells | | | | |
| • Mitogenicity | H4IIe cells | | | | |
| • Metabolic potency | 3T3L1 cells | | | | |
| **Secondary Pharmacodynamics** | | | | | |
| No secondary pharmacology studies of LY2963016 were performed in agreement with EMA guidance [a] | | | | | |
| **Safety Pharmacology** | | | | | |
| No safety pharmacology studies of LY2963016 were performed in agreement with EMA guidance [a] | | | | | |
| **Pharmacodynamic Drug Interactions** | | | | | |
| No pharmacodynamic drug interaction studies of LY2963016 were performed in agreement with EMA guidance [a] | | | | | |

Abbreviations: hIGF-1R = human insulin-like growth factor 1; hIR-A = human insulin receptor isoform A; human IR-B = insulin receptor isoform B

[a] [EMA] European Medicines Agency. 2006. Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues. Guidance on similar medicinal products containing recombinant human soluble insulin. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/ 2009/09/WC500003957.pdf. 6 pp. Accessed 03 March 2013.

### Pharmacokinetics: Overview

| Type of Study | Doses Received[a] | Test System | Route | Testing Facility | Document ID |
|---|---|---|---|---|---|
| **Absorption** | | | | (b) (4) | |
| Multiple-dose TK (0.3, 1, 3/2[b] mg/kg) | 1x/day, 4 weeks | Sprague Dawley Rat | sc | | 8229488 (Table 2.6.7.7A) |
| Multiple-dose TK (0.3, 1, 2 mg/kg) | 1x/day, 4 weeks | Sprague Dawley Rat | sc | | 8259267 (Table 2.6.7.7B) |
| **Distribution** – Not Applicable | | | | | |
| **Metabolism** – Not Applicable | | | | | |
| **Excretion** – Not Applicable | | | | | |
| **Pharmacokinetic Drug Interactions** – Not Applicable | | | | | |
| **Other Studies** – Not Applicable | | | | | |

Abbreviations: sc = subcutaneous, TK = Toxicokinetics, 1x/day = once daily.

[a] Doses received in the toxicokinetic arms of the study; equal dose levels of either LANTUS® or LY2963016.

[b] Animals were dosed at a level of 3 mg/kg/day for Days 1 through 11 of the dosing phase. Beginning on Day 12 of the dosing phase animals at the 3-mg/kg/day dose level were placed on a dosing holiday through Day 14. On Day 15, dosing resumed for these animals at 2 mg/kg/day.

### Toxicology: Overview

| Type of Study | Species/Strain | Route | Duration of Dosing | Dose (mg/kg) of LY2963016 or LANTUS®[a] | GLP | Testing Facility | Document ID |
|---|---|---|---|---|---|---|---|
| **Single Dose Toxicity:** No single dose studies of LY2963016 were performed in agreement with regulatory guidance[c,d] | | | | | | | |
| **Repeat-dose Toxicity** | Rat/ Crl:CD(SD) | Subcutaneous | 1/day; 4 weeks | 0, 0.3, 1, 3/2 [b] | Yes | (b) (4) | Study 8229488 |
| | Rat/ Crl:CD(SD) | Subcutaneous | 1/day; 4 weeks | 0, 0.3, 1, 2 | Yes | | Study 8259267 |

**Genotoxicity:** No genotoxicity studies of LY2963016 were performed in agreement with regulatory guidance[c,d]
**Carcinogenicity:** No carcinogenicity studies of LY2963016 were performed in agreement with regulatory guidance[c,d]
**Reproductive and Developmental Toxicity:** No reproductive and developmental studies of LY2963016 were performed in agreement with regulatory guidance[c]
**Studies in Juvenile Animals:** No studies in juvenile animals of LY2963016 were performed in agreement with regulatory guidance[c]
**Local Tolerance:** No local tolerance studies of LY2963016 were performed in agreement with regulatory guidance[c]

Abbreviation: GLP = Good Laboratory Practices.

[a] Reference material was US-approved LANTUS® in Study 8229488, Reference material was EU-approved LANTUS® in Study 8259267.

[b] Animals were dosed at a level of 3 mg/kg/day for Days 1 through 11 of the dosing phase. Beginning on Day 12 of the dosing phase animals at the 3-mg/kg/day dose level were placed on a dosing holiday through Day 14. On Day 15, dosing resumed for these animals at 2 mg/kg/day as the maximum tolerated dose for hypoglycemia was exceeded at 3 mg/kg.

[c] [EMA] European Medicines Agency. 2006. Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues. Guidance on similar medicinal products containing recombinant human soluble insulin. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003957.pdf. 6 pp. Accessed 03 March 2013.

[d] [ICH] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2011. Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6(R1). http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S6_R1/Step4/S6_R1_Guideline.pdf. Accessed 06 May 2013.

## Toxicokinetics: Overview of Toxicokinetics Studies

| Type of Study | Species/Strain/Sex | Route | Dose (mg/kg) of LY2963016 or LANTUS®[a] | GLP | Document ID |
|---|---|---|---|---|---|
| Repeat dose, 4 weeks | Rat/Crl:CD(SD)/Male and Female | Subcutaneous | 0.3, 1, 3/2 [b]; 1/day x 4 weeks | Yes | Study 8229488 |
| Repeat dose, 4 weeks | Rat/ Crl:CD(SD)/Male and Female | Subcutaneous | 0.3, 1, 2; 1/day x 4 weeks | Yes | Study 8259267 |

[a] Reference material was US-approved LANTUS® in Study 8229488. Reference material was EU-approved LANTUS® in Study 8259267.

[b] Animals were dosed at a level of 3 mg/kg/day for Days 1 through 11 of the dosing phase. Beginning on Day 12 of the dosing phase animals at the 3-mg/kg/day dose level were placed on a dosing holiday through Day 14. On Day 15, dosing resumed for these animals at 2 mg/kg/day as the maximum tolerated dose for hypoglycemia was exceeded at 3 mg/kg.

## Repeat-Dose Toxicity: Nonpivotal Studies

| Species Strain | Route | Duration of Dosing | Doses (mg/kg/d)[a] | Sex #/Group | Noteworthy Findings | Document ID |
|---|---|---|---|---|---|---|
| Rat SD CD/IGS | SC | 14 d | LY2963016 0.375, 0.75, 1.5, 3 | M 5 | 3 mg/kg LANTUS®: BWG/FC exceeded 3 mg/kg LY2963016 | Study 8226-562[b] |
| | | | | | 1.5 mg/kg LY2963016: 1 rat euthanized with clinical signs of severe hypoglycemia. | |
| | | | US-approved LANTUS® 0.375, 0.75, 1.5, 3 | | Glucodynamic profiles of LANTUS® and LY2963016 were similar at all doses on Day 1 and Day 14; dose-related rebound hyperglycemia occurred at all doses after 14 daily doses of LANTUS® and LY2963016. | |

Abbreviations: # = number, BWG = body weight gain, d = day, FC = food consumption, M = male, SC = subcutaneous, SD = Sprague Dawley.

[a] Vehicle = 17 mg/mL glycerin, 2.7 mg/mL meta-cresol, 0.0373 mg/mL of zinc oxide in sterile water, pH 4.

[b] Report available upon request.

## Repeat-Dose Toxicity: 4-Week Study in Rats Given LY2963016 or US-Approved LANTUS®

Report title: A Repeat-Dose Toxicity and Toxicokinetic Study in Rats Given LY2963016 or LANTUS® (US-Approved) by Subcutaneous Injection for 4 Weeks

Species/strain: Rat/Crl:CD(SD)
Initial age: 10 – 14 weeks
Date of first dose: 22 July 2010
NOAEL: 0.3 mg/kg

Duration of dosing: 4 weeks
Duration of reversibility: not applicable
Route: subcutaneous
Vehicle: 17 mg/mL glycerin, 2.7 mg/mL m-cresol, and (b) (4) zinc oxide prepared in Sterile Water for Injection, USP, pH 4.0 (± 0.2).

Document ID: Study 8229488
GLP compliance: Yes
Test Articles: LY2963016, US-approved LANTUS®[a]

Special features: None

(Continued)

| Mean Toxicokinetics (TK) | | | | |
|---|---|---|---|---|
| Analytes: LY2963016 and LANTUS® | Number of TK animals (Main Study Phase): 8/timepoint, 12/sex, 153 total [b]<br>Number of TK animals (Day 1 repeat): 8/timepoint, 16/sex, 192 total [c], | | | |
| Dosing: 1/day x 4 weeks | Feeding condition: fed | Samples: rat serum | Assay/Method: RIA/8225346 | |
| Daily Dose (mg/kg) of LY2963016 or LANTUS® | 0.3[c] | 1[c] | 2 | 2[b] |
| Sex | M+F | M+F | M+F | F |
| Day 15 [b] | | | | |
| LY2963016 | | | | |
| $C_{max}$ (pM) | | | | 19133 |
| $AUC_{0-24hr}$ (pM•hr) | | | | 252377 |
| (SEM) | | | | (12564) |
| $T_{max}$ (hr) | | | | 2 |
| Repeat Day 1 [c] | | | | |
| LY2963016 | | | | |
| $C_{max}$ (pM) | 7903 | 14600 | 23300 | |
| $AUC_{0-24hr}$ (pM•hr) | 24509 | 71986 | 236377 | |
| (SEM) | (4543) | (11900) | (20122) | |
| $T_{max}$ (hr) | 1 | 2 | 4 | |
| US-approved LANTUS® | | | | |
| $C_{max}$ (pM) | 11017 | 19200 | 28060 | |
| $AUC_{0-24hr}$ (pM•hr) | 31679 | 107598 | 241432 | |
| (SEM) | (4440) | (8583) | (19675) | |
| $T_{max}$ (hr) | 1 | 2 | 4 | |

(Continued)

| Daily Dose (mg/kg) | 0 (Control) | | 0.3 mg/kg LY2963016 | | 1 mg/kg LY2963016 | | 3/2 mg/kg[b] LY2963016 | |
|---|---|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | | | |
| Died or euthanized moribund (n[d]) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Body weight gain (g), Days 1-29 (%[e]) | 86 | 28 | ↑12 | ↑36 | ↑13 | ↑29 | ↑43* | ↑143* |
| Food consumption (g), Days 1-29 (%[e]) | 938 | 655 | ↑3 | ↑4 | ↑10 | ↑4 | ↑24* | ↑25* |
| Clinical signs (n[d]) | | | | | | | | |
| Recumbent | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Oral discharge | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Irregular respiration | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Noteworthy Findings | | | | | | | | |
| Clinical chemistry[f] (mg/dL) | | | | | | | | |
| Glucose nadir(time point) Day 1 | 126 (2hr) | 124 (4hr) | 42 (2hr) | 40 (2 hr) | 47 (2hr) | 45 (2hr) | 43 (1hr) | 39 (2hr) |
| Glucose (time point) Day 1 | 137 (24hr) | 145 (24hr) | 134 (24hr) | 148 (24hr) | 144 (24hr) | 139 (24hr) | 149 (24hr) | 119 (24hr) |
| Glucose nadir(time point) Day 15[g] | 125 (1hr) | 125 (4hr) | NC | NC | NC | NC | 26 (4hr) | 30 (4hr) |
| Glucose (time point) Day 15[g] | 159 (24hr) | 146 (24hr) | NC | NC | NC | NC | 171 (24hr) | 140 (24hr) |
| Glucose nadir(time point) Day 29[h] | 106 (1hr) | 106 (18hr) | 38 (2hr) | 35 (2hr) | 30 (2hr) | 35 (1hr) | NC | NC |
| Glucose (time point) Day 29[h] | 117 (24hr) | 124 (24hr) | 131 (24hr) | 141 (24hr) | 146 (24hr) | 149 (24hr) | NC | NC |
| Glucose nadir(time point) Day 32[h] | NC | NC | NC | NC | NC | NC | 18 (8hr) | 23 (4 hr) |
| Glucose (time point) Day 32[h] | NC | NC | NC | NC | NC | NC | 221 (24hr) | 222 (24hr) |
| Histopathology (n[d]) | | | | | | | | |
| Sciatic nerve | | | | | | | | |
| Axonal degeneration | 1 | 0 | 0 | 1 | 0 | 0 | 4 | 4 |
| Skin/subcutis | | | | | | | | |
| Increased adipose tissue | 0 | 0 | 1 | 1 | 2 | 3 | 6 | 10 |
| Pancreas | | | | | | | | |
| Decreased cytoplasm/vacuolation, islet cells | 0 | 0 | 0 | 0 | 2 | 1 | 7 | 7 |

(Continued)

| Daily Dose (mg/kg) | 0.3 mg/kg LANTUS® | | 1 mg/kg LANTUS® | | 3/2 mg/kg[a] LANTUS® | |
|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | |
| Died or euthanized moribund (n[d]) | 0 | 0 | 0 | 0 | 1 | 1 |
| Body weight gain (g), Days 1-29 (%[e]) | ↑17 | ↑14 | ↑13 | ↑7 | ↑36* | ↑157* |
| Food consumption (g), Days 1-29 (%[e]) | ↑5 | ↑1 | ↑5 | ↑1 | ↑22* | ↑32* |
| Clinical signs (n[d]) | | | | | | |
| Hypoactive | 0 | 0 | 0 | 0 | 1 | 0 |
| Recumbent | 0 | 0 | 0 | 0 | 1 | 0 |
| Labored respiration | 0 | 0 | 0 | 0 | 1 | 1 |
| Cold to touch | 0 | 0 | 0 | 0 | 1 | 0 |
| General debilitation | 0 | 0 | 0 | 0 | 1 | 1 |
| Ataxic | 0 | 0 | 0 | 0 | 0 | 1 |
| Clinical chemistry[f] (mg/dL) | | | | | | |
| Glucose nadir(time point) Day 1 | 46 (2hr) | 44 (2hr) | 46 (1hr) | 40 (1hr) | 47 (1hr) | 45 (2hr) |
| Glucose (time point) Day 1 | 141 (24hr) | 147 (24hr) | 147 (24hr) | 148 (24hr) | 153 (24hr) | 132 (24hr) |
| Glucose nadir(time point) Day 15[g] | NC | NC | NC | NC | 29 (4hr) | 31 (4hr) |
| Glucose (time point) Day 15[g] | NC | NC | NC | NC | 187 (24hr) | 166 (24hr) |
| Glucose nadir(time point) Day 29[h] | 35 (2hr) | 40 (1hr) | 32 (2hr) | 33 (1hr) | NC | NC |
| Glucose nadir(time point) Day 29[h] | 131 (24hr) | 140 (24hr) | 158 (24hr) | 165 (24hr) | NC | NC |
| Glucose nadir(time point) Day 32[h] | NC | NC | NC | NC | 25 (4hr) | 23 (2hr) |
| Glucose (time point) Day 32[h] | NC | NC | NC | NC | 248 (24hr) | 243 (24hr) |

(Continued)

| Daily Dose (mg/kg) | 0.3 mg/kg LANTUS® | | 1 mg/kg LANTUS® | | 3/2 mg/kg[a] LANTUS® | |
|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | |
| Histopathology (n[d]) | | | | | | |
| Sciatic nerve | | | | | | |
| Axonal degeneration | 0 | 1 | 1 | 0 | 4 | 3 |
| Skin/subcutis | | | | | | |
| Increased adipose tissue | 0 | 1 | 7 | 5 | 8 | 9 |
| Pancreas | | | | | | |
| ↓cytoplasm/vacuolation, islet cells | 0 | 0 | 2 | 2 | 6 | 8 |

Abbreviations: $AUC_{0\text{-}24hr}$ = area under the plasma concentration-time curve from 0 to 24 hours, $C_{max}$ = maximum observed plasma concentration, F = female, GLP = Good Laboratory Practices, hr = hour, M = male, M+F = male and female combined, NC = not collected, NOAEL = no-observed-adverse-effect level, RIA = radioimmunoassay, SEM= Standard Error of the Mean, $T_{max}$ = time to maximum concentration, TK = toxicokinetics, ↑ = increase, ↓ = decrease.

[a] US-approved LANTUS® was included in the study as a reference compound.

[b] Animals were dosed at a level of 3 mg/kg/day for Days 1 through 11 of the dosing phase. Beginning on Day 12 of the dosing phase animals at the 3-mg/kg/day dose level were placed on a dosing holiday through Day 14. On Day 15, dosing resumed for these animals at 2 mg/kg/day as the MTD for hypoglycemia was exceeded at 3 mg/kg. Nine replacement animals were used for Days 15, 23, or 32.

[c] The study protocol was amended to add additional TK animals. These animals were given a single dose of 0.3, 1, or 2 mg/kg of LY2963016 or LANTUS® in order to obtain associated Day 1 toxicokinetic data (Table 2.6.4.4.2) for this study, and this TK analysis is labeled as occurring on "Repeat Day 1".

[d] Number of animals or tissues.

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

[f] Blood samples for glucose concentration determinations on Days 1 and 29 were obtained from toxicokinetic animals (3/sex/time point).

[g] Blood samples for glucose concentration determinations were collected from 0- and 3/2-mg/kg animals only on Day 15. Day 15 was the first day of dosing at the lowered dose level of 2 mg/kg following the dosing holiday on Day 12.

[h] Due to the dosing holiday from Days 12-14, the dosing phase was extended by 3 days for the 3/2-mg/kg animals. As a result, the last set of glucose samples were collected on Day 32 instead of Day 29.

* $p \leq .05$ (ANOVA and Dunnett's, if applicable).

## Repeat-Dose Toxicity: 4-Week Study in Rats Given LY2963016 and EU-Approved LANTUS®

Report title: A Repeat-Dose Toxicity and Toxicokinetic Study in Rats Given LY2963016 or LANTUS® (European-Approved) by Subcutaneous Injection for 4 Weeks

Species/strain: Rat/Crl:CD(SD)
Initial age: 10 to 11 weeks
Date of first dose: 26 April 2012

Duration of dosing: 1/day x 4 weeks
Duration of reversibility: not applicable
Route: subcutaneous

Document ID: Study 8259267
GLP compliance: Yes
Test Articles: LY2963016, EU-approved LANTUS®[a]

NOAEL: 0.3 mg/kg

Vehicle: 17 mg/mL glycerin, 2.7 mg/mL m-cresol, and (b) (4) zinc oxide prepared in Sterile Water for Injection, USP, pH 4.0 (± 0.2).

Special features: None



Reference ID: 3527465

| Mean Toxicokinetics (TK) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Analytes: LY2963016 and LANTUS® | | | | Number of TK Animals: 6/timepoint, 24/sex,48/dose group 288 total | | | | | |
| Dosing: 1/day x 4 weeks | Feeding condition: fed | | | Sample: rat serum | | | Assay/Method: RIA/8225346 | | |
| Daily Dose (mg/kg) of LY2963016 or LANTUS® | 0.3 | | | 1 | | | 2 | | |
| Sex: | M | F | M+F | M | F | M+F | M | F | M+F |
| Day 1 | | | | | | | | | |
| LY2963016 | | | | | | | | | |
| $C_{max}$(pM) | 4440 | 7813 | 5917 | 16390 | 20133 | 18262 | 26800 | 23200 | 25360 |
| (SD) | (286) | (2075) | (2523) | (9514) | (6507) | (7573) | (3300) | (NA) | (4835) |
| $AUC_{0-24hr}$ (pM•hr) | 15961 | 26376 | 21016 | 95241 | 98567 | 96904 | 223386 | 222888 | 224217 |
| (SEM) | (2526) | (4489) | (2806) | (18560) | (11584) | (10812) | (30179) | (33856) | (20045) |
| $T_{max}$ (hr) | 1 | 2 | 2 | 4 | 4 | 4 | 4 | 4 | 4 |
| EU-approved LANTUS® | | | | | | | | | |
| $C_{max}$(pM) | 6947 | 6310 | 6628 | 14333 | 23000 | 18300 | 17567 | 41500 | 28497 |
| (SD) | (1410) | (2793) | (2009) | (8009) | (4513) | (6084) | (929) | (19419) | (19742) |
| $AUC_{0-24hr}$ (pM•hr) | 19216 | 17095 | 18122 | 82629 | 100983 | 92089 | 174405 | 239692 | 207049 |
| (SEM) | (1376) | (3197) | (1665) | (14735) | (19615) | (11923) | (24420) | (36968) | (26523) |
| $T_{max}$ (hr) | 2 | 2 | 2 | 4 | 2 | 2 | 8 | 4 | 4 |
| Day 29 | | | | | | | | | |
| LY2963016 | | | | | | | | | |
| $C_{max}$(pM) | 11227 | 14033 | 11012 | 25700 | 17967 | 20850 | 106733 | 38600 | 69137 |
| (SD) | (5198) | (4934) | (4879) | (4877) | (907) | (7837) | (98063) | (NA) | (75440) |
| $AUC_{0-24hr}$ (pM•hr) | 58558 | 41784 | 50182 | 142077 | 116315 | 128699 | 688670 | 391906 | 551466 |
| (SEM) | (9811) | (8353) | (8236) | (14424) | (14743) | (11636) | (179199) | (54833 ) | (103131) |
| $T_{max}$ (hr) | 4 | 2 | 2 | 4 | 2 | 4 | 4 | 1 | 4 |
| EU-approved LANTUS® | | | | | | | | | |
| $C_{max}$ (pM) | 12230 | 9537 | 10883 | 30933 | 33900 | 32417 | 38800 | 47433 | 35720 |
| (SD) | (2484) | (2658) | (2733) | (3623) | (18489) | (12026) | (NA) | (10872) | (17805) |
| $AUC_{0-24hr}$ (pM•hr) | 44819 | 31585 | 37545 | 167112 | 136217 | 152919 | 313244 | 478785 | 406818 |
| (SEM) | (5519) | (3064) | (3595) | (22435) | (33638) | (22282) | (38204) | (98168) | (65041) |
| $T_{max}$ (hr) | 1 | 1 | 1 | 2 | 2 | 2 | 8 | 2 | 2 |

(Continued)

| Test Article: Daily Dose (mg/kg) | 0 (Control) | | 0.3 mg/kg LY2963016 | | 1 mg/kg LY2963016 | | 2 mg/kg LY2963016 | |
|---|---|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | | | |
| Died or euthanized moribund (n[b]) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Body weight gain (g), Days 1-29 (%[c]) | 82 | 32 | ↑15 | - | ↑13 | ↑47* | ↑60* | ↑113* |
| Food consumption, Days 1-29 (g) (%[c]) | 883 | 644 | ↑4 | ↑2 | ↑5 | ↑8 | ↑25 | ↑25* |
| Clinical signs (n[b]) | | | | | | | | |
| Hunched | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Limited use, hind legs | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Ataxic | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Recumbent | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Hypoactive | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Cold to touch | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| General debilitation | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Labored respiration | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Clinical chemistry[d] (mg/dL) | | | | | | | | |
| Glucose nadir (time point) Day 1 | 117 (12hr) | 123 (24hr) | 51 (2hr) | 57 (1hr) | 48 (2hr) | 45(1hr) | 47(2hr) | 40 (1hr) |
| Glucose (time point) Day 1 | 128 (24hr) | 123 (24hr) | 128 (24hr) | 128 (24hr) | 135 (24hr) | 127 (24hr) | 133 (24hr) | 135 (24hr) |
| Glucose nadir (time point) Day 29 | 115 (0hr) | 109 (0hr) | 31 (1hr) | 44 (1,2hr) | 30 (4hr) | 28 (4hr) | 20 (4hr) | 23 (4,12hr) |
| Glucose (time point) Day 29 | 128 (24hr) | 117 (24hr) | 128 (24hr) | 127 (24hr) | 172 (24hr) | 157 (24hr) | 221 (24hr) | 272 (24hr) |
| Histopathology (n[b]) | | | | | | | | |
| Sciatic nerve | | | | | | | | |
| Axonal degeneration | 0 | 2 | 1 | 1 | 0 | 0 | 2 | 6 |
| Skin/subcutis | | | | | | | | |
| Increased adipose tissue | 0 | 0 | 0 | 0 | 0 | 1 | 6 | 5 |
| Pancreas | | | | | | | | |
| Decreased cytoplasm/vacuolation, islet cells | 0 | 0 | 0 | 0 | 3 | 1 | 7 | 8 |

(Continued)

| Test Article: Daily Dose (mg/kg) | 0.3 mg/kg LANTUS® | | 1 mg/kg LANTUS® | | 2 mg/kg LANTUS® | |
|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | |
| Died or euthanized moribund (n[b]) | 0 | 0 | 0 | 1 | 2 | 0 |
| Body weight gain (g), Days 1-29 (%[c]) | ↑26 | - | ↑13 | ↑19 | ↑77* | ↑131* |
| Food consumption, Days 1-29 (g) (%[c]) | ↑8 | ↑1 | ↑5 | ↑7 | ↑25* | ↑24* |
| Clinical signs (n[b]) | | | | | | |
| Ataxic | 0 | 0 | 0 | 0 | 1 | 0 |
| General debilitation | 0 | 0 | 0 | 0 | 1 | 0 |
| Audible respiration | 0 | 0 | 0 | 0 | 1 | 0 |
| Labored respiration | 0 | 0 | 0 | 0 | 1 | 0 |
| Clinical chemistry[d] (mg/dL) | | | | | | |
| Glucose nadir(timepoint) Day 1 | 44 (1hr) | 47 (2hr) | 48(1hr) | 37 (4hr) | 46 (4hr) | 47 (2,4hr) |
| Glucose (timepoint) Day 1 | 121 (24hr) | 130 (24hr) | 132 (24hr) | 135 (24hr) | 140 (24hr) | 127 (24hr) |
| Glucose nadir(timepoint) Day 29 | 34 (1hr) | 38 (1hr) | 34 (2hr) | 42 (2hr) | 28 (8hr) | 29 (12hr) |
| Glucose (timepoint) Day 29 | 135 (24hr) | 152 (24hr) | 158 (24hr) | 163 (24hr) | 247 (24hr) | 263 (24hr) |
| Histopathology (n[b]) | | | | | | |
| Sciatic nerve | | | | | | |
| Axonal degeneration | 0 | 1 | 0 | 0 | 0 | 2 |
| Skin/subcutis | | | | | | |
| Increased adipose tissue | 0 | 0 | 0 | 0 | 4 | 6 |
| Pancreas | | | | | | |
| Decreased cytoplasm/vacuolation, islet cells | 0 | 0 | 3 | 2 | 7 | 7 |

Abbreviations: $AUC_{0-24hr}$ = area under the plasma concentration-time curve from 0 to 24 hours, $C_{max}$ = maximum observed plasma concentration, F = female, GLP = Good Laboratoary Practices, hr = hour, M = male, M+F = male and female combined, NA = not applicable, NOAEL = no-observed-adverse-effect level, RIA = radioimmunoassay, SD = standard deviation, SEM = Standard Error of the Mean, TK = toxicokinetics, $T_{max}$ = time to maximum concentration, ↑ = increase, – = no important findings.

a EU-approved LANTUS® was included in the study as a reference compound.

b Number of animals or tissues.

c For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance based on actual data (not on the percent differences).

d Blood samples for glucose concentration determinations on Days 1 and 29 were obtained from toxicokinetic animals (3/sex/time point).

* $p \leq .05$.

## Toxicology: Drug Product

| Lot Number | Purity (%) | Total Impurities | Specified Impurities (%) (b) (4) | Document ID | Type of Study |
|---|---|---|---|---|---|
| Specification: | | NMT | NMT (b) (4) | | |
| (b) (4) | | | (b) (4) | Study 8229488<br>Study 8259267 | Repeat dose, 4 week, rat<br>Repeat dose, 4 week, rat |

Abbreviation: NMT = not more than.

## Lantus's Labeling

### 8. USE IN SPECIFIC POPULATIONS

**8.1 Pregnancy**

Pregnancy Category C: Subcutaneous reproduction and teratology studies have been performed with insulin glargine and regular human insulin in rats and Himalayan rabbits. Insulin glargine was given to female rats before mating, during mating, and throughout pregnancy at doses up to 0.36 mg/kg/day, which is approximately 7 times the recommended human subcutaneous starting dose of 10 Units/day (0.008 mg/kg/day), based on $mg/m^2$. In rabbits, doses of 0.072 mg/kg/day, which is approximately 2 times the recommended human subcutaneous starting dose of 10 Units/day (0.008 mg/kg/day), based on $mg/m^2$, were administered during organogenesis. The effects of insulin glargine did not generally differ from those observed with regular human insulin in rats or rabbits. However, in rabbits, five fetuses from two litters of the high-dose group exhibited dilation of the cerebral ventricles. Fertility and early embryonic development appeared normal.

There are no well-controlled clinical studies of the use of [redacted] (b) (4) in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. It is essential for patients with diabetes or a history of gestational diabetes to maintain good metabolic control before conception and throughout pregnancy. Insulin requirements may decrease during the first trimester, generally increase during the second and third trimesters, and rapidly decline after delivery. Careful monitoring of glucose control is essential in these patients.

**8.3 Nursing Mothers**

It is unknown whether insulin glargine is excreted in human milk. Because many drugs, including human insulin, are excreted in human milk, caution should be exercised when [redacted] (b) (4) is administered to a nursing woman. Use of [redacted] (b) (4) is compatible with breastfeeding, but women with diabetes who are lactating may require adjustments of their insulin doses.

**8.4 Pediatric Use**

The safety and effectiveness of [redacted] (b) (4) have been established in pediatric patients (age 6 to 15 years) with type 1 diabetes *[see Clinical Studies (14)]*. [redacted] (b) (4), [redacted] in pediatric patients younger than 6 years of age with type 1 diabetes. [redacted] (b) (4) in pediatric patients with type 2 diabetes.

[redacted] (b) (4)

## 13. NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In mice and rats, standard two-year carcinogenicity studies with insulin glargine were performed at doses up to 0.455 mg/kg, which was for the rat approximately 10 times and for the mouse approximately 5 times the recommended human subcutaneous starting dose of 10 Units/day (0.008 mg/kg/day), based on mg/m$^2$. The findings in female mice were not conclusive due to excessive mortality in all dose groups during the study. Histiocytomas were found at injection sites in male rats (statistically significant) and male mice (not statistically significant) in acid vehicle containing groups. These tumors were not found in female animals, in saline control, or insulin comparator groups using a different vehicle. The relevance of these findings to humans is unknown.

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

In a combined fertility and prenatal and postnatal study in male and female rats at subcutaneous doses up to 0.36 mg/kg/day, which was approximately 7 times the recommended human subcutaneous starting dose of 10 Units/day (0.008 mg/kg/day), based on mg/m$^2$, maternal toxicity due to dose-dependent hypoglycemia, including some deaths, was observed. Consequently, a reduction of the rearing rate occurred in the high-dose group only. Similar effects were observed with NPH insulin.

## Clinical Studies Conducted for this NDA Submission

| Identifier; Study Type; Location; Status; Report Type | Primary Objective(s) | Design; Control Type | Test and Control Drug(s); Dose, Route, Regimen | Number of Subjects | Diagnosis or Inclusion Criteria | Treatment Duration |
|---|---|---|---|---|---|---|
| I4L-MC-ABEO;<br><br>Comparative BA and BE;<br><br>Section 5.3.4.1;<br>Complete;<br><br>Full CSR | Evaluate PK similarity of LY2963016 to US-approved LANTUS® in HS following single 0.5-U/kg dose, administered SC. | Phase 1, single-site, randomized, double-blind, single-dose (0.5 U/kg), 2-treatment, 4-period, crossover, replicate, treatment euglycemic clamp study. | Test: LY2963016;<br><br>Single 0.5-U/kg dose, administered SC.<br><br>Control: US-approved LANTUS®;<br><br>Single 0.5-U/kg dose, administered SC. | 91 randomized<br>82 completed | Healthy males or females, aged between 21 and 65 years, inclusive, with screening BMI between 18.5 and 29.9 kg/m$^2$, inclusive, and screening fasting glucose <6.0 mmol/L. | Four 24-hour periods, with a 7-day washout between each period. |
| I4L-MC-ABEA;<br><br>Comparative BA and BE;<br><br>Section 5.3.1.2;<br><br>Complete;<br><br>Full CSR | Evaluate PK equivalence of LY2963016 to LANTUS® in HS following 0.5-U/kg single-dose SC administration. | Phase 1, single-site, randomized, double-blind, single-dose (0.5 U/kg), 2-treatment, 4-period, crossover, replicate, euglycemic clamp study. | Test: LY2963016;<br><br>Single 0.5-U/kg dose, administered SC.<br><br>Control: EU-approved LANTUS®;<br><br>Single 0.5-U/kg dose, administered SC. | 80 randomized<br>78 completed | Healthy males or females, aged between 18 and 60 years, inclusive, with screening BMI between 18.5 and 32.0 kg/m$^2$. | Four 24-hour periods, with a 7-day washout between each period. |



Reference ID: 3527465

| Identifier; Study Type; Location; Status; Report Type | Primary Objective(s) | Design; Control Type | Test and Control Drug(s); Dose, Route, Regimen | Number of Subjects | Diagnosis or Inclusion Criteria | Treatment Duration |
|---|---|---|---|---|---|---|
| I4L-MC-ABEI; Comparative BA and BE; Section 5.3.1.2; Complete; Full CSR | Evaluate RBA of LY2963016 to LANTUS® in HS following 0.5-U/kg single-dose SC administration of each. | Phase 1, single-site, randomized, open-label, single-dose, 2-treatment, 2-period, crossover study. | Test: LY2963016; Single 100-U/mL solution, 0.5-U/kg dose, administered SC. Control: EU-approved LANTUS®; Single 100-U/mL solution, 0.5-U/kg dose, administered SC. | 16 randomized 13 completed | Healthy men and women, aged between 21 and 60 years, with BMI between 18.5 and 29.9 kg/m². | Two 24-hour treatment periods, with a 7-day washout between treatment periods. |
| I4L-MC-ABEM; Comparative BA and BE; Section 5.3.1.2; Complete; Full CSR | Compare PK of LY2963016 and LANTUS® in HS following 0.3- and 0.6-U/kg single-dose SC administration. | Phase 1, single-site, randomized, subject- and investigator-blind, 4-treatment, 4-period, crossover, euglycemic clamp study. | Test: LY2963016; Single 0.3- or 0.6-U/kg dose, administered SC. Control: EU-approved LANTUS®; Single 0.3- or 0.6-U/kg dose, administered SC. | 24 randomized 23 completed | Healthy males or females, aged between 21 and 65 years, inclusive, with BMI between 18.5 and 29.9 kg/m², inclusive. | Four 24-hour periods, with a 6-day washout between each period. |

| Identifier; Study Type; Location; Status; Report Type | Primary Objective(s) | Design; Control Type | Test and Control Drug(s); Dose, Route, Regimen | Number of Subjects | Diagnosis or Inclusion Criteria | Treatment Duration |
|---|---|---|---|---|---|---|
| I4L-MC-ABEN; Comparative BA and BE; Section 5.3.1.2; Complete; Full CSR | Evaluate PK equivalence of EU- to US-approved LANTUS® in HS following 0.5-U/kg single-dose SC administration. | Phase 1, single-site, randomized, double-blind, single-dose, 2-treatment, 4-period, crossover, replicate, euglycemic clamp study. | Test: EU-approved LANTUS® Single 0.5-U/kg dose, administered SC. Control: US-approved LANTUS® Single 0.5-U/kg dose, administered SC. | 40 randomized 34 completed | Subjects aged between 21 and 65 years, inclusive, with BMI between 18.5 and 29.9 kg/m², inclusive. | Four 24-hour periods, with a 7-day washout between each period. |
| I4L-MC-ABEE; Patient PD; Section 5.3.4.2; Complete; Full CSR | Assess the duration of action of LY2963016 compared to LANTUS® in subjects with T1DM. | Phase 1, single-site, randomized, subject- and investigator-blind, single-dose, 2-period, crossover, 42-hour postdose, euglycemic clamp study. | Test: LY2963016; Single 0.3-U/kg dose, administered SC. Control: EU-approved LANTUS®; Single 0.3-U/kg dose, administered SC. | 20 randomized 20 completed | Males and females, aged between 18 and 60 years, inclusive, with T1DM for ≥1 year, HbA1c ≤10.0%, fasting C-peptide ≤0.3 nmol/L, and BMI ≤29 kg/m². | Two 2-day treatment periods, with a washout from 7 to 21 days between treatment periods. |



Reference ID: 3527465

| Identifier; Study Type; Location; Status; Report Type | Primary Objective(s) | Design; Control Type | Test and Control Drug(s); Dose, Route, Regimen | Number of Subjects | Diagnosis or Inclusion Criteria | Treatment Duration |
|---|---|---|---|---|---|---|
| I4L-MC-ABEB;<br><br>Efficacy and Safety;<br><br>Section 5.3.5.1;<br><br>Complete;<br><br>Full CSR | To test the hypothesis that LY2963016 QD was noninferior to LANTUS® QD, as measured by change in HbA1c from baseline to 24 weeks, when used SC in combination with premeal insulin lispro administered TID. | Phase 3, prospective, randomized, multicenter, 2-arm, active control, open-label, parallel, 24-week treatment study with a 28-week active control, open-label extension period and 4-week posttreatment follow-up in patients with T1DM. | Test: LY2963016 QD, administered SC.<br><br>LY2963016 was started at the same dose and administered at same timing (i.e., daytime or nighttime) as the patient's prestudy QD basal insulin and individually titrated.<br><br>Control: US- or EU-approved* LANTUS® QD, administered SC.<br><br>LANTUS® was started at the same dose and administered at same timing (i.e., daytime or nighttime) as the patient's prestudy QD basal insulin and individually titrated. | 24-week treatment:<br><br>536 randomized<br>509 completed<br><br>28-week extension:<br><br>490 completed extension | Males and females with T1DM ≥1 year, aged ≥18, BMI ≤35 kg/m², HbA1c ≤11%, on basal-bolus insulin therapy for ≥1 year.<br><br>Basal insulin must be QD injection of NPH, LANTUS®, or detemir ≥3 months prior to study entry and combined with mealtime injections of human regular insulin, insulin analog lispro, aspart, or glulisine. | 24-week treatment and 28-week extension periods (52 weeks) |

| Identifier; Study Type; Location; Status; Report Type | Primary Objective(s) | Design; Control Type | Test and Control Drug(s); Dose, Route, Regimen | Number of Subjects | Diagnosis or Inclusion Criteria | Treatment Duration |
|---|---|---|---|---|---|---|
| I4L-MC-ABEC;<br><br>Efficacy and Safety;<br><br>Section 5.3.5.1;<br><br>Complete;<br><br>Full CSR | To test the hypothesis that LY2963016 QD was noninferior to LANTUS® QD, as measured by change in HbA1c from baseline to 24 weeks, when used in combination with OAMs. | Phase 3, prospective, randomized, multicenter, 2-arm, active control, double-blind, parallel, 24-week treatment, and 4-week posttreatment follow-up study in adult patients with T2DM. | Test: LY2963016 QD;<br><br>Patients on prestudy LANTUS®: Starting LY2963016, at same dose as prestudy LANTUS® administered SC and individually titrated.<br><br>Insulin-naïve patients: Starting LY2963016 QD 10-U dose, administered SC and individually titrated.<br><br>Control: US- or EU-approved* LANTUS® QD;<br><br>Patients on prestudy LANTUS®: Starting LANTUS® QD, at same dose as prestudy LANTUS®, administered SC and individually titrated.<br><br>Insulin-naïve patients: Starting LANTUS® QD 10-U dose, administered SC and individually titrated. | 759 randomized<br>662 completed | Patients with T2DM, aged ≥18, with BMI ≤45 kg/m2, and on ≥2 OAMs (with or without LANTUS®) for ≥12 weeks prior to study entry.<br><br>Insulin-naïve patients: HbA1c between ≥7.0% and ≤11.0%.<br><br>Prestudy LANTUS® patients: HbA1c ≤11.0%. | 24 weeks |

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/s/

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MIYUN M TSAI-TURTON
06/18/2014

KAREN L DAVIS BRUNO
06/23/2014

# Pharmacology/Toxicology Mid-Cycle Deliverables

Drug Information: This NDA 205692 is a 505b2 submission for LY2963016, by Lilly to treat T1DM and T2DM. This long acting insulin analog is formulated in a 3ml cartridge sealed in a prefilled pen injector (KwikPen) for injection.

| Mid-Cycle Deliverable | Goal Date | Status |
|---|---|---|
| **Filing Review Meeting**<br>• **Deliverable: Regulatory history, summary of pharmacology and toxicology findings from preliminary review of existing data, adequacy of NDA submission** | **45- day of NDA submission** | Completed |
| **Genetox Study Review (impurities and drug substance)**<br>• **Deliverable: Draft review, label recommendation/interact with genetox committee** | **2-3 months of NDA submission** | n/a |
| **Carcinogenicity Study Review**<br>• **Deliverable: Identify statistical reviewer** | **1-month of NDA submission** | n/a |
| • **Deliverable: Schedule ECAC meeting** | **1-month of NDA submission** | n/a |
| • **Deliverable: Draft review of Carcinogenicity study with statistical input, *Interact with statistician*** | **6-months of NDA submission** | n/a |
| • **Deliverable: ECAC review of carcinogenicity study, incorporation of eCAC comments in the review, Identification of issues from Carcinogenicity study & related post marketing commitments, labeling recommendation** | **7- month of NDA submission** | n/a |
| **ReproTox Study Review**<br>• **Deliverable: Draft review, Identify issue/s (special studies for post marketing commitments), labeling recommendation, *Interact with Reprotox committee*** | **5- month of NDA submission** | n/a<br><br>Sponsor is seeking Category C labeling (same as Lantus) |
| **Impurity/Extractable Qualification**<br>• **Deliverable: Begin review; *interact with CMC*** | **3-month of NDA submission-will depend on CMC also** | Drug substance impurities in final drug substance close or above the 0.1% were characterized. (b) (4) [redacted] )<br><br>Drug product impurities for full identification were set at (b) (4) %. (b) (4) [redacted] and unidentified |

| | | |
|---|---|---|
| | | modifications were observed.<br><br>Drug product impurities were quantified in 4 week repeat dose rat studies and were below specifications. Two lots were tested. Total impurities were (b) (4) (b) (4)% and (b) (4) was (b) (4) % (with specifications of (b) (4)% for total impurities and (b) (4)% for (b) (4)). |
| • **Deliverable: Identify whether the impurities are qualified, Identify approvability/post marketing commitment issues** | **5-6 month of NDA submission- if sponsor has final formulation** | n/a |
| **Toxicokinetic Study Review**<br>• **Deliverable: Draft review; *Interact with Clinical Pharmacology* for dose and ADME, *Interact with Medical Reviewer* for adverse event comparison and dose multiples** | **6- months of NDA submission** | Under review<br><br>TK groups were included in repeat dose toxicity rat studies. |
| **Chronic Toxicity Study Review**<br>• **Deliverable: Draft review** | **6- months of NDA submission** | Under review<br><br>Study No 8229488 (4 week toxicity rat study vs. US Lantus) was reviewed under IND105423 original submission.<br><br>Study No 8259267 (4 week toxicity rat study vs. EU Lantus) was first submitted under IND105423 eCTD0023. |
| **Pharmacology Study Review**<br>• **Deliverable: Draft review, identify PD issues** | **6- months of NDA submission** | Under review<br><br>DBT93 (in vitro PD study vs. US Lantus) was reviewed under IND105423 original submission.<br><br>DBT149 (in vitro PD study comparing batches with EU or US Lantus) was first submitted under IND105423 eCTD0032. |
| **Tox findings compared in animal chronic studies and human phase 3 studies**<br>• **Deliverable: Input in ODS database** | **8- months of NDA submission** | The final recommendation is pending completion of comprehensive review. |

## *Proposed Labeling*

### 8 USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

Pregnancy Category C. Subcutaneous reproduction and teratology studies have been performed with another insulin glargine product and with regular human insulin in rats and Himalayan rabbits. (b) (4) insulin glargine product was given to female rats before mating, during mating, and throughout pregnancy at dose up to 0.36 mg/kg/day, which is approximately 7 times the recommended human subcutaneous starting dose of 10 units/day (0.008 mg/kg/day) based on mg/m$^2$. In rabbits, doses of 0.072 mg/kg/day, which is approximately 2 times the recommended human subcutaneous starting dose of 10 units/day (0.008 mg/kg/day), based on mg/m$^2$, were administered during organogenesis. The effects of (b) (4) insulin glargine product did not generally differ from those observed with regular human insulin in rats and rabbits. However, in rabbits, five fetuses from two litters of the high-dose group exhibited dilation of the cerebral ventricles. Fertility and early embryonic development appeared normal.

There are no well-controlled clinical studies of the use of insulin glargine in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. It is essential for patients with diabetes or a history of gestational diabetes to maintain good metabolic control before conception and throughout pregnancy. Insulin requirements may decrease during the first trimester, generally increase during the second and third trimesters, and rapidly decline after delivery. Careful monitoring of glucose control is essential in these patients.

### 8.3 Nursing Mothers

It is unknown whether insulin glargine is excreted in human milk. Because many drugs, including human insulin, are excreted in human milk, caution should be exercised when (b) (4) is administered to a nursing woman. Use of (b) (4) is compatible with breastfeeding, but women with diabetes who are lactating may require adjustments of their insulin doses.

### 8.4 Pediatric Use

The safety and effectiveness of (b) (4) have been established in pediatric patients (age 6 to 15 years) with type 1 diabetes *[see Clinical Studies (14)]*. (b) (4) in pediatric patients younger than 6 years of age with type 1 (b) (4) pediatric patients with type 2 diabetes.

(b) (4)

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In mice and rats, standard two-year carcinogenicity studies with another insulin glargine product were performed at doses up to 0.455 mg/kg, which was for the rat approximately 10 times and for the mouse approximately 5 times the recommended human subcutaneous starting dose of 10 units/day (0.008 mg/kg/day), based on mg/m$^2$. The findings in female mice were not conclusive due to excessive mortality in all dose groups during the study. Histiocytomas were found at injection sites in male rats (statistically significant) and male mice (not statistically significant) in acid vehicle containing groups. These tumors were not found in female animals, in saline control, or insulin comparator groups using a difference vehicle. The relevance of these findings to humans is unknown.

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

In a combined fertility and prenatal and postnatal study of another insulin glargine product in male and female rats at subcutaneous doses up to 0.36 mg/kg/day, which was approximately 7

times the recommended human subcutaneous starting dose of 10 units/day (0.008 mg/kg/day), based on mg/m$^2$, maternal toxicity due to dose-dependent hypoglycemia, including some deaths, was observed. Consequently, a reduction of the rearing rate occurred in the high-dose group only. Similar effects were observed with NPH insulin.

Lantus labeling

## 8. USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

Pregnancy Category C: Subcutaneous reproduction and teratology studies have been performed with insulin glargine and regular human insulin in rats and Himalayan rabbits. Insulin glargine was given to female rats before mating, during mating, and throughout pregnancy at doses up to 0.36 mg/kg/day, which is approximately 7 times the recommended human subcutaneous starting dose of 10 Units/day (0.008 mg/kg/day), based on mg/m$^2$. In rabbits, doses of 0.072 mg/kg/day, which is approximately 2 times the recommended human subcutaneous starting dose of 10 Units/day (0.008 mg/kg/day), based on mg/m$^2$, were administered during organogenesis. The effects of insulin glargine did not generally differ from those observed with regular human insulin in rats or rabbits. However, in rabbits, five fetuses from two litters of the high-dose group exhibited dilation of the cerebral ventricles. Fertility and early embryonic development appeared normal.

There are no well-controlled clinical studies of the use of LANTUS in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. It is essential for patients with diabetes or a history of gestational diabetes to maintain good metabolic control before conception and throughout pregnancy. Insulin requirements may decrease during the first trimester, generally increase during the second and third trimesters, and rapidly decline after delivery. Careful monitoring of glucose control is essential in these patients.

### 8.3 Nursing Mothers

It is unknown whether insulin glargine is excreted in human milk. Because many drugs, including human insulin, are excreted in human milk, caution should be exercised when LANTUS is administered to a nursing woman. Use of LANTUS is compatible with breastfeeding, but women with diabetes who are lactating may require adjustments of their insulin doses.

### 8.4 Pediatric Use

The safety and effectiveness of subcutaneous injections of LANTUS have been established in pediatric patients (age 6 to 15 years) with type 1 diabetes *[see Clinical Studies (14)]*. LANTUS has not been studied in pediatric patients younger than 6 years of age with type 1 diabetes. LANTUS has not been studied in pediatric patients with type 2 diabetes.

Based on the results of a study in pediatric patients, the dose recommendation when switching to LANTUS is the same as that described for adults *[see Dosage and Administration (2.3)* and *Clinical Studies (14)]*. As in adults, the dosage of LANTUS must be individualized in pediatric patients based on metabolic needs and frequent monitoring of blood glucose.

## 13. NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

In mice and rats, standard two-year carcinogenicity studies with insulin glargine were performed at doses up to 0.455 mg/kg, which was for the rat approximately 10 times and for the mouse approximately 5 times the recommended human subcutaneous starting dose of 10 Units/day (0.008 mg/kg/day), based on mg/m$^2$. The findings in female mice were not conclusive due to excessive mortality in all dose groups during the study. Histiocytomas were found at injection sites in male rats (statistically significant) and male mice (not statistically significant) in acid vehicle containing groups. These tumors were not found in female animals, in saline control, or insulin comparator groups using a different vehicle. The relevance of these findings to humans is unknown.

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

In a combined fertility and prenatal and postnatal study in male and female rats at subcutaneous doses up to 0.36 mg/kg/day, which was approximately 7 times the recommended human subcutaneous starting dose of 10 Units/day (0.008 mg/kg/day), based on mg/m$^2$, maternal toxicity due to dose-dependent hypoglycemia, including some deaths, was observed. Consequently, a reduction of the rearing rate occurred in the high-dose group only. Similar effects were observed with NPH insulin.

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/s/

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MIYUN M TSAI-TURTON
03/14/2014

KAREN L DAVIS BRUNO
03/14/2014

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

**NDA/BLA Number: 205692** **Applicant: Lilly** **Stamp Date: 10/18/2013**

**Drug Name: (b) (4)™ KwikPen™ (insulin glargine, LY2963016)** **NDA/BLA Type: 505b2**

Lilly submitted NDA 205692 for LY2963016 under 505b2. LY2963016 is a similar version of LANTUS (insulin glargine injection; NDA 021081). Both have similar formulations. It is long acting insulin analog to improve glycemic control in adults and children with T1DM and in adults with T2DM. It is administered as a sc injection and will be available in a 3ml cartridge sealed in a prefilled pen injector (KwikPen™). The nonclinical development program for LY2963016 focused on demonstrating the similarity of LY2963016 to EU-approved or US-approved LANTUS® with regards to primary pharmacodynamics, pharmacokinetics, and animal toxicity.

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

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 1 | Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin? | X | | |
| 2 | Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin? | X | | |
| 3 | Is the pharmacology/toxicology section legible so that substantive review can begin? | X | | |
| 4 | Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)? | X | | Under its 505b2 submission, the sponsor submitted:<br>▪ Primary PD: dbt93 and dbt149<br>▪ PK: 8225346<br>▪ Tox: 8229488 and 8259267 |
| 5 | If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA). | X | | |
| 6 | Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route? | X | | Intended route: sc injection |

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

| | Content Parameter | Yes | No | Comment |
|---|---|---|---|---|
| 7 | Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? | X | | |
| 8 | Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions? | X | | |
| 9 | Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57? | X | | Sections related to pharm/tox were appropriately addressed in the Section 8.1 Pregnancy (category C) and Section 13.1 |
| 10 | Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.) | X | | Impurities in drug product are quantified and are below specifications. No toxicity study is needed. |
| 11 | Has the applicant addressed any abuse potential issues in the submission? | | | n/a |
| 12 | If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted? | | | n/a |

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

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

n/a

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

There are no nonclinical comments for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NONCLINICAL PROGRAM OVERVIEW

## Pharmacology: Overview

| Type of Study | Test System | Route | Testing Facility | Document ID | Section # |
|---|---|---|---|---|---|
| **Primary Pharmacodynamics** | | | | | |
| In vitro pharmacology | | | | | |
| • Binding affinity to hIGF-1R, hIR-A, and hIR-B | 293HEK cells | In vitro | Eli Lilly and Company<br>Lilly Corporate Center<br>Indianapolis, IN 46285 USA | DBT149<br>DBT93 | 2.6.2.2.1.1.1<br>2.6.2.2.1.1.2 |
| • Activation of hIR-A and hIR-B | 293HEK cells<br>SAOS2 cells | | | | |
| • Mitogenicity | H4IIe cells | | | | |
| • Metabolic potency | 3T3L1 cells | | | | |
| **Secondary Pharmacodynamics** | | | | | |
| No secondary pharmacology studies of LY2963016 were performed in agreement with EMA guidance [a] | | | | | |
| **Safety Pharmacology** | | | | | |
| No safety pharmacology studies of LY2963016 were performed in agreement with EMA guidance [a] | | | | | |
| **Pharmacodynamic Drug Interactions** | | | | | |
| No pharmacodynamic drug interaction studies of LY2963016 were performed in agreement with EMA guidance [a] | | | | | |

Abbreviations: hIGF-1R = human insulin-like growth factor 1; hIR-A = human insulin receptor isoform A; human IR-B = insulin receptor isoform B

[a] [EMA] European Medicines Agency. 2006. Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues. Guidance on similar medicinal products containing recombinant human soluble insulin. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/ 2009/09/WC500003957.pdf. 6 pp. Accessed 03 March 2013.

## Pharmacokinetics: Overview

| Type of Study | Doses Received[a] | Test System | Route | Testing Facility | Document ID |
|---|---|---|---|---|---|
| **Absorption** | | | | (b) (4) | |
| Multiple-dose TK (0.3, 1, 3/2[b] mg/kg) | 1x/day, 4 weeks | Sprague Dawley Rat | sc | | 8229488<br>(Table 2.6.7.7A) |
| Multiple-dose TK (0.3, 1, 2 mg/kg) | 1x/day, 4 weeks | Sprague Dawley Rat | sc | | 8259267<br>(Table 2.6.7.7B) |
| **Distribution** – Not Applicable | | | | | |
| **Metabolism** – Not Applicable | | | | | |
| **Excretion** – Not Applicable | | | | | |
| **Pharmacokinetic Drug Interactions** – Not Applicable | | | | | |
| **Other Studies** – Not Applicable | | | | | |

Abbreviations: sc = subcutaneous, TK = Toxicokinetics, 1x/day = once daily.

[a] Doses received in the toxicokinetic arms of the study; equal dose levels of either LANTUS® or LY2963016.

[b] Animals were dosed at a level of 3 mg/kg/day for Days 1 through 11 of the dosing phase. Beginning on Day 12 of the dosing phase animals at the 3-mg/kg/day dose level were placed on a dosing holiday through Day 14. On Day 15, dosing resumed for these animals at 2 mg/kg/day.

## Toxicology: Overview

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

| Type of Study | Species/Strain | Route | Duration of Dosing | Dose (mg/kg) of LY2963016 or LANTUS®[a] | GLP | Testing Facility | Document ID |
|---|---|---|---|---|---|---|---|
| **Single Dose Toxicity:** No single dose studies of LY2963016 were performed in agreement with regulatory guidance[c,d] | | | | | | | |
| **Repeat-dose Toxicity** | Rat/ Crl:CD(SD) | Subcutaneous | 1/day; 4 weeks | 0, 0.3, 1, 3/2 [b] | Yes | (b) (4) | Study 8229488 |
| | Rat/ Crl:CD(SD) | Subcutaneous | 1/day; 4 weeks | 0, 0.3, 1, 2 | Yes | | Study 8259267 |

**Genotoxicity:** No genotoxicity studies of LY2963016 were performed in agreement with regulatory guidance[c,d]
**Carcinogenicity:** No carcinogenicity studies of LY2963016 were performed in agreement with regulatory guidance[c,d]
**Reproductive and Developmental Toxicity:** No reproductive and developmental studies of LY2963016 were performed in agreement with regulatory guidance[c]
**Studies in Juvenile Animals:** No studies in juvenile animals of LY2963016 were performed in agreement with regulatory guidance[c]
**Local Tolerance:** No local tolerance studies of LY2963016 were performed in agreement with regulatory guidance[c]

Abbreviation: GLP = Good Laboratory Practices.

[a] Reference material was US-approved LANTUS® in Study 8229488, Reference material was EU-approved LANTUS® in Study 8259267.

[b] Animals were dosed at a level of 3 mg/kg/day for Days 1 through 11 of the dosing phase. Beginning on Day 12 of the dosing phase animals at the 3-mg/kg/day dose level were placed on a dosing holiday through Day 14. On Day 15, dosing resumed for these animals at 2 mg/kg/day as the maximum tolerated dose for hypoglycemia was exceeded at 3 mg/kg.

[c] [EMA] European Medicines Agency. 2006. Annex to guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues. Guidance on similar medicinal products containing recombinant human soluble insulin. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003957.pdf. 6 pp. Accessed 03 March 2013.

[d] [ICH] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2011. Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6(R1). http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S6_R1/Step4/S6_R1_Guideline.pdf. Accessed 06 May 2013.

## Toxicokinetics: Overview of Toxicokinetics Studies

| Type of Study | Species/Strain/Sex | Route | Dose (mg/kg) of LY2963016 or LANTUS®[a] | GLP | Document ID |
|---|---|---|---|---|---|
| Repeat dose, 4 weeks | Rat/Crl:CD(SD)/Male and Female | Subcutaneous | 0.3, 1, 3/2 [b]; 1/day x 4 weeks | Yes | Study 8229488 |
| Repeat dose, 4 weeks | Rat/ Crl:CD(SD)/Male and Female | Subcutaneous | 0.3, 1, 2; 1/day x 4 weeks | Yes | Study 8259267 |

[a] Reference material was US-approved LANTUS® in Study 8229488. Reference material was EU-approved LANTUS® in Study 8259267.

[b] Animals were dosed at a level of 3 mg/kg/day for Days 1 through 11 of the dosing phase. Beginning on Day 12 of the dosing phase animals at the 3-mg/kg/day dose level were placed on a dosing holiday through Day 14. On Day 15, dosing resumed for these animals at 2 mg/kg/day as the maximum tolerated dose for hypoglycemia was exceeded at 3 mg/kg.

## Repeat-Dose Toxicity: Nonpivotal Studies

| Species Strain | Route | Duration of Dosing | Doses (mg/kg/d)[a] | Sex #/Group | Noteworthy Findings | Document ID |
|---|---|---|---|---|---|---|
| Rat SD CD/IGS | SC | 14 d | LY2963016 0.375, 0.75, 1.5, 3 | M 5 | 3 mg/kg LANTUS®: BWG/FC exceeded 3 mg/kg LY2963016 | Study 8226-562[b] |
| | | | | | 1.5 mg/kg LY2963016: 1 rat euthanized with clinical signs of severe hypoglycemia. | |
| | | | US-approved LANTUS® 0.375, 0.75, 1.5, 3 | | Glucodynamic profiles of LANTUS® and LY2963016 were similar at all doses on Day 1 and Day 14; dose-related rebound hyperglycemia occurred at all doses after 14 daily doses of LANTUS® and LY2963016. | |

Abbreviations: # = number, BWG = body weight gain, d = day, FC = food consumption, M = male, SC = subcutaneous, SD = Sprague Dawley.

[a] Vehicle = 17 mg/mL glycerin, 2.7 mg/mL meta-cresol, (b) (4) of zinc oxide in sterile water, pH 4.

[b] Report available upon request.

## Repeat-Dose Toxicity: 4-Week Study in Rats Given LY2963016 or US-Approved LANTUS®

# PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

Report title: A Repeat-Dose Toxicity and Toxicokinetic Study in Rats Given LY2963016 or LANTUS® (US-Approved) by Subcutaneous Injection for 4 Weeks

Species/strain: Rat/Crl:CD(SD)
Initial age: 10 – 14 weeks
Date of first dose: 22 July 2010
NOAEL: 0.3 mg/kg

Duration of dosing: 4 weeks
Duration of reversibility: not applicable
Route: subcutaneous
Vehicle: 17 mg/mL glycerin, 2.7 mg/mL m-cresol, and (b) (4), zinc oxide prepared in Sterile Water for Injection, USP, pH 4.0 (± 0.2).

Document ID: Study 8229488
GLP compliance: Yes
Test Articles: LY2963016, US-approved LANTUS®[a]

Special features: None

(Continued)

| Mean Toxicokinetics (TK) | | | | |
|---|---|---|---|---|
| Analytes: LY2963016 and LANTUS® | Number of TK animals (Main Study Phase): 8/timepoint, 12/sex, 153 total [b]<br>Number of TK animals (Day 1 repeat): 8/timepoint, 16/sex, 192 total [c], | | | |
| Dosing: 1/day x 4 weeks | Feeding condition: fed | Samples: rat serum | Assay/Method: RIA/8225346 | |
| Daily Dose (mg/kg) of LY2963016 or LANTUS® | 0.3[c] | 1[c] | 2 | 2[b] |
| Sex | M+F | M+F | M+F | F |
| Day 15 [b] | | | | |
| LY2963016 | | | | |
| $C_{max}$ (pM) | | | | 19133 |
| $AUC_{0-24hr}$ (pM•hr) | | | | 252377 |
| (SEM) | | | | (12564) |
| $T_{max}$ (hr) | | | | 2 |
| Repeat Day 1 [c] | | | | |
| LY2963016 | | | | |
| $C_{max}$ (pM) | 7903 | 14600 | 23300 | |
| $AUC_{0-24hr}$ (pM•hr) | 24509 | 71986 | 236377 | |
| (SEM) | (4543) | (11900) | (20122) | |
| $T_{max}$ (hr) | 1 | 2 | 4 | |
| US-approved LANTUS® | | | | |
| $C_{max}$ (pM) | 11017 | 19200 | 28060 | |
| $AUC_{0-24hr}$ (pM•hr) | 31679 | 107598 | 241432 | |
| (SEM) | (4440) | (8583) | (19675) | |
| $T_{max}$ (hr) | 1 | 2 | 4 | |

(Continued)

| Daily Dose (mg/kg) | 0 (Control) | | 0.3 mg/kg LY2963016 | | 1 mg/kg LY2963016 | | 3/2 mg/kg[b] LY2963016 | |
|---|---|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | | | |
| Died or euthanized moribund (n[d]) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Body weight gain (g), Days 1-29 (%[e]) | 86 | 28 | ↑12 | ↑36 | ↑13 | ↑29 | ↑43* | ↑143* |
| Food consumption (g), Days 1-29 (%[e]) | 938 | 655 | ↑3 | ↑4 | ↑10 | ↑4 | ↑24* | ↑25* |
| Clinical signs (n[d]) | | | | | | | | |
| Recumbent | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Oral discharge | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Irregular respiration | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Noteworthy Findings | | | | | | | | |
| Clinical chemistry[f] (mg/dL) | | | | | | | | |
| Glucose nadir(time point) Day 1 | 126 (2hr) | 124 (4hr) | 42 (2hr) | 40 (2 hr) | 47 (2hr) | 45 (2hr) | 43 (1hr) | 39 (2hr) |
| Glucose (time point) Day 1 | 137 (24hr) | 145 (24hr) | 134 (24hr) | 148 (24hr) | 144 (24hr) | 139 (24hr) | 149 (24hr) | 119 (24hr) |
| Glucose nadir(time point) Day 15[g] | 125 (1hr) | 125 (4hr) | NC | NC | NC | NC | 26 (4hr) | 30 (4hr) |
| Glucose (time point) Day 15[g] | 159 (24hr) | 146 (24hr) | NC | NC | NC | NC | 171 (24hr) | 140 (24hr) |
| Glucose nadir(time point) Day 29[h] | 106 (1hr) | 106 (18hr) | 38 (2hr) | 35 (2hr) | 30 (2hr) | 35 (1hr) | NC | NC |
| Glucose (time point) Day 29[h] | 117 (24hr) | 124 (24hr) | 131 (24hr) | 141 (24hr) | 146 (24hr) | 149 (24hr) | NC | NC |
| Glucose nadir(time point) Day 32[h] | NC | NC | NC | NC | NC | NC | 18 (8hr) | 23 (4 hr) |
| Glucose (time point) Day 32[h] | NC | NC | NC | NC | NC | NC | 221 (24hr) | 222 (24hr) |
| Histopathology (n[d]) | | | | | | | | |
| Sciatic nerve | | | | | | | | |
| Axonal degeneration | 1 | 0 | 0 | 1 | 0 | 0 | 4 | 4 |
| Skin/subcutis | | | | | | | | |
| Increased adipose tissue | 0 | 0 | 1 | 1 | 2 | 3 | 6 | 10 |
| Pancreas | | | | | | | | |
| Decreased cytoplasm/vacuolation, islet cells | 0 | 0 | 0 | 0 | 2 | 1 | 7 | 7 |

(Continued)

| Daily Dose (mg/kg) | 0.3 mg/kg LANTUS® | | 1 mg/kg LANTUS® | | 3/2 mg/kg[a] LANTUS® | |
|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | |
| Died or euthanized moribund (n[d]) | 0 | 0 | 0 | 0 | 1 | 1 |
| Body weight gain (g), Days 1-29 (%[e]) | ↑17 | ↑14 | ↑13 | ↑7 | ↑36* | ↑157* |
| Food consumption (g), Days 1-29 (%[e]) | ↑5 | ↑1 | ↑5 | ↑1 | ↑22* | ↑32* |
| Clinical signs (n[d]) | | | | | | |
| Hypoactive | 0 | 0 | 0 | 0 | 1 | 0 |
| Recumbent | 0 | 0 | 0 | 0 | 1 | 0 |
| Labored respiration | 0 | 0 | 0 | 0 | 1 | 1 |
| Cold to touch | 0 | 0 | 0 | 0 | 1 | 0 |
| General debilitation | 0 | 0 | 0 | 0 | 1 | 1 |
| Ataxic | 0 | 0 | 0 | 0 | 0 | 1 |
| Clinical chemistry[f] (mg/dL) | | | | | | |
| Glucose nadir(time point) Day 1 | 46 (2hr) | 44 (2hr) | 46 (1hr) | 40 (1hr) | 47 (1hr) | 45 (2hr) |
| Glucose (time point) Day 1 | 141 (24hr) | 147 (24hr) | 147 (24hr) | 148 (24hr) | 153 (24hr) | 132 (24hr) |
| Glucose nadir(time point) Day 15[g] | NC | NC | NC | NC | 29 (4hr) | 31 (4hr) |
| Glucose (time point) Day 15[g] | NC | NC | NC | NC | 187 (24hr) | 166 (24hr) |
| Glucose nadir(time point) Day 29[h] | 35 (2hr) | 40 (1hr) | 32 (2hr) | 33 (1hr) | NC | NC |
| Glucose nadir(time point) Day 29[h] | 131 (24hr) | 140 (24hr) | 158 (24hr) | 165 (24hr) | NC | NC |
| Glucose nadir(time point) Day 32[h] | NC | NC | NC | NC | 25 (4hr) | 23 (2hr) |
| Glucose (time point) Day 32[h] | NC | NC | NC | NC | 248 (24hr) | 243 (24hr) |

(Continued)

| Daily Dose (mg/kg) | 0.3 mg/kg LANTUS® | | 1 mg/kg LANTUS® | | 3/2 mg/kg[a] LANTUS® | |
|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | |
| Histopathology (n[d]) | | | | | | |
| Sciatic nerve | | | | | | |
| Axonal degeneration | 0 | 1 | 1 | 0 | 4 | 3 |
| Skin/subcutis | | | | | | |
| Increased adipose tissue | 0 | 1 | 7 | 5 | 8 | 9 |
| Pancreas | | | | | | |
| ↓cytoplasm/vacuolation, islet cells | 0 | 0 | 2 | 2 | 6 | 8 |

Abbreviations: $AUC_{0\text{-}24hr}$ = area under the plasma concentration-time curve from 0 to 24 hours, $C_{max}$ = maximum observed plasma concentration, F = female, GLP = Good Laboratory Practices, hr = hour, M = male, M+F = male and female combined, NC = not collected, NOAEL = no-observed-adverse-effect level, RIA = radioimmunoassay, SEM= Standard Error of the Mean, $T_{max}$ = time to maximum concentration, TK = toxicokinetics, ↑ = increase, ↓ = decrease.

[a] US-approved LANTUS® was included in the study as a reference compound.

[b] Animals were dosed at a level of 3 mg/kg/day for Days 1 through 11 of the dosing phase. Beginning on Day 12 of the dosing phase animals at the 3-mg/kg/day dose level were placed on a dosing holiday through Day 14. On Day 15, dosing resumed for these animals at 2 mg/kg/day as the MTD for hypoglycemia was exceeded at 3 mg/kg. Nine replacement animals were used for Days 15, 23, or 32.

[c] The study protocol was amended to add additional TK animals. These animals were given a single dose of 0.3, 1, or 2 mg/kg of LY2963016 or LANTUS® in order to obtain associated Day 1 toxicokinetic data (Table 2.6.4.4.2) for this study, and this TK analysis is labeled as occurring on "Repeat Day 1".

[d] Number of animals or tissues.

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

[f] Blood samples for glucose concentration determinations on Days 1 and 29 were obtained from toxicokinetic animals (3/sex/time point).

[g] Blood samples for glucose concentration determinations were collected from 0- and 3/2-mg/kg animals only on Day 15. Day 15 was the first day of dosing at the lowered dose level of 2 mg/kg following the dosing holiday on Day 12.

[h] Due to the dosing holiday from Days 12-14, the dosing phase was extended by 3 days for the 3/2-mg/kg animals. As a result, the last set of glucose samples were collected on Day 32 instead of Day 29.

* $p \leq .05$ (ANOVA and Dunnett's, if applicable).

## Repeat-Dose Toxicity: 4-Week Study in Rats Given LY2963016 and EU-Approved LANTUS®

Report title: A Repeat-Dose Toxicity and Toxicokinetic Study in Rats Given LY2963016 or LANTUS® (European-Approved) by Subcutaneous Injection for 4 Weeks

Species/strain: Rat/Crl:CD(SD)
Initial age: 10 to 11 weeks
Date of first dose: 26 April 2012

NOAEL: 0.3 mg/kg

Special features: None

Duration of dosing: 1/day x 4 weeks
Duration of reversibility: not applicable
Route: subcutaneous

Vehicle: 17 mg/mL glycerin, 2.7 mg/mL m-cresol, and (b) (4) zinc oxide prepared in Sterile Water for Injection, USP, pH 4.0 (± 0.2).

Document ID: Study 8259267
GLP compliance: Yes
Test Articles: LY2963016, EU-approved LANTUS®[a]

| Mean Toxicokinetics (TK) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Analytes: LY2963016 and LANTUS® | | | | Number of TK Animals: 6/timepoint, 24/sex,48/dose group 288 total | | | | | |
| Dosing: 1/day x 4 weeks | Feeding condition: fed | | | Sample: rat serum | | | Assay/Method: RIA/8225346 | | |
| Daily Dose (mg/kg) of LY2963016 or LANTUS® | 0.3 | | | 1 | | | 2 | | |
| Sex: | M | F | M+F | M | F | M+F | M | F | M+F |
| Day 1 | | | | | | | | | |
| LY2963016 | | | | | | | | | |
| $C_{max}$(pM) | 4440 | 7813 | 5917 | 16390 | 20133 | 18262 | 26800 | 23200 | 25360 |
| (SD) | (286) | (2075) | (2523) | (9514) | (6507) | (7573) | (3300) | (NA) | (4835) |
| $AUC_{0-24hr}$ (pM•hr) | 15961 | 26376 | 21016 | 95241 | 98567 | 96904 | 223386 | 222888 | 224217 |
| (SEM) | (2526) | (4489) | (2806) | (18560) | (11584) | (10812) | (30179) | (33856) | (20045) |
| $T_{max}$ (hr) | 1 | 2 | 2 | 4 | 4 | 4 | 4 | 4 | 4 |
| EU-approved LANTUS® | | | | | | | | | |
| $C_{max}$(pM) | 6947 | 6310 | 6628 | 14333 | 23000 | 18300 | 17567 | 41500 | 28497 |
| (SD) | (1410) | (2793) | (2009) | (8009) | (4513) | (6084) | (929) | (19419) | (19742) |
| $AUC_{0-24hr}$ (pM•hr) | 19216 | 17095 | 18122 | 82629 | 100983 | 92089 | 174405 | 239692 | 207049 |
| (SEM) | (1376) | (3197) | (1665) | (14735) | (19615) | (11923) | (24420) | (36968) | (26523) |
| $T_{max}$ (hr) | 2 | 2 | 2 | 4 | 2 | 2 | 8 | 4 | 4 |
| Day 29 | | | | | | | | | |
| LY2963016 | | | | | | | | | |
| $C_{max}$(pM) | 11227 | 14033 | 11012 | 25700 | 17967 | 20850 | 106733 | 38600 | 69137 |
| (SD) | (5198) | (4934) | (4879) | (4877) | (907) | (7837) | (98063) | (NA) | (75440) |
| $AUC_{0-24hr}$ (pM•hr) | 58558 | 41784 | 50182 | 142077 | 116315 | 128699 | 688670 | 391906 | 551466 |
| (SEM) | (9811) | (8353) | (8236) | (14424) | (14743) | (11636) | (179199) | (54833 ) | (103131) |
| $T_{max}$ (hr) | 4 | 2 | 2 | 4 | 2 | 4 | 4 | 1 | 4 |
| EU-approved LANTUS® | | | | | | | | | |
| $C_{max}$ (pM) | 12230 | 9537 | 10883 | 30933 | 33900 | 32417 | 38800 | 47433 | 35720 |
| (SD) | (2484) | (2658) | (2733) | (3623) | (18489) | (12026) | (NA) | (10872) | (17805) |
| $AUC_{0-24hr}$ (pM•hr) | 44819 | 31585 | 37545 | 167112 | 136217 | 152919 | 313244 | 478785 | 406818 |
| (SEM) | (5519) | (3064) | (3595) | (22435) | (33638) | (22282) | (38204) | (98168) | (65041) |
| $T_{max}$ (hr) | 1 | 1 | 1 | 2 | 2 | 2 | 8 | 2 | 2 |

(Continued)

| Test Article: Daily Dose (mg/kg) | 0 (Control) | | 0.3 mg/kg LY2963016 | | 1 mg/kg LY2963016 | | 2 mg/kg LY2963016 | |
|---|---|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | | | |
| Died or euthanized moribund (n[b]) | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Body weight gain (g), Days 1-29 (%[c]) | 82 | 32 | ↑15 | - | ↑13 | ↑47* | ↑60* | ↑113* |
| Food consumption, Days 1-29 (g) (%[c]) | 883 | 644 | ↑4 | ↑2 | ↑5 | ↑8 | ↑25 | ↑25* |
| Clinical signs (n[b]) | | | | | | | | |
| Hunched | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Limited use, hind legs | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Ataxic | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Recumbent | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Hypoactive | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Cold to touch | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| General debilitation | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Labored respiration | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Clinical chemistry[d] (mg/dL) | | | | | | | | |
| Glucose nadir (time point) Day 1 | 117 (12hr) | 123 (24hr) | 51 (2hr) | 57 (1hr) | 48 (2hr) | 45(1hr) | 47(2hr) | 40 (1hr) |
| Glucose (time point) Day 1 | 128 (24hr) | 123 (24hr) | 128 (24hr) | 128 (24hr) | 135 (24hr) | 127 (24hr) | 133 (24hr) | 135 (24hr) |
| Glucose nadir (time point) Day 29 | 115 (0hr) | 109 (0hr) | 31 (1hr) | 44 (1,2hr) | 30 (4hr) | 28 (4hr) | 20 (4hr) | 23 (4,12hr) |
| Glucose (time point) Day 29 | 128 (24hr) | 117 (24hr) | 128 (24hr) | 127 (24hr) | 172 (24hr) | 157 (24hr) | 221 (24hr) | 272 (24hr) |
| Histopathology (n[b]) | | | | | | | | |
| Sciatic nerve | | | | | | | | |
| Axonal degeneration | 0 | 2 | 1 | 1 | 0 | 0 | 2 | 6 |
| Skin/subcutis | | | | | | | | |
| Increased adipose tissue | 0 | 0 | 0 | 0 | 0 | 1 | 6 | 5 |
| Pancreas | | | | | | | | |
| Decreased cytoplasm/vacuolation, islet cells | 0 | 0 | 0 | 0 | 3 | 1 | 7 | 8 |

(Continued)

| Test Article: Daily Dose (mg/kg) | 0.3 mg/kg LANTUS® | | 1 mg/kg LANTUS® | | 2 mg/kg LANTUS® | |
|---|---|---|---|---|---|---|
| Sex:Number of Animals | M:10 | F:10 | M:10 | F:10 | M:10 | F:10 |
| Noteworthy Findings | | | | | | |
| Died or euthanized moribund (n[b]) | 0 | 0 | 0 | 1 | 2 | 0 |
| Body weight gain (g), Days 1-29 (%[c]) | ↑26 | - | ↑13 | ↑19 | ↑77* | ↑131* |
| Food consumption, Days 1-29 (g) (%[c]) | ↑8 | ↑1 | ↑5 | ↑7 | ↑25* | ↑24* |
| Clinical signs (n[b]) | | | | | | |
| Ataxic | 0 | 0 | 0 | 0 | 1 | 0 |
| General debilitation | 0 | 0 | 0 | 0 | 1 | 0 |
| Audible respiration | 0 | 0 | 0 | 0 | 1 | 0 |
| Labored respiration | 0 | 0 | 0 | 0 | 1 | 0 |
| Clinical chemistry[d] (mg/dL) | | | | | | |
| Glucose nadir(timepoint) Day 1 | 44 (1hr) | 47 (2hr) | 48(1hr) | 37 (4hr) | 46 (4hr) | 47 (2,4hr) |
| Glucose (timepoint) Day 1 | 121 (24hr) | 130 (24hr) | 132 (24hr) | 135 (24hr) | 140 (24hr) | 127 (24hr) |
| Glucose nadir(timepoint) Day 29 | 34 (1hr) | 38 (1hr) | 34 (2hr) | 42 (2hr) | 28 (8hr) | 29 (12hr) |
| Glucose (timepoint) Day 29 | 135 (24hr) | 152 (24hr) | 158 (24hr) | 163 (24hr) | 247 (24hr) | 263 (24hr) |
| Histopathology (n[b]) | | | | | | |
| Sciatic nerve | | | | | | |
| Axonal degeneration | 0 | 1 | 0 | 0 | 0 | 2 |
| Skin/subcutis | | | | | | |
| Increased adipose tissue | 0 | 0 | 0 | 0 | 4 | 6 |
| Pancreas | | | | | | |
| Decreased cytoplasm/vacuolation, islet cells | 0 | 0 | 3 | 2 | 7 | 7 |

Abbreviations: $AUC_{0-24hr}$ = area under the plasma concentration-time curve from 0 to 24 hours, $C_{max}$ = maximum observed plasma concentration, F = female, GLP = Good Laboratoary Practices, hr = hour, M = male, M+F = male and female combined, NA = not applicable, NOAEL = no-observed-adverse-effect level, RIA = radioimmunoassay, SD = standard deviation, SEM = Standard Error of the Mean, TK = toxicokinetics, $T_{max}$ = time to maximum concentration, ↑ = increase, – = no important findings.

a EU-approved LANTUS® was included in the study as a reference compound.

b Number of animals or tissues.

c For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance based on actual data (not on the percent differences).

d Blood samples for glucose concentration determinations on Days 1 and 29 were obtained from toxicokinetic animals (3/sex/time point).

* $p \leq .05$.

## Toxicology: Drug Product

| Lot Number | Purity (%) | Specified Impurities (%) | | | Document ID | Type of Study |
|---|---|---|---|---|---|---|
| | | Total Impurities | (b) (4) | | | |
| | | NMT | NMT | | | |
| Specification: | | (b) (4) | | | | |
| (b) (4) | | | | | Study 8229488 | Repeat dose, 4 week, rat |
| | | | | | Study 8259267 | Repeat dose, 4 week, rat |

Abbreviation: NMT = not more than.

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

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/s/

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MIYUN M TSAI-TURTON
12/03/2013

KAREN L DAVIS BRUNO
12/03/2013