CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125469Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

From: Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
BLA: 125469
Agency receipt date: September 18, 2013
Drug: TRULICITY (Dulaglutide)
Sponsor: Lilly

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

Reviewing Division: Division of Metabolism and Endocrinology Products

Introductory Comments: The pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data support approval of dulaglutide for the indication listed above.

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

Dulaglutide consists of two identical, disulfide-linked chains, each containing a human GLP-1 analog sequence covalently linked to a modified human immunoglobulin G4 (IgG4) heavy chain fragment (Fc) by a small peptide linker. The GLP-1 analog portion of the molecule has approximately 90% sequence similarity to endogenous human GLP-1 (7-37), (b) (4), (b) (4)

A complete general toxicology program was conducted in rats and monkeys. Prominent findings included body weight loss or decreased body weight gain that correlated with decreased food and water consumption. This is an expected pharmacodynamic effect that is also observed in the clinic. Observed gastrointestinal effects were transient.

Genetic toxicity studies were not applicable for this program. A carcinogenicity study in rats demonstrated a risk for thyroid C-cell hyperplasia and tumorigenesis, similar to other GLP-1 agonists, at exposures that were 7-fold or greater than the clinical AUC. A 6-month transgenic assay in mice was negative. The totality of the available data indicates that rodents are more sensitive to the C-cell proliferative effects than nonrodents and presumably humans. Although the human relevance of GLP-1 receptor agonist-induced C-cell tumorigenesis in rodents is unknown, human relevance to drug-induced C-cell tumors cannot be discounted and therefore a boxed warning and appropriate epidemiological monitoring continue to be warranted for GLP-1 agonists.

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A complete battery of reproductive and developmental toxicity studies was conducted in rats and rabbits. Key findings in rats included prolonged diestrus, decreased number of corpora lutea, implantation sites, and viable embryos in a female fertility study, and increased post-implantation loss and decreased viable fetuses, an increase in mean litter proportions of unossified sternebrae, reduced skull ossification, unossified hyoid, and reduced vertebral arch ossification in an embryo-fetal development study. In a peri-/post-natal study, observations included an increased incidence of pups showing small stature, paleness, or cool to touch, reduced body weight, and slightly longer mean time to balano-preputial separation in male pups, and neurotoxicity (as demonstrated by an increase in mean Biel maze straight channel swim trial escape time) in female pups. Fetal malformations and skeletal variations were also observed in rabbits. These findings occurred at exposures that were 13- to 17-fold the anticipated clinical AUC and are described in the product label. The recommended "Pregnancy Category C" for this product is consistent with the current labels for Byetta, Victoza and Tanzeum.

Conclusion:
I agree with the division pharmacology/toxicology conclusion that dulaglutide can be approved from the pharmacology/toxicology perspective. I have reviewed the proposed labeling and agree with the recommendations made by the division regarding the relevant nonclinical sections.

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/s/

TIMOTHY J MCGOVERN
09/16/2014

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	125469
Review number	1
Supporting document:	1
Applicant's letter date:	17 September 2013
CDER stamp date:	18 September 2013
Product:	Dulaglutide
Indication:	Type 2 Diabetes Mellitus
Applicant:	Eli Lilly and Company
Review Division:	Metabolism and Endocrinology Products
Reviewer:	B. Timothy Hummer, PhD, DABT
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1 Executive Summary

1.1 Introduction

LY2189265 (dulaglutide) is a long-acting human glucagon-like peptide-1 (GLP-1) receptor agonist that has been developed for the treatment of type 2 diabetes mellitus (T2DM). The molecule consists of two identical, disulfide-linked chains, each containing a human GLP-1 analog sequence covalently linked to a modified human immunoglobulin G4 (IgG4) heavy chain fragment (Fc) by a small peptide linker (see diagram in Section 2 below). The GLP-1 analog portion of the molecule has approximately 90% sequence similarity to endogenous human GLP-1 (7-37), (b) (4)

1.2 Brief Discussion of Nonclinical Findings

In vitro and in vivo pharmacology studies have demonstrated that LY2189265 potently activates the human GLP-1 receptor; increases insulin secretion in a dose-dependent and glucose-dependent fashion, and decreased glucose levels in rats and monkeys.

Standard toxicology studies were conducted in rats and monkeys for up to 6 and 9 months, respectively, with doses being administered by subcutaneous injection twice weekly. A 4-week study was conducted in mice as a dose range-finding study for the 6-month transgenic mouse carcinogenicity study. One-year studies were conducted in rats and monkeys as part of an enhanced analysis of potential drug-related effects on the thyroid and pancreas (monkey only).

Animals treated with moderate to high dose levels of LY2189265 experienced an initial body weight loss or decreased body weight gain compared with controls that generally correlated with decreased food and water consumption. At higher doses, effects on food and water consumption occasionally resulted in signs of dehydration and anorexia. After the first week or two of treatment, body weight gains became more similar to the control group, although final body weights were generally still lower than controls. During the treatment-free recovery period body weights typically rebounded to values similar to or greater than control values. The effect on body weight and food consumption is related to the pharmacodynamic activity of LY2189265, which is also observed with other GLP-1 receptor agonists.

As noted in monkeys treated with other GLP-1 receptor agonists, increased mean testicular weights were noted at ≥1.63 mg/kg (≥85X clinical AUC). Similarly, increased mean weights were noted for epididymis, prostate, and seminal vesicles. In the 9-month monkey study, increased mean absolute and relative prostate (up to 2-fold) and testis weights (up to 2.5-fold) were noted for all male treatment groups (≥0.41 mg/kg;

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≥26X clinical AUC). There were no correlative histopathology findings for male reproductive organs. In the 9-month study, decreased mean ovary weights were noted at ≥0.41 mg/kg (up to 39% for absolute weight and 31% for relative weight). At the end of the recovery period, increased testis weights and decreased ovary weights were still noted for the 8.15 mg/kg groups (474X clinical AUC).

Microscopic findings were generally limited to effects related to decreased body weight gain, such as hypocellularity and fat atrophy of the bone marrow and lymphoid depletion in the spleen and mesenteric and submandibular lymph nodes in monkeys. General toxicology studies did not reveal target organs of toxicity.

Because of known safety concerns for other GLP-1 receptor agonists, the sponsor conducted 1-year toxicity studies to evaluate potential treatment-related effects on thyroid in rats and monkeys and on pancreas in monkeys. A 3-month study in a diabetic rat model was also conducted to evaluate potential effects on the pancreas. In male Sprague-Dawley rats treated with 5 mg/kg LY2189265 (~58X clinical AUC), an age-related increase in plasma calcitonin was observed in both control and treated animals, with no difference in mean values between the two groups. Additionally, no difference in CaCl-stimulated calcitonin was observed between control and treated groups. There were no noteworthy differences in the incidence or severity of C-cell hyperplasia after treatment for up to 9 months. After 1 year of treatment, an increase in incidence and severity of focal/multifocal C-cell hypertrophy/hyperplasia was noted for the treated group. No difference in incidence or severity of diffuse C-cell hyperplasia or tumors was observed between the two groups. Tissue morphometery showed no statistically significant difference between the two groups for percent calcitonin positive cells, volume of calcitonin positive tissue, thyroid tissue volume, or thyroid organ volume. A consistent relationship between the number/size of calcitonin positive cells and plasma calcitonin values was not observed for individual animals.

In male Cynomolgus monkeys treated with 8.15 mg/kg LY2189265 (~474X clinical AUC), mean serum calcitonin values remained below the level of quantitation for both the control and treated groups throughout the study. Calcitonin secretion with calcium gluconate also did not show a biologically meaningful difference between control and treated animals. No treatment-related adverse microscopic findings were noted for the thyroid or pancreas, including signs of cell proliferation through Ki-67 staining. A slight increase in the severity of increased pancreatic goblet cells in interlobular ducts was noted for the treated group. This finding did not correlate with increases with serum amylase or lipase. A group of pathologists who peer reviewed the slides as part of a pancreas working group concluded that the increased pancreatic goblet cells were not adverse or associated with inflammatory or proliferative changes including pancreatitis, ductal dilatation, ductal blockage, or PanIN.

In male ZDF rats treated with up to 5 mg/kg LY2189265 twice weekly for 3 months, a dose-related increase in mean serum pancreatic-specific amylase was observed for all dose groups; no increases in serum lipase were observed. Microscopically, an increased incidence and severity (minimal to moderate) of increased ductal epithelium

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was observed at all dose levels (≥3X clinical AUC). Animals treated with ≥1.5 mg/kg (≥8X clinical AUC) had an increased incidence of minimal to slight acinar atrophy, with or without mixed cell inflammation. An increased incidence of minimal to slight neutrophilic acinar inflammation occurred at 5 mg/kg (30X clinical AUC). These findings were observed as background lesions in the control group at low incidence (1 to 2 animals) and at minimal severity. An increase in the severity, but not incidence, of neutrophilic periductal inflammation (minimal to moderate) and islet cell hyperplasia was observed for treated groups. With the exception of a few animals, there generally was not a good correlation for individual animals having both a high percentage of Ki-67 positive duct cells and an increased incidence/severity of increased ductal epithelium. Although the microscopic changes observed in this study might imply that some diabetic patients taking LY2189265 could be more susceptible to morphological changes in the exocrine pancreas, the observed changes in ZDF rats were not sufficient or severe enough to classify the effects as drug-induced pancreatitis or premalignant lesions.

The carcinogenic potential of LY2189265 was studied in a 6-month TgRas transgenic mouse study and a 2-year rat study. Twice-weekly subcutaneous injections to transgenic mice for 26 weeks did not affect survival and there was no evidence of drug-related neoplasms at the highest dose administered (5X clinical AUC). An increase in cytoplasmic volume of calcitonin-positive thyroid C-cells was observed for all treated groups (≥1X clinical AUC). In Sprague-Dawley rats, 2 years of twice weekly treatment resulted in an increase in the incidence of thyroid C-cell adenomas at ≥0.5 mg/kg (≥7X clinical AUC). A non-statistically significant, slight numerical increase in thyroid C-cell carcinomas was observed at 5 mg/kg (58X clinical AUC). An increased incidence of diffuse (males and females) and focal (females only) thyroid C-cell hyperplasia was also evident at ≥0.5 mg/kg. The NOEL for increased C-cell tumors was 0.05 mg/kg (0.5X clinical AUC).

Treatment of male Sprague-Dawley rats every third day for 4 weeks prior to mating and during a 2-week cohabitation period did not have an adverse effect on male mating performance or fertility; on sperm concentration, motility, or morphology; or on early embryonic viability at doses up to 16.3 mg/kg (~150X clinical AUC). Female Sprague-Dawley rats treated with LY2189265 every third day for 2 weeks prior to mating, during the cohabitation period until mating was confirmed, and through gestational day (GD) 4, 5, or 6 showed prolonged diestrus and a statistically significant decrease in the number of corpora lutea, implantation sites, and viable embryos at ≥4.9 mg/kg, which occurred in the presence of maternal toxicity (decreased body weight/body weight gain). There were no compound-related effects on embryonic survival or maternal mating, fertility, or conception. The NOAEL for female reproductive performance was considered to be 1.6 mg/kg (~15X clinical AUC).

In a fetal development study in which pregnant Sprague-Dawley rats received LY2189265 every third day from GD 6 through GD15, a significant reduction in maternal body weight gain was noted for all test article groups, with body weight loss occurring at ≥1.63 mg/kg (14X clinical AUC) from GD 6 to 9. An increase in percent post-implantation loss and a decrease in percent viable fetuses were noted for the 4.9 mg/kg

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group (44X clinical AUC). Mean fetal body weights were significantly decreased at ≥1.63 mg/kg. An increase in mean litter proportions of unossified sternebrae, reduced skull ossification, unossified hyoid, and reduced vertebral arch ossification were observed at 4.9 mg/kg. The NOAEL for fetal development was 0.49 mg/kg (4X clinical AUC).

In a fetal development study in which pregnant New Zealand white rabbits received LY2189265 every third day from GD 7 through GD 19, an initial significant maternal weight loss followed by decreased body weight gain occurred at ≥0.12 mg/kg (≥4X clinical AUC); decreased body weight gain was also noted for the 0.04 mg/kg (1X clinical AUC) group throughout the treatment period. There were no effects on maternal reproductive parameters. Increased fetal malformations (lung lobular agenesis [upper end of historical control range], vertebral anomaly with or without associated rib anomaly, and costal cartilage anomaly) occurred at 0.41 mg/kg (13X clinical AUC). Increased fetal skeletal variations occurred at 0.41 mg/kg (increased incidence of 27 presacral vertebrae, 13th full rib, bent hyoid arches, malaligned sternebrae, and reduced ossification of the ribs. Also, a decreased incidence of having a 13th rudimentary rib was noted for kits from the 0.12 and 0.41 mg/kg litters. The NOAEL for fetal development was determined to be 0.12 mg/kg.

In a peri- and post-natal developmental toxicology study in which pregnant Sprague-Dawley rats received LY2189265 every third day from GD 6 through lactation day (LD) 19, a dose-related decrease in mean maternal body weight gain was noted for all F_0 treated groups. The NOAEL for maternal toxicity was considered to be 0.2 mg/kg. Treatment did not affect mean values for gestation duration, litter size, live litter size, number of implantation sites, or pup sex ratio. Survival rates during the lactation period for F_1 pups from HD dams were lower than historical control values, but were similar to the concurrent control values. An increased incidence of pups showing small stature, paleness, or cool to touch was noted for pups from HD dams. Mean body weights for male and female pups from HD dams were statistically significantly lower than controls at all time points from LDs 1 to 21. HD males had a slightly longer mean time to balano-preputial separation (47.0 days vs. 45.6 days for control), which was likely secondary to the smaller size of the HD male pups. The NOAEL for neonatal toxicity was considered to be 0.49 mg/kg (4X clinical AUC). A statistically significant increase in mean Biel maze straight channel swim trial escape time was noted for HD female pups on PND 70 (6.8 sec vs. 5.0 sec for control). An increase in mean Biel maze memory escape time was also noted for HD female pups during trial number 12 (62.6 sec vs. 31.9 sec for control), which was associated with a greater number of memory errors during this trial. No effects in the Biel maze tests were noted for male pups. Accordingly, the NOAEL for developmental neurotoxicity was considered to be 0.49 mg/kg for females and 1.63 mg/kg for males (16X clinical AUC). There were no effects on F_1 generation mating, fertility, or reproductive organs (microscopic findings or organ weights). There were no effects on the mean number of F_2 pups born, live litter size, sex ratio, body weight, or postnatal survival (to PND 1). There were no external findings suggestive of a test article effect. The NOAEL for F_1 reproductive parameters was 1.63 mg/kg.

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1.3 Recommendations

1.3.1 Approvability

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

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Draft labeling for applicable nonclinical sections is presented below. This draft is primarily taken from the sponsor's proposed draft, although some suggested edits were made. A boxed warning is recommended because of dulaglutide-induced thyroid C-cell tumors in rats for which human relevance is unknown; inclusion of a boxed warning is consistent with the labels of the other long-acting GLP-1 receptor agonists that have been granted marketing approval. Clinical exposure margins achieved in rat fertility studies were calculated based on Day 92 AUC values from the 3-month rat toxicity study.

WARNING: RISK OF THYROID C-CELL TUMORS

See full prescribing information for complete boxed warning.

- **Dulaglutide causes thyroid C-cell tumors in rats** (b) (4) **It is unknown whether TRULICITY causes thyroid C-cell tumors, including medullary thyroid carcinoma (MTC), in humans, as human relevance could not be determined by clinical or nonclinical studies (5.1).**
- **TRULICITY is contraindicated in patients with a personal or family history of MTC and in patients with Multiple Endocrine Neoplasia syndrome type 2 (MEN 2) (5.1).**

5 WARNINGS AND PRECAUTIONS

5.1 Risk of Thyroid C-cell Tumors

(b) (4)

(b) (4) It is unknown whether TRULICITY will cause thyroid C-cell tumors, including MTC, in humans, as the human relevance (b) (4) could not be determined by clinical or nonclinical studies.

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8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies of TRULICITY in pregnant women. The risk of birth defects, loss of other adverse outcomes is increased in pregnancies complicated by hyperglycemia and may be decreased with good metabolic control. It is essential for patients with diabetes to maintain good metabolic control before conception and throughout pregnancy.

(b) (4)

In rats and rabbits, dulaglutide administered during the major period of organogenesis produced fetal growth reductions and/or skeletal anomalies and ossification deficits in association with decreased maternal weight and food consumption attributed to the pharmacology of dulaglutide.

In pregnant rats given subcutaneous doses of 0.49, 1.63, or 4.89 mg/kg dulaglutide on Gestation Days 6, 9, 12, and 15 (organogenesis), reduced fetal weights associated with decreased maternal food intake and decreased weight gain attributed to the pharmacology of dulaglutide were observed at ≥1.63 mg/kg, a systemic exposure (b) (4) fold the MRHD based on AUC. Irregular skeletal ossifications and increases in post implantation loss also were observed at 4.89 mg/kg, a systemic exposure (b) (4)-fold the MRHD based on AUC. No developmental adverse effects were observed at 4-fold the MRHD based on AUC.

In pregnant rabbits given subcutaneous doses of 0.04, 0.12, or 0.41 mg/kg dulaglutide on Gestation Days 7, 10, 13, 16, and 19 (organogenesis), fetal skeletal malformations of the vertebrae and/or ribs were observed in conjunction with decreased maternal food intake and decreased weight gain attributed to the pharmacology of dulaglutide at 0.41 mg/kg, a systemic exposure 13-fold the MRHD based on AUC. No developmental adverse effects were observed at 4-fold the MRHD based on AUC.

In a prenatal-postnatal study in F_0 maternal rats given subcutaneous doses of 0.2, 0.49, or 1.63 mg/kg every third day from implantation through lactation, F_1 pups from F_0 maternal rats given 1.63 mg/kg dulaglutide had statistically significantly lower mean body weight from birth through post-natal day 63 for males and post-natal day 84 for females. These findings occurred in conjunction with F_0 decreased maternal food intake and decreased weight gain, attributed to the pharmacologic activity at 1.63 mg/kg, a systemic exposure 16-fold the MRHD based on AUC. F_1 offspring from F_0 maternal rats receiving 1.63 mg/kg dulaglutide had decreased forelimb and hindlimb grip strength and males had delayed balano-preputial separation. Females had decreased startle response. These physical findings may relate to the decreased size of the offspring relative to controls as they appeared at early postnatal assessments but were not observed at a later assessment. F_1 female offspring from F_0 maternal rats receiving 1.63 mg/kg of dulaglutide had a longer mean escape time and a higher mean

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number of errors relative to the concurrent control in the memory evaluation portion of the Biel water maze. The human relevance of these memory deficits seen in female F_1 rats is unknown.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

A 2-year carcinogenicity study was conducted with dulaglutide in male and female rats at doses of 0.05, 0.5, 1.5, and 5.0 mg/kg (0.5-, 7-, 20-, and 58-fold the MRHD of 1.5 mg once weekly based on AUC) administered by subcutaneous injection twice weekly. In rats, dulaglutide caused a dose-related and treatment-duration-dependent increase in the incidence of thyroid C-cell tumors (adenomas and/or carcinomas) compared with controls at ≥0.5 mg/kg (≥7-fold the MRHD based on AUC). A statistically significant increase in C cell adenomas was observed in rats of both genders receiving dulaglutide at ≥0.5 mg/kg. Numerical increases in thyroid C-cell carcinomas occurred at 5 mg/kg and were considered to be treatment-related despite the absence of statistical significance. Human relevance of thyroid C-cell tumors in rats is unknown and could not be determined by clinical studies or nonclinical studies [see Boxed Warning and Warnings and Precautions (5.1)].

A 6-month carcinogenicity study was conducted with dulaglutide in rasH2 transgenic mice at doses of 0.3, 1.0, and 3.0 mg/kg administered by subcutaneous injection twice weekly. Dulaglutide did not produce increased incidences of thyroid C cell hyperplasia or neoplasia at any dose.

Dulaglutide is a recombinant protein; no genotoxicity studies have been conducted.

In fertility and early embryonic development studies in male and female rats, no adverse effects of dulaglutide on sperm morphology, mating, fertility, conception, and embryonic survival were observed at up to 16.3 mg/kg (130-fold the MRHD based on AUC). In female rats, an increase in the number of females with prolonged diestrus and a dose-related decrease in the mean number of corpora lutea, implantation sites, and viable embryos were observed at ≥4.9 mg/kg (≥32-fold the MRHD based on AUC), which occurred in the presence of decreased maternal food consumption and body weight gain.

13.2 Animal Toxicology and/or Pharmacology

Zucker diabetic fatty (ZDF) rats were given 0.5, 1.5, or 5.0 mg/kg of dulaglutide (3-, 8-, and 30-fold the MRHD based on AUC) twice weekly for 3 months. Increases of 12% to 33% in total and pancreatic amylase, but not lipase, were observed at all doses without microscopic pancreatic inflammatory correlates in individual animals. Other changes in the dulaglutide-treated animals included increased interlobular ductal epithelium without active ductal cell proliferation (≥0.5 mg/kg), increased acinar atrophy with/without inflammation (≥1.5 mg/kg), and increased neutrophilic inflammation of the acinar pancreas (5 mg/kg).

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Treatment of monkeys with 8.15 mg/kg of dulaglutide (nearly 500 fold the MRHD based on AUC) twice weekly for 12 months demonstrated no evidence of pancreatic inflammation [redacted] (b) (4). In 4 of 19 monkeys on dulaglutide treatment, there was an increase in goblet cells within the pancreatic ducts, but no differences from the control group in total amylase or lipase at study termination.

2 Drug Information

2.1 Drug

CAS Registry Number:	923950-08-7
Proprietary Name:	Trulicity™
Generic Name:	Dulaglutide
Code Name:	LY2189265
Chemical Name:	7-37-glucagon-like peptide I [8-glycine,22-glutamic acid,36-glycine] (synthetic human) fusion protein with peptide (synthetic 16-amino acid linker) fusion protein with immunoglobulin G4 (synthetic human Fc fragment), dimer [Gly^{8},Glu^{22},Gly^{36}]human glucagon-like peptide 1-(7-37)-peptidyltetraglycyl-L-seryltetraglycyl-L-seryltetra-glycyl-L-seryl-L-alanyldes-Lys^{229}-[Pro^{10},Ala^{16},Ala^{17}] human immunoglobulin heavy constant γ4 chain H-CH2-CH3 fragment, (55-55':58-58')-bisdisulfide dimer
Molecular Formula:	$C_{2646}H_{4044}O_{836}N_{704}S_{18}$ ([redacted] (b) (4)
Molecular Weight:	59,672 Da [redacted] (b) (4)

Structure or Biochemical Description:

(b) (4)

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

2.2 Relevant INDs and NDAs

Dulaglutide - IND 70,930

Exendin-4 analogs
NDA 022200 / IND 67,092 - exenatide, Astra Zeneca, once weekly formulation
NDA 021773 / IND 57,725 - exenatide, BYETTA, Astra Zeneca
NDA 021919 / IND 57,725 - exenatide, BYETTA, Astra Zeneca, monotherapy
IND (b) (4)
IND
IND
IND
IND
IND

GLP-1 analogs
NDA 22-341 / IND 61,040 - GLP-1 analog, liraglutide, Novo Nordisk
IND [redacted] (b) (4)
BLA 125431 / IND 65,177 - GLP-1-albumin fusion protein, albiglutide, GlaxoSmithKline
IND [redacted] (b) (4)
IND
IND
IND
IND
IND
IND

2.3 Drug Formulation

A summary of the formulation is presented in the sponsor-generated table below. In clinical trials, dulaglutide has been formulated at two dosage strengths: 0.75 mg in 0.5 mL and 1.5 mg in 0.5 mL. For marketing, the sponsor has proposed a recommended dose of 1.5 mg only. The dulaglutide injection product will be available as a single-use pen and a prefilled syringe.

Table 2.3.P.1.1-1 Composition of Dulaglutide Injection, 0.75 mg/0.5 mL and 1.5 mg/0.5 mL

Ingredient	Quantity (mg)per Syringe		Function	Reference to Standards
	Low Strength	High Strength		
Active Ingredient				
Dulaglutide	0.75	1.5	Active Ingredient	Internal Standard: See Section S.4.1, Specifications
Other Ingredients				
Trisodium Citrate Dihydrate	1.37	1.37	[redacted] (b) (4)	USP, Ph.Eur., JP
Citric Acid Anhydrous	0.07	0.07		USP, Ph.Eur., JP
Mannitol	23.2	23.2		USP, Ph.Eur., JP
Polysorbate 80	0.10	0.10		NF, Ph.Eur., JP
Water for Injection	[redacted] (b) (4)			USP, Ph.Eur., JP

2.4 Comments on Novel Excipients

No novel excipients will be used in the product formulation.

2.5 Comments on Impurities/Degradants of Concern

No impurities or degradants of concern have been identified. Product-related substances and impurities as well as potential process-related impurities have been characterized throughout the development history. The proposed clinical drug substance acceptance criteria and batch analysis summaries are shown in sponsor-generated Table 2.3.S.4.4.1-1 below. The major impurities were qualified in toxicology studies. The impurity profiles of the drug substances used in the toxicology program are presented in sponsor-generated Table 2.6.7.4.

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Table 2.3.S.4.4.1-1 Batch Analyses

Batches		Validation Batches	Primary Stability Batches	Clinical Batches (Produced between 2007 – 2011)
Test	Acceptance Criteria[1]	Results		
Identity (b) (4)	(b) (4)	Pass	Pass	Pass
Identity (b) (4)		Pass	Pass	Pass
Quantity (b) (4)		(b) (4)		
Potency (Bioassay) (b) (4)				
Related Substances/Impurities (b) (4)				
(b) (4) Purity (b) (4)				
Total Aggregates (b) (4)				

Table 2.6.7.4: Impurity Profiles of Drug Substance Batches Used in Toxicology Studies

(b) (4)

Abbreviation: ND = not determined

2.6 Proposed Clinical Population and Dosing Regimen

Dulaglutide is proposed to be used as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus (T2DM). Dulaglutide drug product will be supplied as a once weekly solution for subcutaneous administration. The proposed commercial dulaglutide products will be a 1.5 mg prefilled syringe and a 1.5 mg single-use pen that has an auto-injection function. Currently the sponsor recommends that the 1.5 mg dose level be both the starting and maintenance doses and that a dose titration strategy using the lower dose level of 0.75 mg is not necessary.

Using clinical trial PK meta-analysis, mean C_{max}, $AUC_{0\text{-}168h}$, and T_{max} values for dulaglutide at steady state after multiple 1.5 mg doses in patients with T2DM were calculated to be 114 ng/mL, 14,000 ng•h/mL, and 48 hours (range of 24 to 72 hours). Steady state was reached between the second and fourth doses. Accumulation after multiple doses of 1.5 mg dulaglutide was approximately 1.6-fold. After multiple doses of 1.5 mg dulaglutide, the mean half-life value for patients with T2DM was 4.7 days. Absolute bioavailability for the 1.5 mg dose in healthy volunteers was approximately 47%.

3 Studies Submitted

3.1 Studies Reviewed

Type of Study	Test System	Route	Testing Facility	Document ID	Section #
Primary Pharmacodynamics					
Cell-Based In Vitro Binding	HEK293 or CHO cells transfected with GLP-1, GIP, or glucagon receptor	In vitro	Eli Lilly and Company Indianapolis, IN, USA and San Diego, CA, USA	bTDR225	2.6.2.2.1
Cell-Based In Vitro Activity	HEK293 cells, transfected with human GLP-1 receptor and reporter gene	In vitro	Eli Lilly and Company Indianapolis, IN, USA	bTDR01	2.6.2.2.2
Insulinotropic Activity	INS-1 832/3 rat insulinoma cells Rodent and Primate pancreatic islet cells	In vitro		DBT56	2.6.2.2.3
Cell-Based ADCC Activity	Purified human PBMC, HEK cells	In vitro		bTDR04	2.6.2.2.4
Intravenous Glucose Tolerance Test	Rat, Sprague Dawley	SC		bTDR02	2.6.2.2.6
Intravenous Glucose Tolerance Test	Monkey, Cynomolgus	SC		OAAX03	2.6.2.2.7
Stepped Glucose Infusion	Rat, Sprague Dawley	SC		bTDR03	2.6.2.2.8
Stepped Glucose Infusion	Monkey, Cynomolgus	SC	(b) (4)	6180-702	2.6.2.2.9
Stepped Glucose Infusion	Monkey, Cynomolgus	SC		6180-773	2.6.2.2.10
Analysis Of T-Cell Epitopes	In silico	In silico	Eli Lilly and Company San Diego, CA, USA	bTDR224	2.6.2.2.5
Secondary Pharmacodynamics – Not Applicable					
Safety Pharmacology					
hERG Ion Channel	HEK 293 cells transfected with hERG clone	In vitro	(b) (4)	LLY05_07	2.6.2.4.1.1
Cardiovascular[a]	Monkey, Cynomolgus	In vivo	(b) (4)	010.08	2.6.2.4.1.2
			Eli Lilly and Company Greenfield, IN, USA	P00054	2.6.2.4.1.3
			(b) (4)	7608-192	2.6.2.4.1.4
				7608-235	2.6.2.4.1.5
Pharmacodynamic Drug Interactions – Not Applicable					

Abbreviations: # = number, ADCC = antibody-dependent cell mediated cytotoxicity, CHO = Chinese hamster ovary, HEK = human embryonic kidney, hERG = human ether-à-go-go-related gene, PBMC = peripheral blood mononuclear cell, SC = subcutaneous.

[a] Contains a GLP compliance statement.

Reference ID: 3527260

Type of Study	Species	Route	Duration of Dosing	Doses (mg/kg)	GLP	Testing Facility	Document ID
Single dose – Not applicable							
Repeat-dose toxicity							
	Mouse	Subcutaneous	1 month	0, 1, 10, 100	Yes	(b) (4)	8224143
	Rat	Subcutaneous	1 month	0, 2, 6, 20	Yes	Vienna, Virginia Eli Lilly and Company, Greenfield, Indiana	R00359
	Rat	Subcutaneous	3 months	0, 1.63, 4.89, 16.29	Yes	(b) (4)	7608-191
	Rat	Subcutaneous	6 months with 1 month of recovery	0, 1.63, 4.89, 16.29	Yes		7608-236
	Monkey	Subcutaneous	1 month	0, 1, 3, 10	Yes	Eli Lilly and Company, Greenfield, Indiana	P00054
	Monkey	Subcutaneous	3 months	0, 0.41, 1.63, 8.15	Yes	(b) (4)	7608-192
	Monkey	Subcutaneous	9 months with 2 months of recovery	0, 0.41, 1.63, 8.15	Yes		7608-235
Carcinogenicity							
	Mouse	Subcutaneous	6 months	0, 0.3, 1, 3	Yes		8224144
	Rat	Subcutaneous	2 years	0, 0.05, 0.5, 1.5, 5	Yes		8203405
Reproductive and Developmental Toxicity							
Female Fertility	Rat	Subcutaneous	Up to 5 weeks	0, 1.63, 4.89, 16.3	Yes		(b) (4) -353112
Male Fertility	Rat	Subcutaneous	6 weeks	0, 1.63 4.89, 16.3	Yes		-353113
Embryo Fetal	Rat	Subcutaneous	GD 6, 9, 12 and 15	0, 0.49, 1.63, 4.89	Yes		-353116
Embryo Fetal	Rabbit	Subcutaneous	GD 7, 10, 13, 16, and 19	0, 0.04, 0.12, 0.41	Yes		-353119
Pre/postnatal	Rat	Subcutaneous	GD6, 9, 12, 15, and 18; LD 1, 4, 7, 10, 13, 16, and 19	0, 0.2, 0.49, 1.63	Yes		-353253
Mechanistic							
	Rat	Subcutaneous	3 months	0, 0.5, 1.5, 5	Yes		504758
	Rat	Subcutaneous	1 year	0, 5	Yes		8236888
	Monkey	Subcutaneous	1 year	0, 8.15	Yes		8252199

3.2 Studies Not Reviewed

None

4 Pharmacology

Mechanism of action:
LY2189265 binds to and activates the human glucagon-like peptide 1 (GLP-1) receptor. This is anticipated to enhance glucose-dependent insulin biosynthesis and secretion; inhibition of glucagon secretion; slowing of gastric emptying; reduction of appetite; possible regulation of pancreatic islet cell apoptosis and neogenesis; and possibly weight loss in humans.

Drug activity related to proposed indication:
Pharmacology studies were conducted to examine the potential of LY2189265 to activate the GLP-1 receptor in vitro and the insulinotropic activity in vivo in rodent and non-human primate models of stepped glucose infusion (SGI). The results of these experiments demonstrated that: LY2189265 potently activated the human GLP-1 receptor; increased insulin secretion in a dose-dependent fashion after a single subcutaneous administration in a rodent model of SGI; increased insulin secretion after a single subcutaneous administration in a nonhuman primate model of SGI; increased insulin secretion after multiple weekly subcutaneous administrations in a non-human primate model of SGI; and decreased glucose levels in rats and monkeys.

4.1 Primary Pharmacology

Study title: In vitro activity of LY2189265 (Study bTDR01)
The ability of LY2189265 to activate the human GLP-1 receptor was evaluated by using HEK-293 cells that stably express both the human GLP-1 receptor and an intracellular reporter gene (CRE4-luciferase) whose expression is coupled to GLP-1 receptor signaling through cAMP production. Results were compared with a control peptide, which is a DPP-4 protected GLP-1 analog (LY307161). EC_{50} values were determined in 19 individual experiments.

Results:
LY2189265 activated the reporter gene with approximately 2-fold greater potency than the control peptide (sponsor-generated Table 1). Although native GLP-1 was not tested in this assay, the control peptide demonstrated approximately 60% less potency when compared with native GLP-1 (7-37) in a similar assay system (EC_{50} of 21.2 and 7.9 pM, respectively). These data suggest that the potency of LY2189265 is similar to native GLP-1 under the conditions of this in vitro reporter gene assay.

Table 1: Activity of LY2189265 in a Cell-Based Reporter Gene Assay System

	Control Peptide	LY2189265
EC_{50} [pM]	26.5	12.5
SEM [pM]	1.6	2.5
Repeats [n]	19	19

Abbreviations: EC50=Half-Maximal Effect Concentration, SEM=Standard Error of Mean

Reference ID: 3527260

Study title: In vitro binding activity of dulaglutide, LY2189265 (Study bTDR225)

The ability of LY2189265 to compete with ^{125}I-labeled native peptide ligands for binding the GLP-1, GIP, and glucagon receptors was characterized in competitive receptor/ligand binding experiments using a scintillation proximity assay (SPA) format. The binding assay was conducted with HEK-293 cells stably expressing the human GLP-1 or glucagon receptor and with CHO cells stably expressing the human GIP receptor. Results were compared with a control compound (GLP-1 7-36 amide; LY306263)

Results:

LY2189265 binds specifically to the human GLP-1 receptor with a K_i of 4.2 nM, while binding to the human GIP and glucagon receptors was very weak, with K_i values greater than 697 nM and 1360 nM, respectively (sponsor-generated Table 1). In this assay, LY2189265 had an approximately 10-fold higher K_i value for the human GLP-1 receptor compared with native GLP-1.

Table 1: In Vitro Receptor Binding Affinity (K_i) in Membranes Prepared from Cells Over Expressing the Recombinant Human GLP-1, Glucagon, or GIP Receptors

	Receptor Binding Affinity, K_i, nM (SEM, n)		
Serial No.	Human GLP-1R	Human GlucR	Human GIPR
2189265	4.20 (0.45, n=3)	>1360 (n=1/3)	>697 (n=1/3)
306263	0.388 (0.043, n=22)	>23700 (n=1/11)	94.5 (1/14)

Means are expressed as Geometric means with the standard error of the mean (SEM) and the number of replicates (n) indicated in parenthesis. SEM is represented with the same number of decimal points as the Geometric mean.

A qualifier (>) indicates that the data did not reach 50% inhibition and the Ki is calculated using the highest concentration tested in the assay.

Replicate numbers (n) expressed as x/y indicate that the Ki value is determined from x replicates out of a total of y tests. This means that some of the Ki results had qualifiers (>) associated with them and are not used in the mean calculation, or that all of the replicates had qualifiers and the mean shown is the highest test result out of all replicates.

Study title: LY2189265 glucose-dependently stimulates insulin secretion from rodent and primate pancreatic islets through interaction with the GLP-1 receptor (Study (b) (4)

Isolated rat and monkey pancreatic islet cells were isolated and tested in vitro for insulin secretion after incubation with 3 or 30 nM LY2189265 in the presence of low (2.8 mM) or high (16.8 mM) glucose. The rat INS-1 cell line was also used. Unmodified human GLP-1 was used as a positive control and the receptor antagonist, exendin-4 (9-39), was used as a negative competitor.

Reference ID: 3527260

Results:
Insulin secretion from isolated rat islets was potently (2.5- to 3-fold) enhanced by the inclusion of 3 or 30 nM LY2189265 in the presence of high (16.8 mM) glucose but not in the presence of low (2.8 mM) glucose. Unmodified human GLP-1 (3 nM) produced a 4-fold enhancement of insulin secretion from rat islets elicited by 16.8 mM glucose (Figure 1A). The EC_{50} of insulin secretion by LY2189265 was 4.2 ± 2.9 nM (4 individual experiments) with maximal stimulation (4-fold) occurring at 300 nM LY2189265 (Figure 1B; N=4). The inclusion of 1 μM of the GLP-1 receptor antagonist exendin-4 (9-39) reversed the glucose-dependent stimulation of insulin secretion induced by LY2189265, providing evidence that LY2189265 acts through the GLP-1 receptor (Figure 1C). LY2189265 also dose-dependently stimulated insulin secretion from INS-1 832/3 cells when stimulated by 16.8 mM glucose, with EC_{50} values of 8.3 and 34 nM in two individual experiments (data not shown). In islets isolated from Cynomolgus monkeys, LY2189265 also dose-dependently increased insulin secretion in the presence of a high glucose concentration (Fig. 1D).

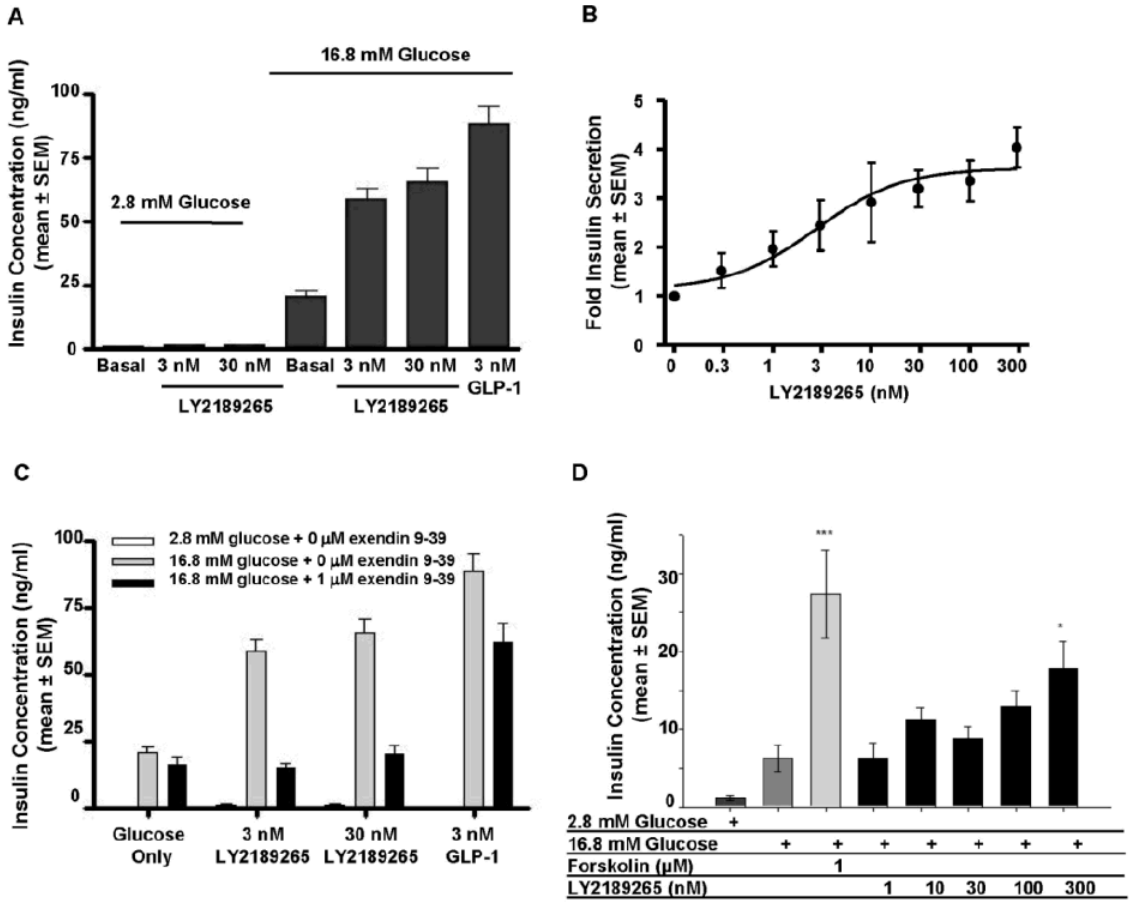


Figure 1: **LY2189265 induces glucose-dependent insulin secretion through activation of the GLP-1 receptor in rat and monkey islets.**

Study title: Assessment of the time action of insulinotropic activity of LY2189265 in an intravenous glucose tolerance test (IVGTT) in Sprague-Dawley rats
(Study bTDR02)

Male Sprague-Dawley rats (4-5/group/time point) were given an intravenous glucose challenge 24 and 72 hours after subcutaneous administration of vehicle (saline), 0.179, or 1.79 mg/kg (3 and 30 nmol/kg) LY2189265, respectively. Plasma insulin levels were assessed as a measure for the insulinotropic activity of GLP-1. After a 16 hour fast on Day 2 and Day 4, animals received 0.5 g/kg glucose via a bolus infusion. Blood samples were collected just before the glucose infusion and at 2, 4, 6, 10, 20, and 30 minutes after infusion.

Results:

Statistical significant decreases in mean plasma glucose AUC was noted at both dose levels 24 hours after treatment and 72 hours after treatment (0-30 min sampling period). A statistically significant increase in mean plasma insulin AUC was only noted for the lower dose level 24 hours after dosing and was only noted for the higher dose level 72 hours after dosing (sponsor-generated Table 1).

Table 1: Mean (± SEM) Glucodynamic and Insulinotropic Activity of LY2189265 24 and 72 hours After Subcutaneous Administration to Sprague Dawley Rats

Compound	Glucose AUC (0-10 min) [mg *min/dl]	Glucose AUC (0-30 min) [mg *min/dl]	Insulin AUC (0-10 min) [ng *min/ml]	Insulin AUC (0-30 min) [ng *min/ml]
Vehicle 24h	1448.4 ± 37.5 n=5	2073.4 ± 109.8 n=5	31.2 ± 5.2 n=5	40.5 ± 4.9 n=5
LY2189265 3 nmol/kg 24h	1475 ± 36 n=5	1600 ± 57 * n=5	71.1 ± 8.0 * n=5	82.1 ± 8.0 * n=5
LY2189265 30 nmol/kg 24h	1407 ± 40* n=5	1536 ± 130* n=5	43.6 ± 3.8 n=5	45.1 ± 4.9 n=5
Vehicle 72h	1588 ± 55 n=4	2295±222 n=4	32 ± 5 n=4	44 ± 5 n=4
LY2189265 3 nmol/kg 72h	1422 ± 63 n=5	1722 ± 113* n=5	31 ± 4 n=5	41 ± 6 n=5
LY2189265 30 nmol/kg 72h	1457 ± 161 n=5	1374±161* n=5	46 ± 4 * n=5	58 ± 8 n=5

Abbreviation: SEM = standard error of mean.
*: statistical significance compared to vehicle $p<0.05$

Reference ID: 3527260

Study title: Assessment of the dose-dependency of insulinotropic activity of LY2189265 in a stepped glucose infusion (SGI) model in Sprague-Dawley rats (Study bTDR03)

This study evaluated the dose-response of insulinotropic activity of LY2189265 in male Sprague-Dawley rats (3-7/group). Rats received glucose infusions at rates of 50 and 150 mg/kg/min, raising plasma glucose levels from 120 mg/dL to 340 and 700 mg/dL, respectively. LY2189265 was administered by subcutaneous injection at dose levels of 0.0179, 0.06, 0.179, or 1.79 mg/kg 24 hours before the glucose infusion. Plasma glucose levels were measured as a biomarker for the insulinotropic activity of GLP-1. Plasma samples were collected at -20, -10, and 0 minutes before glucose infusion (saline infusion) and 10, 20, 30 (50 mg/kg/min glucose infusion), 40, 50, and 60 minutes (150 mg/kg/min glucose infusion).

Results:

As shown in sponsor-generated Figure 1 below, LY2189265 caused a dose-related increase in plasma insulin levels under both infusion rates. Statistical significance was achieved at 0.179 and 1.79 mg/kg.

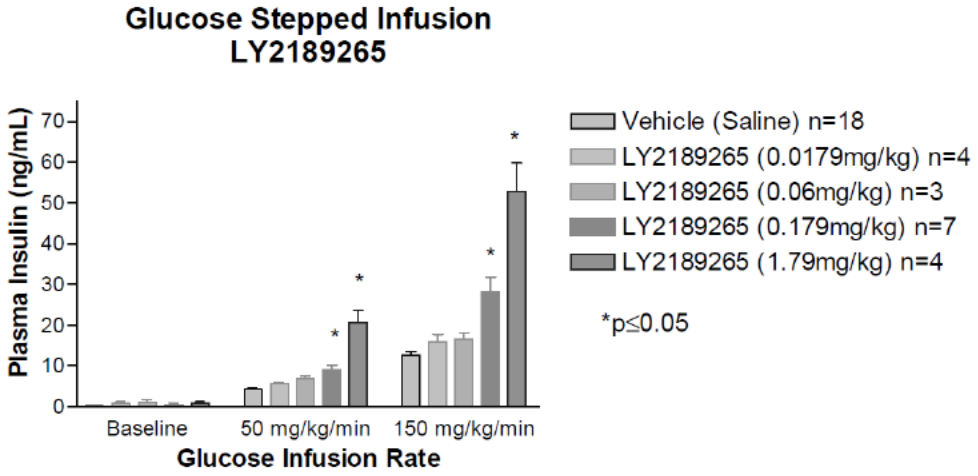


Figure 1: **Mean (+SEM) effect of LY2189265 on insulin concentrations in rats during a stepped glucose infusion 24 hours after subcutaneous administration.**

Study title: Evaluation of the activity of subcutaneously administered 2189265 (b) (4) in normal, Cynomolgus monkeys in the fasting state and during an intravenous glucose tolerance test (Study OAAX03)

Six sedated male Cynomolgus monkeys underwent an in vitro glucose tolerance test (IVGTT) on Day 1 after receiving the vehicle control and then again 4 and 7 days after receiving 0.1 mg/kg LY2189265 by subcutaneous injection. Glucose was administered as a 50% dextrose solution (0.5 g/kg) by a bolus intravenous injection (1 mL/kg) over 30 seconds. Prior to the IVGTT, animals were fasted overnight for 16 hours. Serum glucose, insulin, C-peptide, glucagon, and GLP-1 were measured at 15 minutes before, immediately before, and 2.5, 5, 7.5, 10, 15, 20, 30, 45, and 60 minutes after glucose injection.

Results:
In this study, the sponsor states that uncharacteristic glucose profiles were observed for 5/6 animals on Day 4 and for 4/6 animals on Day 7. Generally, these responses included rapid, transient decreases in serum glucose concentrations followed by either a return to normal glucose or rebound hyperglycemia (sponsor-generated Figure 2). Overall, a slight decrease in mean glucose $AUC_{0\text{-}60min}$ and slight increase in insulin $AUC_{0\text{-}60min}$ was observed 4 and 7 days after LY2189265 treatment, which was primarily due to the effects observed within the first 5 or 10 minutes (sponsor-generated Figure 7).

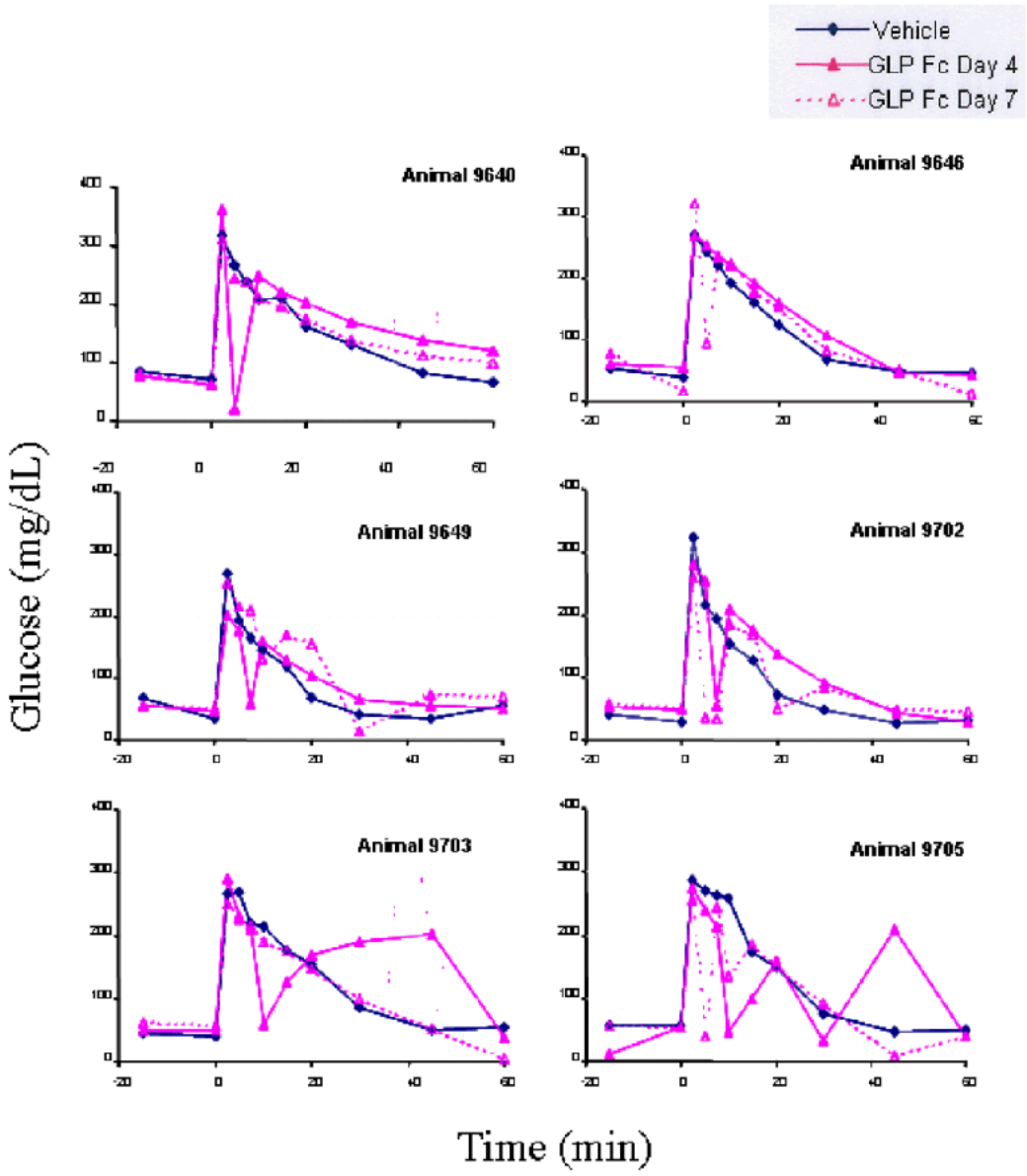


Figure 2: **Effect of 2189265 on glucose concentrations in individual cynomolgus monkeys during an IVGTT performed 4 and 7 days after a subcutaneous injection of 0.1 mg/kg 2189265.**

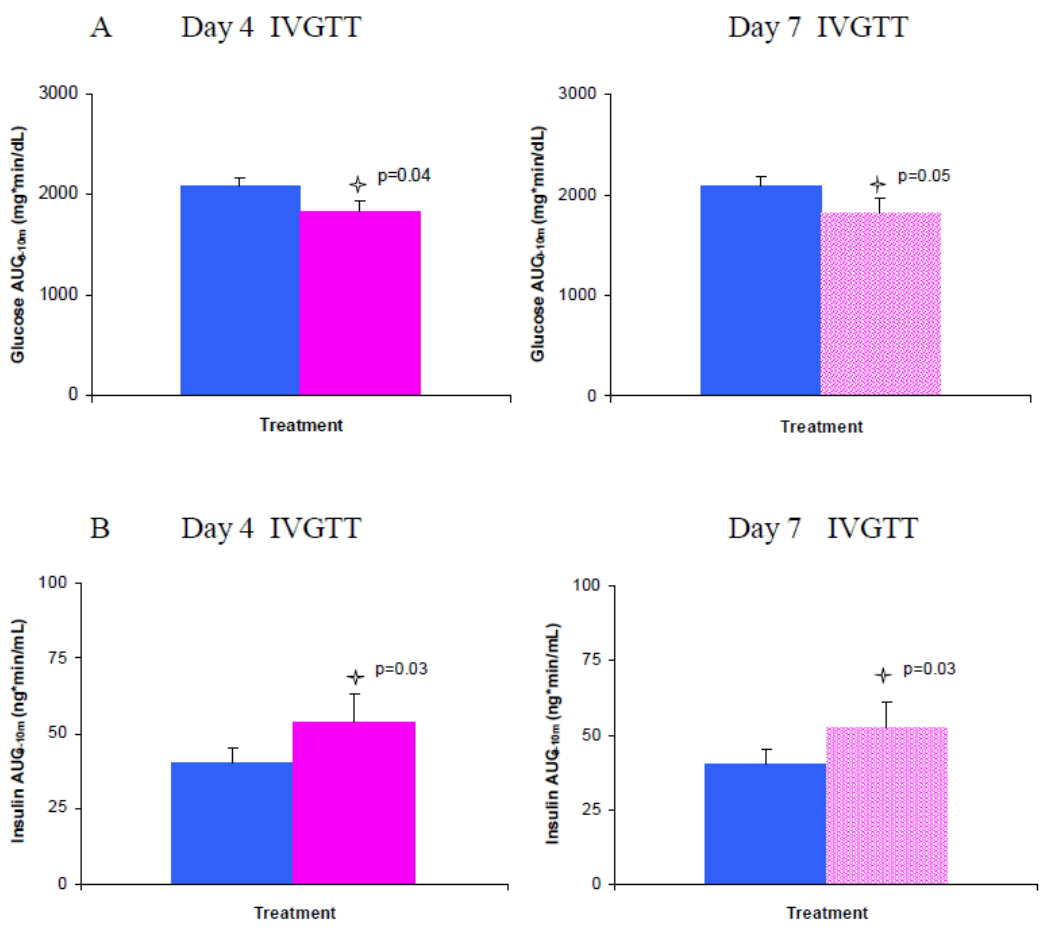


Figure 7: **Mean (+SEM) effect of 2189265 on A) serum glucose, and B) serum insulin exposure ($AUC_{0\text{-}10min}$) in cynomolgus monkeys during and IVGTT performed following a single subcutaneous injection of vehicle (■) or during an IVGTT performed 4 and 7 days after a subcutaneous injection of 0.1 mg/kg 2189265 (■). Values are means ± SEM of 6 animals.**

Study title: Effect of LY2189265 on glucodynamics/insulinotropics in normal Cynomolgus monkeys subjected to repeat graded intravenous glucose infusions (Study 6180-702)

Six male Cynomolgus monkeys underwent a stepped IV glucose infusion immediately after SC administration of vehicle or 0.1 mg/kg LY2189265 on Day 1 and Day 3, respectively. The duration of glucose infusion was approximately 40 minutes, with glucose given at 10 mg/kg/min for the first 20 minutes and 25 mg/kg/min for the last 20 minutes. Glucose infusions also occurred 5 and 7 days after the SC injection of test article to evaluate the duration of pharmacodynamics after a single dose. The PK profile of LY2189265 was also evaluated over the 7-day post-dose period.

Results:
Administration of LY2189265 resulted in significant insulinotropic activity for at least 7 days (sponsor-generated Figure 1) and was also associated with significant increases in C-peptide levels (sponsor-generated Figure 2). There were no clear effects on glucose, GLP-1, or glucagon levels (relative to placebo). Mean serum LY2189265 concentrations were 325, 47, and 24 ng/mL at 1, 5, and 7 days after administration, respectively.

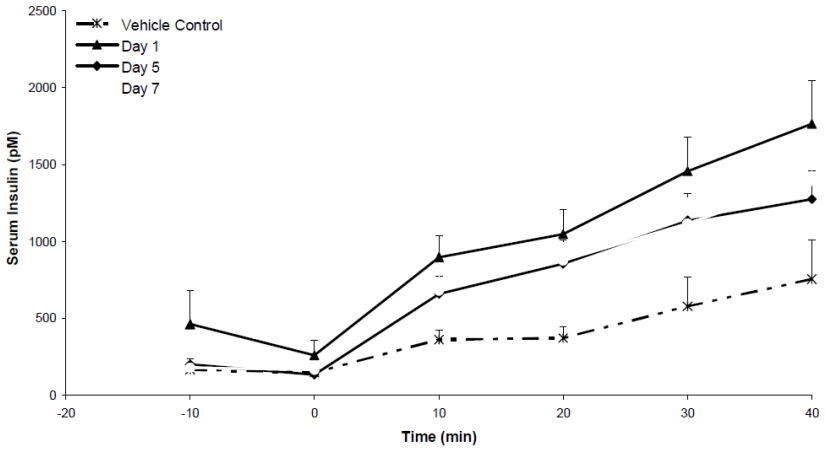


Figure 1: **Mean (+SEM) effect of LY2189265 on insulin concentrations in normal cynomolgus monkeys during a stepped glucose infusion 1, 5, and 7 days after a single subcutaneous injection of 0.1 mg/kg LY2189265.**

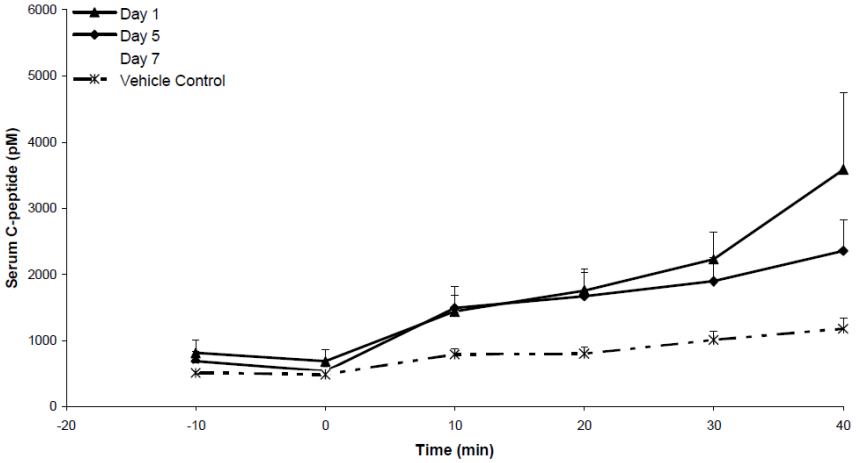


Figure 2: **Mean (+SEM) effect of LY2189265 on C-peptide concentrations in normal cynomolgus monkeys during a stepped glucose infusion 1, 5, and 7 days after a single subcutaneous injection of 0.1 mg/kg LY2189265.**

Study title: Repeat-dose effect of LY2189265 on glucodynamics/insulinotropics in normal Cynomolgus monkeys subjected to graded intravenous glucose infusions (Study 6180-773)

Six male Cynomolgus monkeys underwent a stepped IV glucose infusion immediately after SC administration of vehicle on Day 1. Monkeys received a SC dose of 0.1 mg/kg LY2189265 every 7 days for a total of 4 doses, starting on Day 3. Monkeys then underwent a stepped glucose infusion on Study Day 28, which was 25 days after first dose and 4 days after the final dose. The duration of glucose infusion was approximately 40 minutes, with glucose given at 10 mg/kg/min for the first 20 minutes and 25 mg/kg/min for the last 20 minutes. LY2189265 exposure was also measured on Study Day 28.

Results:

Administration of LY2189265 resulted in significant insulinotropic activity 4 days after the final dose of four once weekly doses (sponsor-generated Figure 1) and was also associated with significant increases in C-peptide levels (sponsor-generated Figure 2). On Study Day 28, mean triglyceride levels were lower than on Day 1 (Table 4) and mean GLP-1 levels were higher than on Day 1 (Table 6), suggesting an LY2189265-related effect on these endpoints. There were no clear effects on glucose or glucagon levels (relative to placebo). Mean serum LY2189265 concentrations 4 days after the final administration was 53 ng/mL.

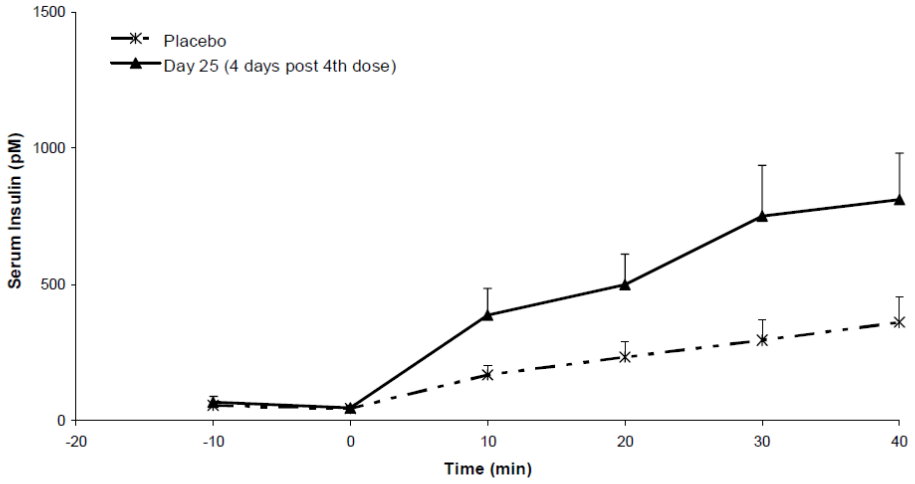


Figure 1: **Mean (+SEM) effect of LY2189265 on insulin concentrations in normal cynomolgus monkeys during a stepped glucose infusion 4 days after 4 weekly subcutaneous injections of 0.1 mg/kg LY2189265.**

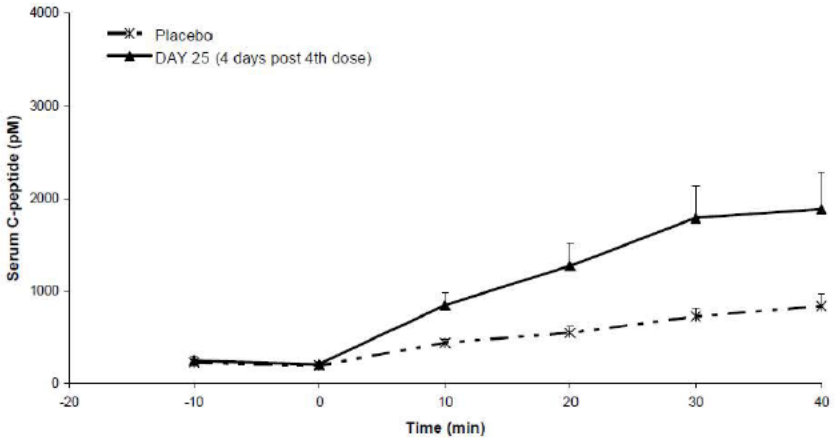


Figure 2: **Mean (+SEM) effect of LY2189265 on C-peptide concentrations in normal cynomolgus monkeys during a stepped glucose infusion 4 days after 4 weekly subcutaneous injections of 0.1 mg/kg LY2189265.**

Attachment 4: **Triglyceride concentrations (mg/dL) in normal cynomolgus monkeys during a stepped glucose infusion predose and 4 days after 4 weekly subcutaneous injections of 0.1 mg/kg LY2189265.**

TRIG (Placebo) mg/dL

Time (min)	736	737	738	739	740	741	AVE	SD	SE
-10.	51	64	57	52	50	36	52	9	4
0.	53	66	55	50	47	43	52	8	3
10.	42	61	56	49	40	37	48	10	4
20.	40	56	57	48	34	36	45	10	4
30.	43	50	52	49	31	34	43	9	4
40.	40	59	54	49	33	29	44	12	5

TRIG (Day 25) mg/dL

Time (min)	736	737	738	739	740	741	AVE	SD	SE
-10.	32	54	45	34	29	43	40	9	4
0.	31	46	43	35	32	41	38	6	3
10.	NR	51	37	39	39	33	40	7	3
20.	30	49	36	26	27	NR	34	9	4
30.	30	46	40	26	24	30	33	9	3
40.	26	40	36	22	21	24	28	8	3

Abbreviations: AVE = arithmetic average; NR = no result; SD = standard deviation; SE = standard error of the mean.

Attachment 6: **GLP-1 concentrations (pM) in normal cynomolgus monkeys during a stepped glucose infusion predose and 4 days after 4 weekly subcutaneous injections of 0.1 mg/kg LY2189265.**

GLP (Placebo)	pM								
Time (min)	736	737	738	739	740	741	AVE	SD	SE
-10.	11	22	9	17	0	7	11	8	3
0.	9	18	8	15	0	0	8	7	3
10.	9	16	8	13	0	11	10	5	2
20.	6	12	0	0	0	0	3	5	2
30.	7	12	7	7	0	0	6	5	2
40.	0	10	0	7	0	0	3	4	2

GLP (Day 25)	pM								
Time (min)	736	737	738	739	740	741	AVE	SD	SE
-10.	15	26	20	26	0	13	17	10	4
0.	15	23	20	19	0	13	15	8	3
10.	13	22	20	18	0	13	14	8	3
20.	13	21	20	14	0	14	14	8	3
30.	10	19	20	19	0	10	13	8	3
40.	15	21	20	12	0	9	13	8	3

Abbreviations: AVE = arithmetic average; SD = standard deviation; SE = standard error of the mean.

4.2 Secondary Pharmacology

Study title: In silico evaluation of T-cell epitopes for dulaglutide, LY2189265 (Study bTDR224)

LY2189265 is a fusion protein of a GLP-1 analog and a modified IgG4-Fc part of a human antibody, (b) (4) Because LY2189265 has an Fc region, it was assessed for its ability to evoke the formation of anti-drug antibodies (ADAs) in humans. T-cell epitopes are required for the formation of ADAs and can be predicted with in silico tools, such as the EpiMatrix algorithm. Therefore, the purpose of this assay was to evaluate whether changes introduced into the GLP-1 moiety and/or the fusion to a modified IgG4-Fc part of a human antibody had generated non-native epitopes that would be recognized by T-cells. The sequence of native GLP-1 (7-37), the (b) (4) amino acids of a precursor of LY2189265 (b) (4), and the (b) (4) amino acids of LY2189265 were analyzed for potential T-cell epitopes (sequences shown in sponsor-generated Figure 1). The remainder of the sequence of the GLP-1-Fc fusion proteins (CH2 and CH3 domains of human IgG4) was not analyzed because those regions represent native human sequence with "no risk of non-native T-cell epitopes being introduced".

```
GLP-1 (7-37)
    7 HAEGTFTSDV SSYLEGQAAK EFIAWLVKGR G
```

(b) (4)

Figure 1: Amino acid sequence of native human GLP-1 (7-37), the (b) (4) amino acids of (b) (4) (a precursor of LY2189265), and the (b) (4) amino acids of LY2189265.

(b) (4)

Results:
Only one potential T-cell epitope was identified for LY2189265, which is also present in native GLP-1. LY2189265 was not found to possess any non-native potential T-cell epitopes. (b) (4)

Study title: In vitro evaluation of ADCC effector functions of LY2189265 and related proteins (Study bTDR04)
LY2189265 is a fusion protein of a GLP-1 analog and a modified IgG4-Fc part of a human antibody, (b) (4) . Because LY2189265 has an Fc region, it was assessed for its ability to induce ADCC in a fully human in vitro test system. The test system comprised human HEK-293 cells expressing human GLP-1 receptor as target cells and human peripheral blood mononuclear cells (PBMCs) as effector cells. Cell cytotoxicity in response to LY2189265 was assessed by using a cytotoxicity detection kit, which measures lactate dehydrogenase in the culture medium. HEK/GLP-1R cells were seeded with PBMCs at effector to target ratios of 20:1, 10:1, or 7:1. Test article solutions ranged from 0.4 to 50 µg/mL and were incubated with the HEK cells for 20 minutes before the PBMCs were added. Media was measured for lactate dehydrogenase (LDH) 3 to 4 hours after the addition of PBMCs. Each assay included positive control, unmodified IgG1 and IgG4 antibodies that would be expected to induce ADCC.

Results:

Experiment 1: Varying ratios of effector cells to target cells (A = 20:1; B = 10:1, and C = 7:1)

The results indicate that under all 3 cell ratios, no significant dose-dependent increase of cellular lysis was observed for LY2189265 or its IgG4 isotype; however some cytotoxicity was observed for the IgG1 isotype (sponsor-generated Figure 1)

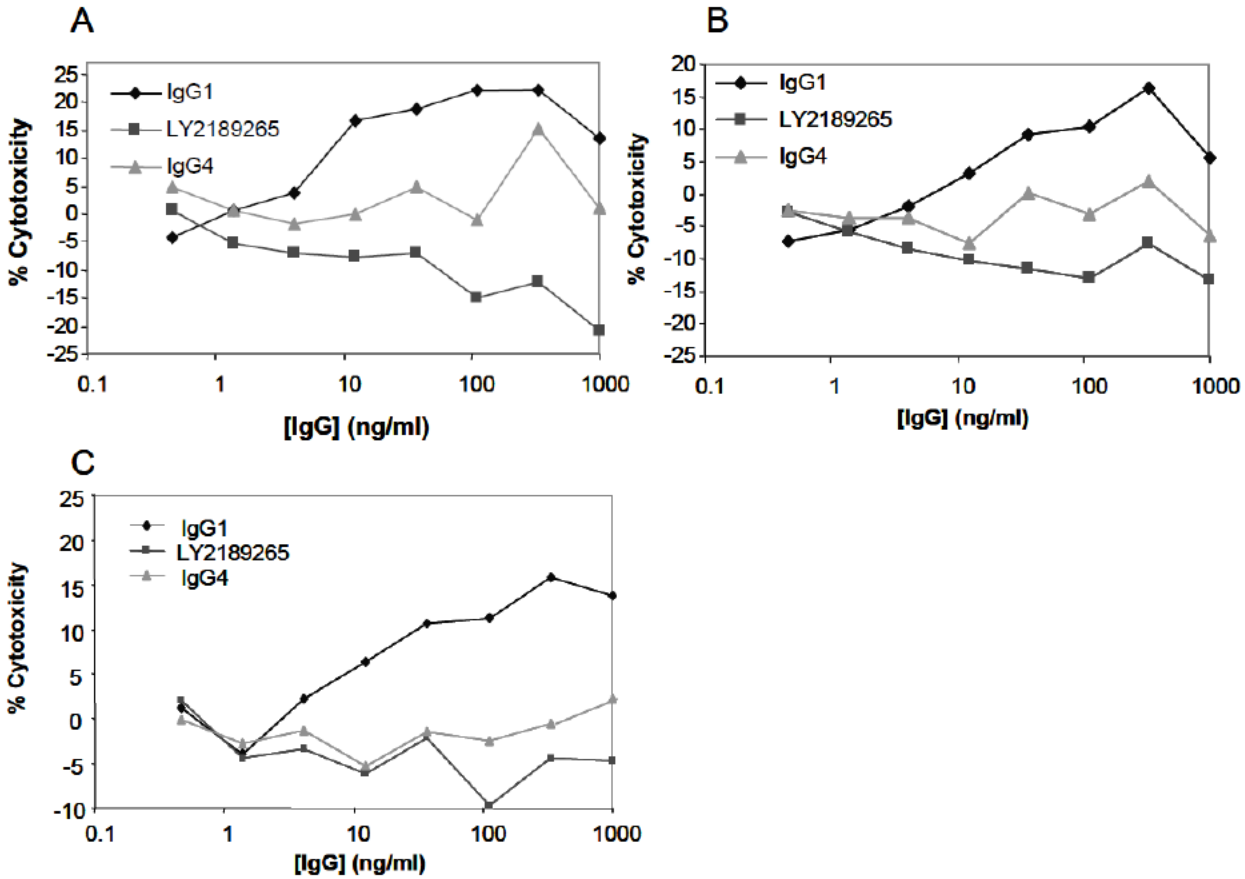


Figure 1: **Lysis of HEK293 hGLP-1R cells in response to various doses of LY2189265 and its IgG1 and IgG4 isotypes (single point determinations).**

Experiment 2: (20:1 effector to target cell ratio)

Sponsor-generated Figure 2 shows that the IgG4 isotype of LY2189265 did not induce cell lysis, whereas the IgG1 isotype induced a dose-dependent increase in cell lysis. The apparent increase in cytotoxicity with 1000 ng/mL IgG4 (left panel) was thought to represent an “edge effect” rather than direct Ab-mediated cell lysis.

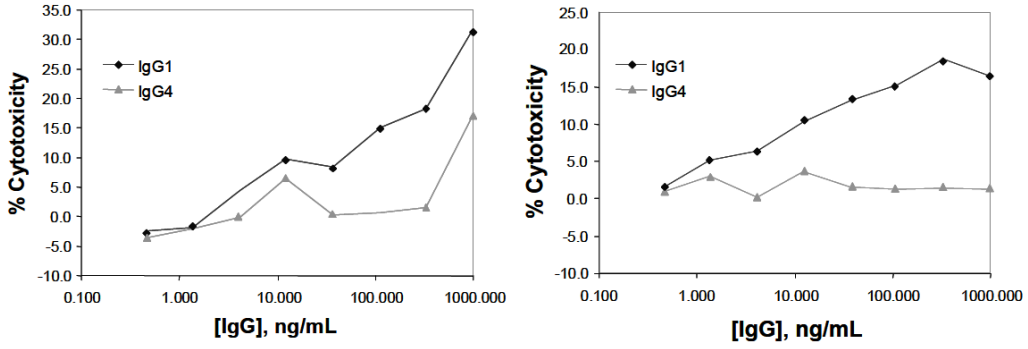


Figure 2: **Lysis of HEK293 hGLP-1R cells in response to various doses of the IgG1 and IgG4 isotypes of LY2189265 (Mean of four data points).**

Experiment 3:
This experiment was conducted to evaluate the variability in PBMC activity among different donors. The concentration range was increased to 50 µg/mL. As with the previous experiments, sponsor-generated Figure 3 shows that the IgG4 isotype of LY2189265 did not induce cell lysis, whereas the IgG1 isotype induced a dose-dependent increase in cell lysis. No meaningful donor-to-donor variability was observed. The sponsor stated that the bell shaped curve generated for the IgG1 isotype has been observed for other antibodies and it is not believed to be antibody specific, target cell specific, or LDH specific.

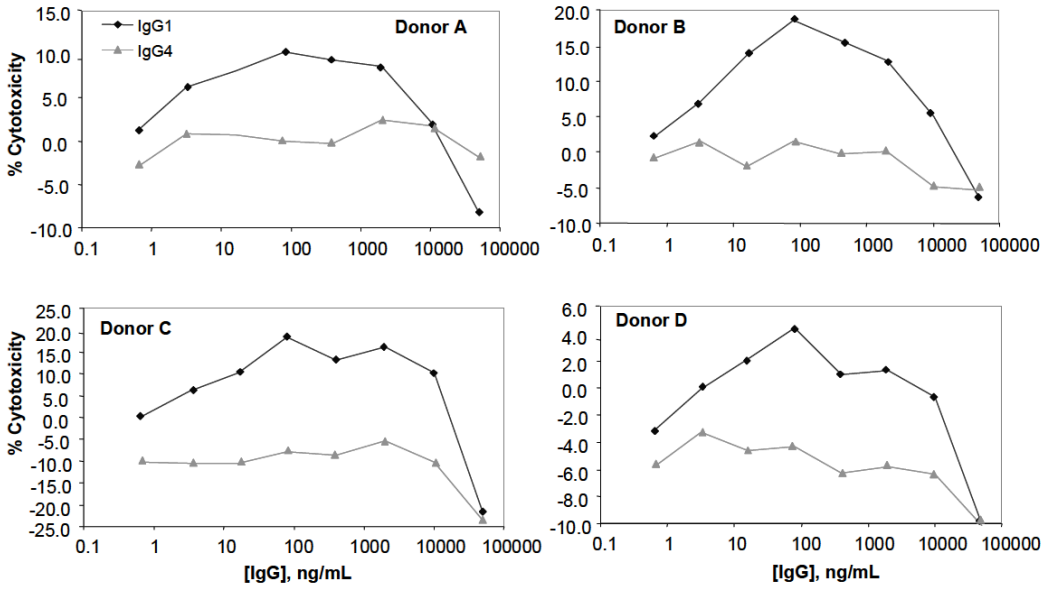


Figure 3: **Lysis of HEK293 hGLP-1R cells in response to various doses of the IgG1 and IgG4 isotypes of LY2189265 (Mean of duplicate data points).**

Effect of higher doses and donor-to-donor variability.

4.3 Safety Pharmacology

Cardiovascular

hERG Assay

Study title: LY2189265: In vitro effect on hERG current (I_{kr}) expressed in human embryonic kidney (HEK) cells (Study LLY05_07)

The potential of LY2189265 to inhibit hERG channel current was evaluated in a stably transfected HEK cell line at concentrations of 0.51, 1.52, 5.1, and 15.2 µg/mL. E-4031 (0.1 µM) was used as a positive control. Channel currents were measured using the whole-cell variant of the patch clamp method. After current kinetics and amplitudes stabilized, rate-dependent effects were determined by a train of depolarizing voltage steps at 3 Hz from a holding potential of -75 mV.

Results:

A concentration-dependent increase in hERG current inhibition was noted, with 33% inhibition at the highest concentration of 15.2 µg/mL (sponsor-generated Table 1). The positive control cells (n=2) showed 93% and 100% channel blockades.

Table 1: Block of hERG Current at 0.1 Hz by LY2189265

% reduction from each cell is shown.

Cell Number	0.51µg/ml	1.52µg/ml	5.1µg/ml	15.2µg/ml
1	9.5	12.8	16.3	rupture
2	5.7	8.2	14.4	31.2
3	8.8	13.2	20.2	rupture
4	4.2	5.4	14.7	27.3
5	7.2	------	------	------
6	------	------	21.6	rupture
7	6.2	8.0	25.5	41.8
X ± SEM	6.9 ± 0.8	9.5 ± 1.5	18.8 ± 1.8	33.4 ± 4.3

Note: % change was calculated from the peak current amplitudes after a steady-state level of drug block was achieved relative to the current amplitude before drug was introduced (control). Pacing rate was 0.1 Hz. hERG current was elicited by a voltage pulse to +10 mV (500 ms) from a holding potential of -75 mV. hERG tail currents were measured upon repolarization to -40 mV (500 ms).

Values on bottom row are X ± SEM.

------ Indicates concentration was not attempted.

Reference ID: 3527260

In vivo in monkeys

Study title: Cardiovascular telemetry study in conscious Cynomolgus monkeys administered LY2189265 by subcutaneous injection (Study (b) (4) .010.08)

Male and female, naïve and non-naïve Cynomolgus monkeys (6/group; 2-4/sex/group), implanted with cardiovascular telemetry devices, were evaluated for LY2189265-related effects on cardiovascular function. Monkeys received a single subcutaneous dose of 1 or 10 mg/kg LY2189265 or vehicle ((b) (4) citrate buffer pH (b) (4) polysorbate 80, (b) (4) NaCl). Cardiovascular parameters (systolic pressure, diastolic pressure, mean pressure, pulse pressure, left ventricular inotropic state, heart rate, and ECG intervals) were collected continuously for at least 2 hours before dose administration and at least 8 hours after dose administration. Data were also acquired for at least every 10 minutes every hour thereafter through 74 hours post-dose.

Results:

Clinical signs

On Day 2, emesis was noted for 2/6 animals receiving 1 mg/kg and 4/6 animals receiving 10 mg/kg, compared with 0/6 animals for the control group. Marked decreases in food consumption were observed for 4/6 animals receiving 1 mg/kg and all animals receiving 10 mg/kg. Decreased food consumption was noted until 3 days after dosing for the 1 mg/kg dose and throughout the 4-day monitoring period for the HD group.

Blood pressure

No physiological important test article-related effects occurred for systolic, diastolic, pulse, or mean pressures.

Left ventricular inotropic state (dP/dtmax)

Dose responsive test article-related increases on dP/dtmax occurred in both the 1 and 10 mg/kg dose groups. These effects were considered to be physiologically important because of both the magnitude and duration of these effects. At 1 mg/kg, increases in dP/dtmax occurred from 33 to 44 hours post dosing compared with the control group. At 10 mg/kg, increases occurred from 11 to 20 hours post dose, 33 to 44 hours post dose, and 57 to 74 hours post dose compared with the control group. In general, the greatest differences from control occurred during the dark cycles, in which values decreased for control animals but remained elevated for treated animals.

Reference ID: 3527260

Table 32. Left Ventricular Inotropic State (sponsor-generated tables)

Phase 1

Group	Statistics	Baseline	Overall	1 Hour	2 Hour	3 Hour	4 Hour	5 Hour	6 Hour	7 Hour	8 Hour
1	Mean	2736.7	3093.5	3201.3	2956.7	2686.7	3082.1	2848.5	3432.4	3371.2	3168.8
	N	6	6	6	6	6	6	6	6	6	6
	LSMean	2736.7	3233	3340.9	3096.3	2826.3	3221.7	2988.1	3572	3510.8	3308.3
	LSM s.e.	340.7	222.7	282.7	282.7	282.7	282.7	282.7	282.7	282.7	282.7
2	Mean	2758.9	2869.3	2869.2	2902.1	2882.6	2777.4	2899.8	2892.4	2965	2765.9
	N	6	6	6	6	6	6	6	6	6	6
	LSMean	2758.9	2989	2988.9	3021.8	3002.3	2897.1	3019.5	3012.1	3084.7	2885.6
	LSM s.e.	340.7	222.4	282.4	282.4	282.4	282.4	282.4	282.4	282.4	282.4
	Trend p-value+	NT	NT								
3	Mean	3181.3	3122	3266.8	3162.2	3110	3015.8	3009.3	3023	3104.6	3284.6
	N	6	6	6	6	6	6	6	6	6	6
	LSMean	3181.3	2862.7	3007.5	2902.9	2850.7	2756.5	2750	2763.7	2845.3	3025.3
	LSM s.e.	340.7	226.2	285.4	285.4	285.4	285.4	285.4	285.4	285.4	285.4
	Trend p-value+	0.371	0.268								
All	Grp F-test p-val#	0.593	0.515								
Intn	Grp*Time p-val#		0.17								
	GrpTrend*LinTime p-val+		0.496								
	GrpTrend*QuadTime p-val+		0.298								

Phase 2

Group	Statistics	Baseline	Overall	11 Hour	14 Hour	17 Hour	20 Hour
1	Mean	2736.7	2232.6	2251.6	2291.5	2118.1	2269.1
	N	6	6	6	6	6	6
	LSMean	2736.7	2331.3	2350.3	2390.2	2216.8	2367.9
	LSM s.e.	340.7	232.2	246	246	246	246
2	Mean	2758.9	2727.4	2406.7	2615.3	2743.7	3144.1
	N	6	6	6	6	6	6
	LSMean	2758.9	2812.1	2491.3	2699.9	2828.3	3228.8
	LSM s.e.	340.7	231.8	245.6	245.6	245.6	245.6
	Trend p-value+	NT	0.163				
3	Mean	3181.3	3242	3037.5	3296.4	3327.6	3306.4
	N	6	6	6	6	6	6
	LSMean	3181.3	3058.6	2854.1	3113	3144.2	3123
	LSM s.e.	340.7	236	249.6	249.6	249.6	249.6
	Trend p-value+	0.371	0.048*				
All	Grp F-test p-val#	0.593	0.121				
Intn	Grp*Time p-val#		0.032				
	GrpTrend*LinTime p-val+		0.252				
	GrpTrend*QuadTime p-val+		0.143				

Phase 3

Group	Statistics	Baseline	Overall	23 Hour	26 Hour	29 Hour	32 Hour
1	Mean	2736.7	2917.8	2786	2735.7	3106.8	3042.6
	N	6	6	6	6	6	6
	LSMean	2736.7	3016.9	2885.1	2834.8	3205.9	3141.7
	LSM s.e.	340.7	170.3	208.3	208.3	208.3	208.3
2	Mean	2758.9	3117.1	3325.2	2821.2	3174.5	3147.7
	N	6	6	6	6	6	6
	LSMean	2758.9	3202.1	3410.2	2906.2	3259.5	3232.7
	LSM s.e.	340.7	170	208	208	208	208
	Trend p-value+	NT	NT				
3	Mean	3181.3	3481.5	3538.7	3327.9	3601.7	3457.7
	N	6	6	6	6	6	6
	LSMean	3181.3	3297.3	3354.6	3143.7	3417.5	3273.6
	LSM s.e.	340.7	173	210.5	210.5	210.5	210.5
	Trend p-value+	0.371	0.273				
All	Grp F-test p-val#	0.593	0.522				
Intn	Grp*Time p-val#		0.609				
	GrpTrend*LinTime p-val+		0.34				
	GrpTrend*QuadTime p-val+		0.139				

Phase 4

Group	Statistics	Baseline	Overall	35 Hour	38 Hour	41 Hour	44 Hour
1	Mean	2736.7	2252.4	2254.5	2226.8	2101.9	2426.5
	N	6	6	6	6	6	6
	LSMean	2736.7	2317.1	2319.1	2291.4	2166.6	2491.1
	LSM s.e.	340.7	182.3	206.6	206.6	206.6	206.6
2	Mean	2758.9	2994.2	2727.4	3003.7	3086.8	3158.7
	N	6	6	6	6	6	6
	LSMean	2758.9	3049.6	2782.8	3059.1	3142.2	3214.1
	LSM s.e.	340.7	181.9	206.4	206.4	206.4	206.4
	Trend p-value+	NT	0.013*				
3	Mean	3181.3	3484.7	3294.8	3696.9	3457.7	3489.2
	N	6	6	6	6	6	6
	LSMean	3181.3	3364.6	3174.8	3576.9	3337.7	3369.1
	LSM s.e.	340.7	185.2	209.2	209.2	209.2	209.2
	Trend p-value+	0.371	0.001*				
All	Grp F-test p-val#	0.593	0.004*				
Intn	Grp*Time p-val#		0.103				
	GrpTrend*LinTime p-val+		0.961				
	GrpTrend*QuadTime p-val+		0.406				

Phase 5

Group	Statistics	Baseline	Overall	47 Hour	50 Hour	53 Hour	56 Hour
1	Mean	2736.7	3076.4	2959.8	2806.8	3409.8	3129.3
	N	6	6	6	6	6	6
	LSMean	2736.7	3158.4	3041.8	2888.7	3491.7	3211.2
	LSM s.e.	340.7	181.3	215.2	215.2	215.2	215.2
2	Mean	2758.9	3000	3093.9	2913.2	3073	2920
	N	6	6	6	6	6	6
	LSMean	2758.9	3070.3	3164.2	2983.5	3143.2	2990.3
	LSM s.e.	340.7	181	214.9	214.9	214.9	214.9
	Trend p-value+	NT	NT				
3	Mean	3181.3	3428.3	3535.7	3391.1	3379.5	3407
	N	6	6	6	6	6	6
	LSMean	3181.3	3276.1	3383.5	3238.9	3227.3	3254.8
	LSM s.e.	340.7	184.4	217.7	217.7	217.7	217.7
	Trend p-value+	0.371	0.66				
All	Grp F-test p-val#	0.593	0.737				
Intn	Grp*Time p-val#		0.249				
	GrpTrend*LinTime p-val+		0.081				
	GrpTrend*QuadTime p-val+		0.987				

Phase 6

Group	Statistics	Baseline	Overall	59 Hour	62 Hour	65 Hour	68 Hour	71 Hour	74 Hour
1	Mean	2736.7	2470.2	2216.1	2217.3	2066.6	2337.3	2974.7	3009.5
	N	6	6	6	6	6	6	6	6
	LSMean	2736.7	2553.4	2299.2	2300.4	2149.7	2420.4	3057.8	3092.6
	LSM s.e.	340.7	105.8	145	145	145	145	145	145
2	Mean	2758.9	2560.1	2418.3	2393.1	2433.3	2740.9	2761.6	2613.4
	N	6	6	6	6	6	6	6	6
	LSMean	2758.9	2631.4	2489.6	2464.3	2504.6	2812.2	2832.9	2684.7
	LSM s.e.	340.7	105.6	144.9	144.9	144.9	144.9	144.9	144.9
	Trend p-value+	NT	0.608						
3	Mean	3181.3	3111.7	2874.9	3070	2963.6	3220.4	3250.4	3290.8
	N	6	6	6	6	6	6	6	6
	LSMean	3181.3	2957.3	2720.5	2915.6	2809.2	3066	3096	3136.4
	LSM s.e.	340.7	107.5	146.2	146.2	146.2	146.2	146.2	146.2
	Trend p-value+	0.371	0.019*						
All	Grp F-test p-val#	0.593	0.045						
Intn	Grp*Time p-val#		0.016						
	GrpTrend*LinTime p-val+		0.066						
	GrpTrend*QuadTime p-val+		0.089						

Heart rate

A dose-related increase in heart rate was observed. These effects were considered to be physiologically important due to both the magnitude and duration of these effects. At 1 mg/kg, statistically significant increases (21% and 22%) in heart rate were observed at 3 and 5 hours post dosing, respectively, compared with control. Overall increases (15% to 28%) were also noted for the other data collection periods, except for Phase 5. At 10 mg/kg, a statistically significant increase in heart rate (26%) was observed at 3 hours

after dosing compared with control. Overall statistically significant increases (19% to 44%) were observed in Phases 2 through 6. The difference in pre-treatment baseline values between the HD group and control was 16.5 beats per minute (133.9 vs. 117.4 beats per minute for HD and control, respectively), which slightly increased the overall difference between the treated and control animals during each phase of the study. However, differences from control were also noted for the LD group, which had a similar baseline value as control (116.8 vs. 117.4 beats per minute), and differences between post-treatment and baseline values within each treatment group were observed, indicating a treatment-related effect. The biggest effects appeared to occur during the sleeping hours in which heart rates for control monkeys were lower compared with baseline whereas heart rates for treated monkeys remained elevated or were similar to baseline values.

Table 38. Statistical Evaluation of Heart Rate (sponsor-generated table; beats/min)

Group	Statistics	Baseline	Overall	1 Hour	2 Hour	3 Hour	4 Hour	5 Hour	6 Hour	7 Hour	8 Hour
1	Mean	117.4	126.3	129.4	121.4	113.3	127.8	119.6	135.7	134.5	128.8
	N	6	6	6	6	6	6	6	6	6	6
	LSMean	117.4	131.4	134.5	126.5	118.4	132.9	124.7	140.8	139.6	133.9
	LSM s.e.	9.6	6.1	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
2	Mean	116.8	141.1	126.5	130.9	137.9	140.4	146.7	148.5	153.7	144.6
	N	6	6	6	6	6	6	6	6	6	6
	LSMean	116.8	146.9	132.2	136.6	143.6	146.1	152.4	154.2	159.4	150.3
	LSM s.e.	9.6	6.1	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
	Trend p-value+	NT	NT								
	Bonferroni p-value+		0.184	1	0.687	0.039*	0.434	0.021*	0.419	0.128	0.252
3	Mean	133.9	159.3	148.3	156.3	160.5	161.9	160.3	160.8	163.9	162.4
	N	6	6	6	6	6	6	6	6	6	6
	LSMean	133.9	148.4	137.4	145.4	149.6	151.1	149.4	149.9	153	151.5
	LSM s.e.	9.6	6.3	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7
	Trend p-value+	0.243	0.077								
	Bonferroni p-value+		0.154	1	0.173	0.010*	0.198	0.051	0.809	0.441	0.22
All	Grp F-test p-val#	0.383	0.132								
Intn	Grp*Time p-val#		0.006*								
	GrpTrend*LinTime p-val+		0.92								

Summary of Mean Heart Rate (beats/minute)

	Study Phase					
	1 (1-8 hr)	**2 (11-20 hr)**	**3 (23-32 hr)**	**4 (35-44 hr)**	**5 (47-56 hr)**	**6 (59-74 hr)**
Control						
Diff from baseline	8.9	-12.9	3.9	-16.9	6.0	-14.1
1 mg/kg						
Diff from control	14.8	29.6*	18.9*	28.9*	5.7	15.5
Diff from baseline	24.3	17.3	23.4	12.6	12.3	2.0
10 mg/kg						
Diff from control	33.0	47.9*	34.4*	52.4*	32.4*	48.0
Diff from baseline	25.4	18.5	21.8	19.0	21.9	17.4

Electrocardiographic Evaluation
Noteworthy ECG effects were noted for the 10 mg/kg group at 24 hours post dose, with a statistically significant 11% increase in mean QTc_B (31 msec from baseline and 28 msec from 24-hour control value). At the 24-hour time point (dark cycle), the control value decreased compared with the other time points for this group, whereas the HD group value remained elevated from baseline, similar to the other time points for this group. No other physiologically relevant effects on ECG parameters were noted.

Table 47: Statistical Evaluation of Bazett's-Corrected QT Interval
(sponsor-generated table; milliseconds)

Group	Statistics	Baseline	Overall	6 Hour	12 Hour	24 Hour	36 Hour	72 Hour
1	Mean	348.7	346.8	336.8	360.2	334	355.2	347.7
	N	6	6	6	6	6	6	6
	LSMean	348.7	348.2	338.3	361.6	335.4	356.6	349.1
	LSM s.e.	14.2	6.5	7.5	7.5	7.5	7.5	7.5
2	Mean	375	361.5	357.8	365.5	365.2	359.8	359
	N	6	6	6	6	6	5	6
	LSMean	375	349.4	345.3	353	352.7	349.3	346.5
	LSM s.e.	14.2	7.1	8.1	8.1	8.1	8.3	8.1
	Trend p-value+	NT	NT					
	Bonferroni p-value+		1	1	0.887	0.252	1	1
3	Mean	330.7	354.1	345.3	351.2	362	360.2	351.3
	N	6	6	6	5	6	6	6
	LSMean	330.7	364.7	356.3	361.1	372.9	371.1	362.3
	LSM s.e.	14.2	7	7.9	8.2	7.9	7.9	7.9
	Trend p-value+	0.383	0.103					
	Bonferroni p-value+		0.206	0.206	1	0.002*	0.375	0.461
All	Grp F-test p-val#	0.117	0.218					
Intn	Grp*Time p-val#		0.006*					
	GrpTrend*LinTime p-val+		0.78					
	GrpTrend*QuadTime p-val+		0.85					

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Methods of Analysis
Validated bioanalytical methods (ELISA) were used to quantify the amount of LY2189265 in mouse, rat, rabbit, and monkey plasma collected during toxicology studies. A summary of the validation data and the toxicology studies associated with the validated method is presented in the sponsor-generated table below.

Reference ID: 3527260

Table 2.6.4.2.1.1: Nonclinical Method Validation Data for Dulaglutide in Mouse, Rat, Rabbit, and Monkey Plasma

Method Doc ID/Validation Method Date	Associated Study	Species	Validation Range (ng/mL)	Inter-Assay Precision (%RSD)	Inter-Assay Accuracy (%RE)	Stability data
09-131 01 April 2010	8224143 8224-144	Mouse	2 to 30	2.6 to 13.2	4.6 to 22.7[a]	12 months at -20°C 13 months at -70°C 24 hours at 2 to 8°C and 15 to 30°C 5 f/t cycles
0002294 08 January 2005	R00359	Rat	1.0 to 10	7.53 to 9.97	4.47 to 9.51	70 days at -70°C 4 hours on ice 3 f/t cycles
06-034 28 February 2007	7608-191 7608-236 8203-405 (b) (4)353116	Rat	0.8 to 40.0	4.5 to 15.1	-16.6 to -6.5	6 months at -20°C 24 hours at 2 to 8°C and 20 to 25°C 4 f/t cycles
ICD 373.2 18 January 2011	(b) (4)353253 504758 8236888	Rat	0.8 to 40.0	2.83 to 7.01	-6.24 to 2.27	190 days at -20°C and -80°C 24 hours at room temp 6 f/t cycles
06-070 17 October 2006	(b) (4)-353119	Rabbit	0.5 to 50.0	3.5 to 11.1	-8.8 to 1.2	3 months at -20°C 24 hours at 2 to 8°C and 20 to 25°C 5 f/t cycles
06-033 28 February 2007	7608-235 7608-192	Monkey	0.8 to 40.0	6.4 to 21.3[a]	-17.0 to 7.2	6 months at -20°C 24 hours at 2 to 8°C and 20 to 25°C 4 f/t cycles
0002299 26 January 2005	P00054	Monkey	0.8 to 16	10.84 to 17.34	-2.07 to 5.56	29 days at -70°C 4 hours on ice 3 f/t cycles
MJW4 21 April 2011	8252-199	Monkey	2.00 to 160	2.06 to 3.14	-7.32 to -1.91	184 days at -20°C and -80°C 24 hours at room temp 5 f/t cycles

Abbreviations: Doc ID = Document Identification; f/t = freeze/thaw; LLOQ = lower limit of quantitation; %RSD = percent relative standard deviation; %RE percent relative error.

[a] Result is for the LLOQ (acceptance criteria = 25% for LLOQ and 20% for rest of the concentrations).

Validated methods (surface plasmon resonance [SPR] or ELISA) were used to determine the amount of anti-LY2189265 antibodies produced after repeated dosing in monkeys. In monkey study P00054, the SPR assay that was used had an assay drug tolerance level of ≤0.01 µg/mL and a sensitivity of 0.881 µg/mL anti-LY2189265. Samples from this study were also assessed for anti-GLP-1 antibodies using a validated SPR assay. This assay had a drug tolerance level of ≤0.01 µg/mL and an estimated sensitivity of 1 relative µg/mL. For monkey studies 7608-192 and 7608-235, anti-LY2189265 antibodies were measured by using a validated ELISA method. Assay sensitivity was estimated to be 0.658 µg/mL anti-LY2189265 and drug tolerance was demonstrated to be ≤1 µg/mL for detection of high Ab concentrations (13.5 µg/mL) and ≤0.163 µg/mL for detection of low antibody concentrations (3.4 µg/mL).

Toxicokinetics: Overview

An overview of mean C_{max} and AUC values at various dose levels for mice, rats, rabbits, and monkeys is shown in the sponsor-generated table below. Elimination half-life values were not calculated for mice, rats, or rabbits. The elimination half-life for monkeys was approximately 7 days, which is comparable to the 4.7 day half-life value that was calculated for type 2 diabetics.

	Steady State C_{max} (µg/mL) and AUC (µg•hr/mL)													
	Mice				Rats				Monkey				Rabbits	
Dose	C_{max}		AUC		C_{max}		AUC		C_{max}		AUC		C_{max}	AUC
(mg/kg)	M	F	M	F	M	F	M	F	M	F	M	F	F	F
0.04													0.17[k]	7.05[k]
0.05					0.0914[i]	0.120[i]	4.05[i]	4.07[i]						
0.12													0.56[k]	26.52[k]
0.2						0.497[l]		15.3[l]						
0.3	0.414[h]	0.391[h]	6.76[h]	10.5[h]										
0.41									2.70[e]	2.95[e]	136.67[e]	172.99[e]	1.74[k]	77.94[k]
0.41									3.28[g]	4.33[g]	174.13[g]	236.85[g]		
0.49						0.96[j]		35.00[j]						
0.49						1.04[l]		35.7[l]						
0.5					1.11[i]	1.16[i]	62.4[i]	56.7[i]						
0.5					0.591[m]		21.2[m]							
1	3.25[a]	2.70[a]	118[a]	80.6[a]					8.34[f]	8.59[f]	349.39[f]	370.71[f]		
1	0.645[h]	0.769[h]	24.2[h]	22.4[h]										
1.5					2.88[i]	3.25[i]	162[i]	155[i]						
1.5					1.67[m]		65.4[m]							
1.63					2.92[c]	2.41[c]	114.28[c]	134.00[c]	9.01[e]	13.03[e]	505.93[e]	857.41[e]		
1.63					3.58[d]	2.46[d]	136.90[d]	104.68[d]	10.79[g]	13.94[g]	690.56[g]	747.29[g]		
1.63						2.79[j]		110.06[j]						
1.63						3.95[l]		131[l]						
2					6.30[b]	4.85[b]	237.18[b]	236.54[b]						
3	1.17[h]	1.69[h]	33.1[h]	40.9[h]					25.24[f]	22.26[f]	1137.61[f]	1006.95[f]		
4.89					8.10[c]	7.77[c]	299.72[c]	254.54[c]						
4.89					9.76[d]	13.15[d]	301.08[d]	293.70[d]						
4.89						10.31[j]		348.14[j]						
5					10.0[i]	8.46[i]	366[i]	563[i]						
5					7.1[m]		238[m]							
6					15.10[b]	14.23[b]	749.22[b]	728.18[b]						
8.15									55.64[e]	47.58[e]	3019.54[e]	3048.68[e]		
8.15									74.47[g]	72.25[g]	3412.21[g]	4248.58[g]		
10	32.5[a]	21.5[a]	1394[a]	896[a]					93.00[f]	81.81[f]	4624.89[f]	3217.42[f]		
16.29					22.32[c]	30.87[c]	1042.34[c]	1318.94[c]						
16.29					37.55[d]	33.56[d]	1203.26[d]	1386.72[d]						
20					37.81[b]	37.00[b]	1774.14[b]	1673.20[b]						
100	264[a]	193[a]	9292[a]	8100[a]										

Abbreviations: AUC = area under the concentration time curve; C_{max} = maximum observed concentration; F = female; M = male.

[a] C_{max} and $AUC_{0\text{-}96hr}$ at Day 29 (Study 8224143).
[b] C_{max} and $AUC_{0\text{-}last}$ at Day 32 (Study R00359)
[c] C_{max} and $AUC_{0\text{-}last}$ at Day 92 (Study 7608-191).
[d] C_{max} and $AUC_{0\text{-}last}$ on Day 183 (Study 7608-236).
[e] C_{max} and $AUC_{0\text{-}96hr}$ on Day 92 (Study 7608-192).
[f] C_{max} and $AUC_{0\text{-}last}$ on Day 28 (Study P00054).
[g] C_{max} and $AUC_{0\text{-}96hr}$ on Day 274 (Study 7608-235).
[h] C_{max} and $AUC_{0\text{-}96hr}$ on Day 176 (Study 8224-144).
[i] C_{max} and $AUC_{0\text{-}96hr}$ on Week 52 (Study 8203-405).
[j] C_{max} and $AUC_{0\text{-}96hr}$ on Gestation Day 15 (Study (b) (4)353116).
[k] C_{max} and $AUC_{0\text{-}last}$ on Gestation Day 19 (Study (b) (4)353119); parameters reported exclude animals with likely antibody formation.
[l] C_{max} and $AUC_{0\text{-}last}$ on Lactation Day 4 (Study (b) (4)353253).
[m] C_{max} and $AUC_{0\text{-}96hr}$ at Day 88 (Study 504758).

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: A repeat-dose toxicity and toxicokinetic study in 001178-W (wild type) mice given LY2189265 twice weekly by subcutaneous injection for 4 weeks

Key study findings:

- There were no unscheduled deaths. One HD male and 2 HD females were noted as being hunched on Day 4.
- Mean body weight gain was statistically significantly decreased at the MD and HD. This effect was most severe during the first week of treatment, which correlated with decreased food consumption. After Week 1, weight gain was still decreased for MD and HD animals compared with controls, but not to the same extent as during Week 1, which was consistent with food consumption being more similar to controls after Day 4.
- Slight dose-related decreases in erythrocyte parameters (up to 12%) were noted for males. A decrease in reticulocytes was noted for all treated animals, up to 47% for HD females.
- Increased mean AST (↑270%) and ALT (↑272%) were noted for HD females, which was primarily due to increases for 2/5 animals. A slight, statistically significant increase in ALP was noted for MD (↑23%) and HD (↑37%) males.
- Statistically significant decreases in absolute and relative liver weights were noted for MD and HD animals. Statistically significant decreases in absolute and relative heart weights were noted for MD animals and HD females. Statistically significant decrease in absolute and relative uterus weights was noted for HD females.
- Microscopically, decreased vacuolation (glycogen content) was noted in the liver for MD and HD animals; decreased granulation of the granular secretory duct epithelium of the mandibular salivary gland was noted for HD females; decreased zymogen granules in the pancreas was noted for MD and HD males; decreased dilation and increased atrophy were noted in the uterus of MD and HD females; and increased inflammation and erosion/ulceration was noted at the injection site for MD males and females.
- Exposure increased with dose in a near linear fashion. Mean exposures were higher on Day 29 compared with Day 1 suggesting either accumulation because of the long half-life or possibly because of antibody production (observed with other GLP-1R agonists). However, Ab production was not assessed because the pharmacological effect on body weight gain and food consumption was maintained throughout the dosing period.
- Most findings appeared to be secondary to decreased body weight gain, which is an expected pharmacological effect of the drug. Although increases in AST and ALT were observed, adverse microscopic findings were not identified in the liver. In the absence of definitive drug-induced systemic toxicity, the NOAEL for this study was considered to be 100 mg/kg twice weekly. This dose level resulted in mean AUC_{0-96} values of 9292 µg•h/mL in males and 8100 µg•h/mL in females on Day 29.

Reference ID: 3527260

Study number: 8224143
Report location: Module 4.2.3.2
Conducting laboratory: [redacted] (b) (4)
Date of study initiation: 02 June 2010
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: LY2189265, Lot #PP0105-09 (drug substance), Lot #17804-1 (final bulk API), 87.7% pure, 40.3 mg/mL potency/content
Formulation: [redacted] (b) (4) citrate (pH [redacted] (b) (4) and [redacted] (b) (4) polysorbate 80 (the process description for formulating the drug does not mention that mannitol was added, but it is present in the vehicle control).
Vehicle control: [redacted] (b) (4) citrate (pH [redacted] (b) (4) mannitol (w/v), and [redacted] (b) (4) polysorbate 80.

Methods

Species/strain: Mouse/ Model 001178-W, CByB6F1-Tg(HRAS)2Huc (wild type for Tg.rasH2 transgenic)

Study design: (sponsor-generated table)

Group	Number of Animals Male	Number of Animals Female	Dose Level (mg LY2189265/kg/dose)[a]	Dose Concentration (mg LY2189265/mL)[a]
Toxicity Animals				
1 (Control)[b]	10	10	0	0
2 (Low)	10	10	1	0.1
3 (Mid)	10	10	10	1
4 (High)	10	10	100	10
Toxicokinetic Animals[c,d]				
5 (Control)[b]	8	8	0	0
6 (Low)	38	38	1	0.1
7 (Mid)	38	38	10	1
8 (High)	38	38	100	10

a The dose volume was10 mL/kg. Concentrations were corrected for lot specific test article content.
b Animals in Groups 1 and 5 received only [redacted] (b) (4) sodium citrate [redacted] (b) (4) pH [redacted] (b) (4) [redacted] (b) (4) mannitol (w/v), and [redacted] (b) (4) w/v) polysorbate 80, prepared in sterile water for injection, which served as the vehicle for LY2189265 formulations.
c Toxicokinetic animals were included solely for the purpose of blood sample collections.
d Groups 6 through 8 included 2 animals/sex for immunogenicity analysis.

Recovery: None
Route and regimen: Subcutaneous, twice weekly
Dosing volume: 10 mL/kg
Age: 7 weeks
Weight: 21.4 to 27.2 g (males) and 17.4 to 23.6 g (females)
TK sampling times: Control - predose and 24 hours postdose on Days 1 and 29. (2 mice/sex/time point)
Treated - predose, 4, 12, 24, 48, and 96 hours postdose on Days 1 and 29 and at 24 hours postdose on Days 8, 15, and 22. (2 mice/sex/time point)
Ab sampling times: 96 hours postdose on Day 29 (2/sex for Groups 6, 7, and 8)

Dose formulation analysis: Week 1 preparations were assessed for concentration. The results showed that concentrations were within 1% of target.

Observation and Times:

Clinical signs: Twice daily
Detailed exams: Once weekly
Body weights: Twice weekly
Food consumption: Twice weekly (quantitatively)
Ophthalmoscopy: Predose (all groups) and during Week 5 (toxicity groups)
Hematology: First 5 animals/group from nonfasted toxicity animals
Clinical chemistry: Second 5 animals/group from nonfasted toxicity groups
Urinalysis: Not conducted
Gross pathology: All toxicity animals
Organ weights: All toxicity animals; see histopathology table at end of section
Histopathology: Control and high-dose toxicity animals only, except for the following organs that were examined for all groups based on findings at the high dose; see histopathology table at end of section for tissue collection list:
Females: liver and uterus
Males: liver and pancreas
Adequate Battery: Yes
Peer review: Yes, performed by sponsor on 3 mice/sex from control and high-dose groups. Additionally, liver and pancreas from all mice and ovary, uterus, cervix, and vagina from all female mice in all dose groups were examined.

Results:

Mortality: There were no unscheduled deaths.

Clinical signs: One HD male and 2 HD females were noted as being hunched on Day 4. Mice from the LD (1 male and 1 female) and the HD groups (4 males and 5 females) had sores/scabs on the dorsal abdomen (back), whereas no control or HD animals were noted as having this finding.

Body Weight Gain: Day 1 to Day 32

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Weight (g): Day 1	24.7	19.6	24.6	20.1	24.5	19.2	24.1	19.4
Weight (g): Day 32	27.8	23.3	27.7	23.9	25.5*	20.3*	24.5*	19.6*
Weight gain (g)	3.1	3.7	3.1	3.8	1.0*	1.1*	0.4*	0.2*
Diff from control (g)			0	0.1	-2.1	-2.6	-2.7	-3.5
% diff from control			-	↑3%	↓68%	↓70%	↓87%	↓95%

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

Body Weight Gain: Day 8 to Day 32

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Weight (g): Day 8	25.4	20.5	25.4	21.5	24.0	19.1	22.3	18.0
Weight (g): Day 32	27.8	23.3	27.7	23.9	25.5*	20.3*	24.5*	19.6*
Weight gain (g)	2.4	2.8	2.3	2.4	1.5	1.2	2.2	1.6
Diff from control (g)			-0.1	-0.4	-0.9	-1.6	-0.2	-1.2
% diff from control			↓4%	↓14%	↓38%	↓57%	↓8%	↓43%

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

Figure 1
Mean Body Weight - Males

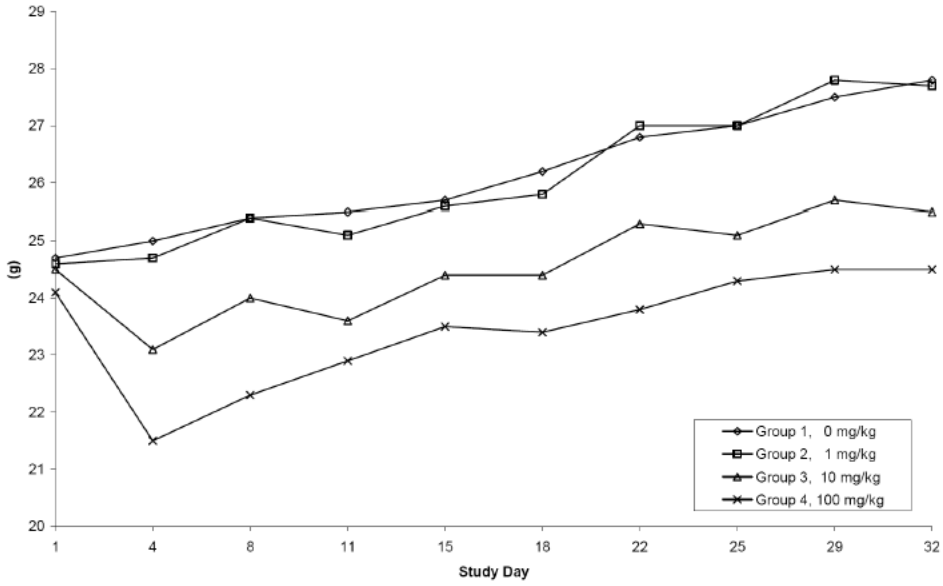
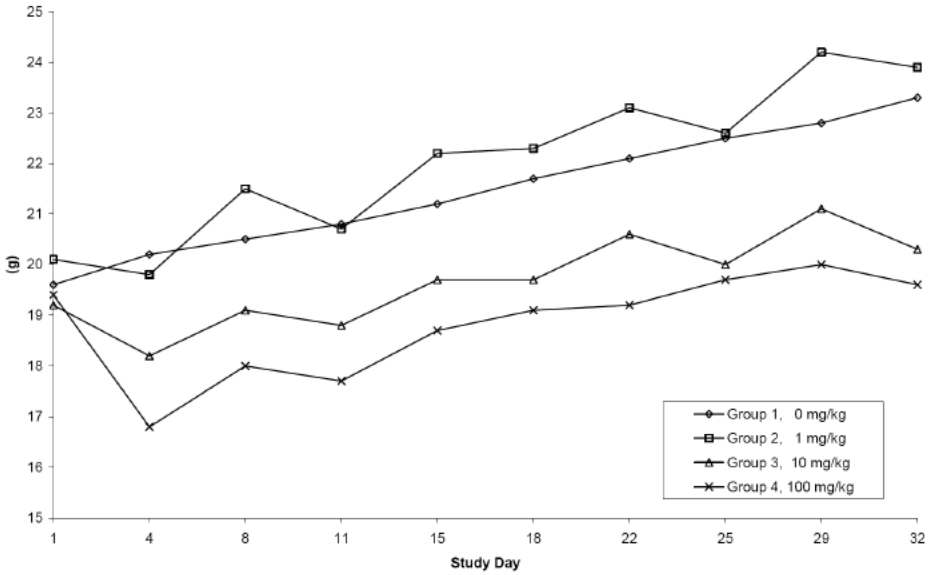


Figure 2
Mean Body Weight - Females



Food consumption: Day 1 to Day 3

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Daily intake (g/mouse)	15.9	13.5	11.9*	9.9*	9.0*	7.8*	6.4*	6.1*
Diff from control (g)			-4.0	-3.6	-6.9	-5.7	-9.5	-7.4
% diff from control			↓25%	↓27%	↓43%	↓42%	↓60%	↓55%

F = female; M = male.
*p≤0.05

Food consumption: Day 4 to Day 31

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Daily intake (g/mouse†)	18.8	17.5	18.7	18.1	18.1	16.8	16.3	14.0*
Diff from control (g)			-0.1	0.6	-0.7	-0.7	-2.5	-3.5
% diff from control			↓1%	↑3%	↓4%	↓4%	↓13%	↓20%

F = female; M = male.
†mean of the eight 3 or 4-day food measurement periods between Days 4 and 31.
*p≤0.05

Figure 3
Mean Food Consumption - Males

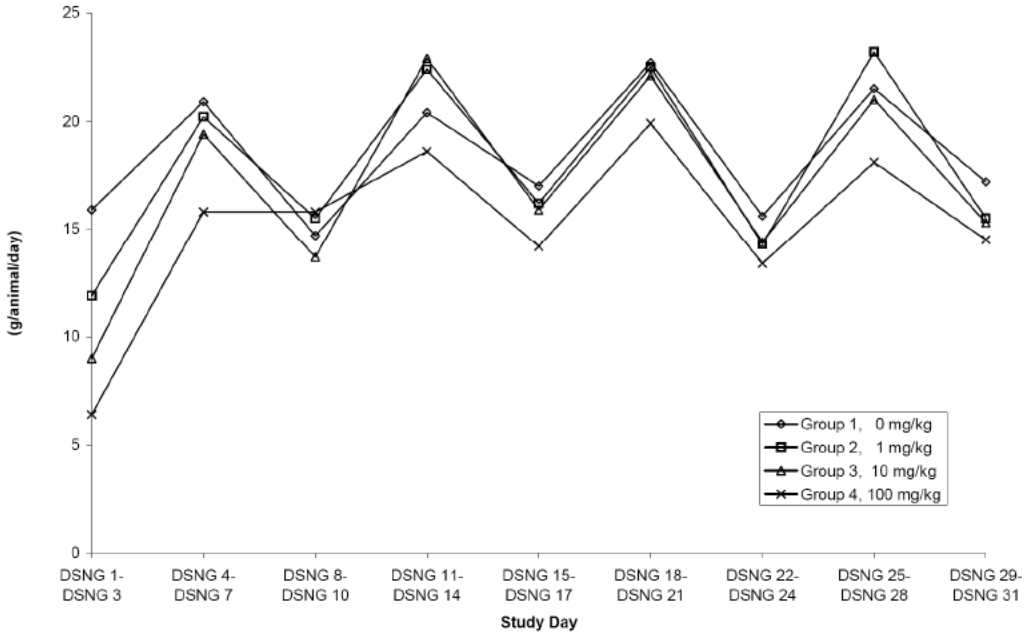
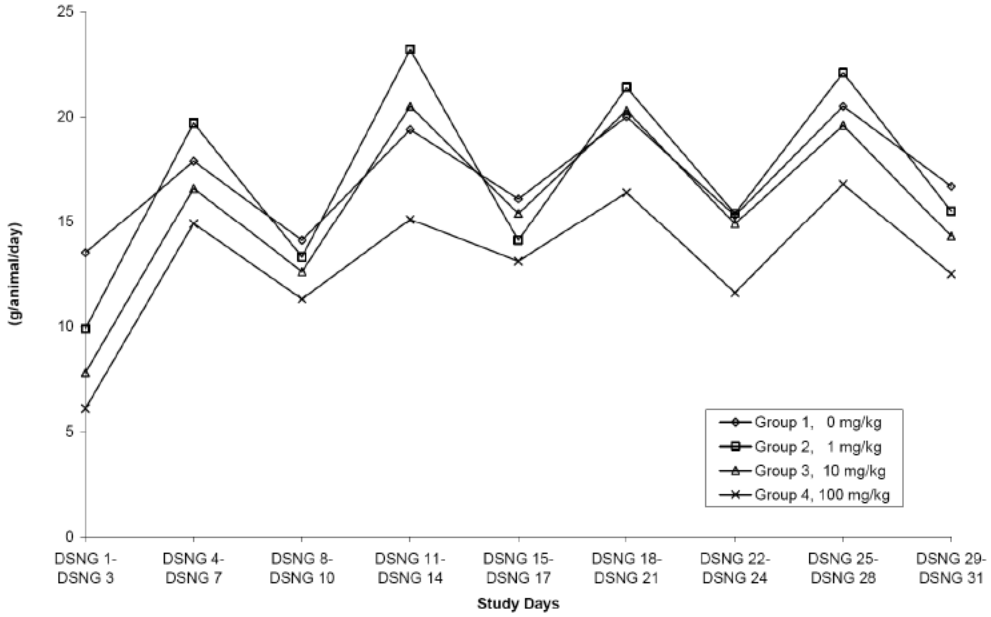


Figure 4
Mean Food Consumption – Females



Ophthalmoscopy: There were drug-related findings noted.

Hematology:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Red blood cells (1×10^6/µL)	10.60	9.84	9.81*	9.27*	9.71*	9.33*	9.55* (↓10%)	9.81
Hemoglobin (g/dL)	16.0	15.3	14.8*	14.3*	14.6*	14.2*	14.1* (↓12%)	14.8
Hematocrit (%)	51.0	50.2	48.3	46.5	47.5	46.0*	46.5* (↓9%)	47.9*
Mean cell hemoglobin conc. (g/dL)	31.5	30.5	30.7	30.8	30.8	30.9	30.5 (↓3%)	31.0
Reticulocytes (1×10^3/µL)	312.3	329.9	244.7*	250.1	248.8	201.6*	250.4 (↓20%)	176.3* (↓47%)
White blood cells (1×10^3/µL)	3.61	1.76	3.09	2.68	2.80	4.00*	2.12 (↓41%)	2.22
Neutrophils (1×10^3/µL)	0.46	0.28	0.59	0.39	0.42	0.66*	0.30 (↓35%)	0.42
Lymphocytes (1×10^3/µL)	2.97	1.36	2.28	2.11	1.75*	3.03*	1.65* (↓44%)	1.63

*p≤0.05; F = female; M = male.

Clinical chemistry:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Glucose (mg/dL)	190	199	140	182	138	138	160	176
AST† (U/L)	-	44	-	53	-	50	-	163*† (↑270%)
ALT† (U/L)	-	18	-	26	-	19	-	67† (↑272%)
ALP (U/L)	82	-	96	-	101* (↑23%)	-	112* (↑37%)	-

*p<0.05; **p<0.01; "-" = no noteworthy difference from control; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; F = female; M = male.
†Mean increase due to individual increase for 2/5 animals.

Gross pathology:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Uterus								
-Distended		1/8		4/5		1/5		0/9
-Thickened		1/8		0/5		0/5		0/9

Organ weights:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Liver/gall bladder (g)	1.439	1.217	1.453	1.292	1.194*	1.056*	1.110* (↓23%)	0.940* (↓23%)
Rel. to BW	5.253	5.359	5.196	5.260	4.582*	4.883	4.463* (↓15%)	4.728* (↓12%)
Rel. to BrW	288.42	234.17	294.95	260.06	245.75*	219.17	233.57* (↓19%)	196.06* (↓16%)
Heart (g)	0.183	0.167	0.179	0.154	0.151*	0.137*	0.165	0.126* (↓31%)
Rel. to BW	0.670	0.735	0.640	0.631*	0.580*	0.639*	0.662	0.635* (↓14%)
Rel. to BrW	36.74	32.08	36.411	31.074	31.136*	28.571	34.654	26.354* (↓18%)
Uterus (g)		0.188		0.242		0.166		0.106* (↓44%)
Rel. to BW		0.825		0.982		0.764		0.533 (↓35%)
Rel. to BrW		36.152		48.648		34.579		22.243 (↓38%)

*p<0.05; **p<0.01; "-" = no noteworthy difference from control; BrW = brain weight; BW = body weight; F = female; M = male; NA = not applicable.

Histopathology:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Liver -Decreased vacuolation (glycogen content)	0/10	0/10	0/10	0/10	**2/10**	**2/10**	**5/10**	**3/10**
Salivary gland (mandibular) -Decreased granulation; granular secretory duct epithelium	0/10	0/10	NE	NE	NE	NE	0/10	**2/10**
Pancreas -Decreased zymogen granules	0/10	0/10	0/10	NE	**5/10**	NE	**6/10**	0/10
Uterus -Dilation -Atrophy	NA	4/10 0/10	NA	6/10 0/10	NA	1/10 2/10	NA	1/10 4/10
Subcutan site -Acute inflammation -Erosion/ulceration -Serocellular exudate	0/10 0/10 0/10	1/10 0/10 0/10	NE	NE	2/3 1/3 1/3	2/2 2/2 2/2	0/10 0/10 0/10	0/10 0/10 0/10

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

Toxicokinetics:

Parameter	Administered Dose (mg/kg)					
	1		10		100	
Sex	M	F	M	F	M	F
LY2189265						
Day 1						
Cmax (µg/mL)	2.87	2.87	27.2	24.5	180	170
$AUC_{0\text{-}96hr}$ (ug·hr/mL)	62.0	62.4	724	604	6385	5379
SEM $AUC_{0\text{-}96hr}$ (ug·hr/mL)	2.06	3.01	18.0	7.98	1359	1162
Day 29						
Cmax (µg/mL)	3.25	2.70	32.5	21.5	264	193
$AUC_{0\text{-}96hr}$ (ug·hr/mL)	118	80.6	1394	896	9292	8100
SEM $AUC_{0\text{-}96hr}$ (ug·hr/mL)	5.05	2.07	43.0	89.1	584	415

Immunogenicity:

Samples collected for the assessment of anti-drug antibodies were discarded without analysis because analysis was not warranted based on the exposure data.

Study title: Repeat-dose toxicology and toxicokinetic studies in Fischer 344 rats given LY2189265 twice weekly by subcutaneous injection for a total of 10 doses (Report R00359)

In this GLP-compliant study, vehicle or 2, 6, or 20 mg/kg LY2189265 was administered twice weekly by subcutaneous injection to Fischer 344 rats (10/sex/group) for 5 weeks. During the treatment period, animals were evaluated for adverse effects on clinical signs, body weight, food consumption, and eyes (ophthalmoscopy). At the end of the study, samples were collected for hematology, coagulation, serum chemistry, and urinalysis. At necropsy, all surviving animals underwent gross examination and selected organs were weighed. Histopathology was conducted on a complete list of tissues for control and HD groups. Potential target organs were also evaluated in the MD and LD animals. Samples for toxicokinetics were collected predose and at 4, 12, 24, 48, and 96 hours postdose on Day 0 and 32 (9/sex/group; 3/sex/time point).

One male rat in the 20 mg/kg group was found dead 2 days after the first dose, which was attributed to non-drug-related duodenal and gastric ulceration. The primary drug-related effect was an initial body weight loss followed by decreased body weight gain at all dose levels that correlated with decreased food consumption. Histopathology revealed an increased incidence of seminiferous tubule atrophy, bilateral epididymal luminal cell debris, oligospermia, and decreased prostate and seminal vesicle secretion in males given 20 mg/kg; an increased incidence of Diestrus II in 20 mg/kg females; and thymic atrophy in males given 6 or 20 mg/kg. The sponsor stated that these changes were consistent with a generalized stress response due to decreased food intake and weight loss (Levin et al. 1991; Yuan 1991) and often correlated with decreased organ size and weight, but none of these findings were considered adverse. The target organs of toxicity included the epididymis, prostate, seminal vesicle, testes, thymus, and vagina. TK results are presented in the sponsor-generated table below.

The sponsor concluded that the NOAEL was 20 mg/kg; however, FDA reviewers felt that the treatment-related effects on body weight and reproductive organs at 20 mg/kg were adverse, and therefore, the NOAEL was considered to be 6 mg/kg.

Toxicokinetics

Dose (mg/kg):	2	6	20
Parameter Sex:	M + F	M + F	M + F
Day 0			
C_{max} (µg/mL)	5.34	11.90	31.18
T_{max} (h)	12	12	12
$AUC_{0\text{-last}}$ (µg•h/mL)	170.13	434.55	1103.39
Day 32			
C_{max} (µg/mL)	5.58	14.67	37.41
T_{max} (h)	12	14	12
$AUC_{0\text{-last}}$ (µg•h/mL)	236.86	738.70	1723.67

Abbreviations: M + F = males and females combined, C_{max} = maximum plasma concentration, T_{max} = time to achieve C_{max}, $AUC_{0\text{-last}}$ = area under the plasma concentration curve from 0 to last time point.

Reference ID: 3527260

Study title: A repeat-dose toxicology study in Cynomolgus monkeys given LY2189265 twice weekly by subcutaneous injection for a total of 9 doses (Report P00054, Amendment 1)

In this GLP-compliant study, Cynomolgus monkeys (3/sex/group) were dosed twice weekly by subcutaneous administration with vehicle or 1, 3, or 10 mg/kg LY2189265 for 1 month (9 doses). All monkeys survived until scheduled necropsy. During the treatment period, animals were evaluated for adverse effects on clinical signs, body weight, food consumption, and eyes (ophthalmoscopy). Neurobehavioral and cardiovascular (ECG only) safety pharmacology endpoints were incorporated into the study. Neurological assessments were made pretreatment and after 1 (Day 0), 4 (Day 11), and 9 (Day 28) doses. ECG assessments were taken under sedation prior to dosing and approximately 2 hours after dosing on Days 4 and 28. Samples were collected for hematology, coagulation, and serum chemistry twice prior to initiation of dosing and prior to dosing on Days 0, 14, and 28. Urine samples were collected prior to initiation of dosing and near the end of the live phase. Blood samples were also collected before dosing on Day 0, on Day 15 (~24 hours after the Day 14 dose), and on Day 32 (~96 hours after the Day 28 dose for complement activation assessments (Bb and C3a). At necropsy, all surviving animals underwent gross examination and selected organs were weighed. Histopathology was conducted on a complete list of tissues for all study animals. Samples for toxicokinetics were collected predose and at 2 (Day 0 only), 4, 12, 24, 48, and 96 hours postdose on Days 0 and 28.

A slight increase in the incidence of vomiting occurred in the drug-treated groups. LY2189265 caused minimal weight loss in males given 1 mg/kg and moderate weight loss in monkeys given 3 or 10 mg/kg during the first 2 weeks of treatment. Dose-related decreases in food consumption occurred in all compound-treated groups, but were generally limited to the first week of the study. After the first dose, a single 10 mg/kg female exhibited abnormal gait (dragging of hind limb), curling of toes, and decreased skin elasticity. After repeated dosing, the signs for this female were consistent, but of lesser frequency. Starting approximately 24 hours after the first dose, a single 3 mg/kg group female exhibited decreased activity, grip strength, muscle strength, motor coordination, skin elasticity, and body temperature, as well as increased responsiveness to handling, light sensitivity, and vomiting. The signs were first noted approximately 24 hours after dosing and progressed through 96 hours after dosing. This female required supportive care (subcutaneous fluids and heat lamp) and did not receive the second dose. The third dose was administered and symptoms were milder on Days 8 and 9 (only hunched posture). The sponsor contended that the effects in these two female monkeys were not related to the test article because the severity did not occur in a dose-related manner and occurred in only one animal per group. However, given the known effects of GLP-1R agonists on food and water consumption and vomiting, it is likely that these effects were secondary to dehydration and decreased food consumption.

Increases in rate-corrected QT interval (QTcL; Lilly correction method) in relation to control group values occurred in all drug-treated groups. The increases were

Reference ID: 3527260

statistically significant at 3 of 4 time points for the 3 and 10 mg/kg groups and at a single time point for the 1 mg/kg group. Prior to dosing and 2 hours after dosing on Day 4, the effects did not occur in a dose-related manner and the mid-dose group had a similar value as on Day -16. The largest difference from control or baseline on Day 4 occurred for LD males (+59 ms and +32 ms, respectively). Prior to dosing on Day 28, the MD group had the largest difference from control, although the mean value was similar to the Day -16 baseline value for this group. The most noteworthy change on Day 28 was for HD females, which had a 34 ms increase from control and a 30 ms increase from Day -16. Males had a similar value as baseline and a 19 ms increase from control. Two hours after dosing on Day 28, mean values from all groups were higher than control; however, most values were similar to Day-16 baseline values. MD females had a 34 ms and 19 ms increase and HD females had a 58 ms and 45 ms increase from control and baseline, respectively.

The immunogenicity of LY2189265 could not be accurately assessed because the plasma LY2189265 concentrations in the compound-treated groups on Day 32 (Day 28 + 96 hours) were above the interference level of the assay. There were no compound-related differences in C3a and Bb complement split products by enzyme ELISA. Microscopic findings included the bone marrow (hypocellularity, atrophy of fat [≥3 mg/kg for females]), mesenteric, submandibular lymph nodes, spleen, thymus (lymphoid depletion [all doses]), and injection sites (inflammation [all doses], hemorrhage [1 HD male]). These effects, other than injection site findings, were considered to be secondary to the effects on food consumption and body weight. The NOAEL was considered to be less than 1 mg/kg based on the prolonged QTc observed at all dose levels.

Toxicokinetics

Dose (mg/kg):	1	3	10
Parameter Sex:	M + F	M + F	M + F
Day 0			
C_{max} (µg/mL)	5.26[a]	11.37	52.97
T_{max} (h)	5.60[a]	10.00	10.67
$AUC_{0\text{-last}}$ (µg•h/mL)	224.40[a]	403.45	1999.90
Day 28			
C_{max} (µg/mL)	8.47	23.75	87.41
T_{max} (h)	8.00	6.67	10.67
$AUC_{0\text{-last}}$ (µg•h/mL)	360.05	1072.28	3921.16

Abbreviations: M + F = males and females combined, C_{max} = maximum plasma concentration, T_{max} = time to achieve C_{max}, $AUC_{0\text{-last}}$ = area under the plasma concentration curve from 0 to last time point.

[a] Data represent results of n = 5 animals. Study Day 0 data for Group 02 Animal 100202 were not included in calculations of mean or SD due to incomplete dosing.

Study title: A repeat-dose toxicology and toxicokinetic study in Sprague Dawley rats given LY2189265 twice weekly by subcutaneous injection for a total of 27 doses

Study number: 7608-191, Amendment 2
Report location: Module 4.2.3.2
Conducting laboratory: (b) (4)
Date of study initiation: 19 June 2006
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: LY2189265, Lot # (b) (4) C1301-78, 57.6 mg/mL
Formulation/vehicle: Trisodium citrate dehydrate, citric acid anhydrous, sodium chloride, and polysorbate 80.

Key study findings:

- There were no test article-related deaths.
- Decreased feces, thinness, and rough haircoat were noted for HD males and females.
- Body weights were statistically significantly decreased from control values at all dose levels for both males (↓15% to 21%) and females (↓10% to 13%). The effects on body weight generally associated with decreased food consumption.
- A statistically significant decrease in reticulocytes (up to 30% decrease) was noted for males at all doses. A slight decrease was also noted for HD females.
- Absolute and relative mean testis weights were slightly decreased by 4% to 5% in HD males.
- Microscopically, minimal to slight degeneration of the seminiferous tubules (2/10), slight inflammation of the epididymis (1/10), and minimal hypospermia (1/10) were noted for HD males.
- Exposure increased in an approximate dose-proportional manner and no significant accumulation was noted after repeated dosing.
- Because no overt toxicity was observed, the NOAEL was determined to be the high dose level of 16.3 mg/kg twice weekly; at this dose level, the mean C_{max} and $AUC_{0\text{-last}}$ values at Day 92 were 32.6 µg/mL and 1450 µg·h/mL, respectively.

Methods

Species/strain: Rat / Sprague Dawley
Administration route: Subcutaneous
Frequency of dosing: Twice weekly
Age: 6 to 7 weeks
Weight: 218 to 279 g (males); 161 to 213 g (females)
Recovery groups: None
TK sampling times: Days 1 and 92 – predose (Day 92 only) and 4, 12, 24, 48, and 96 hours postdose.
Ab sampling times: Immunogenicity not assessed
Protocol deviations: No GLP or protocol deviations occurred that impacted the validity or interpretability of the study data. After the study report was finalized, it was determined that

Reference ID: 3527260

the extinction coefficient of the test article was different than previously calculated resulting in lower administered dose levels than planned. The actual dose levels were 0, 1.63, 4.89, and 16.3 mg/kg rather than 2, 6, and 20 mg/kg as planned.

Study design: (sponsor-generated table)

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg LY2189265/kg/dose)[a]	Dose Concentration (mg LY2189265/mL)[a]
Toxicity Animals				
1 (Control)[b]	10	10	0	0
2 (Low)	10	10	1.63	1.63
3 (Mid)	10	10	4.89	4.89
4 (High)	10	10	16.29	16.29
Toxicokinetic Animals[c]				
5 (Control)[b]	5	5	0	0
6 (Low)	11	11	1.63	1.63
7 (Mid)	11	11	4.89	4.89
8 (High)	11	11	16.29	16.29

a The dose volume was 1 mL/kg.
b Animals in Groups 1 and 5 received the control article only.
c Toxicokinetic animals were included solely for the purpose of blood sample collections. Two animals/sex/group served as replacement animals but were not needed.

Results:

Mortality: (Twice daily)

Two deaths occurred in the high-dose female group during or following blood collection during week 4, which were not considered to be test article-related because they coincided with clinical pathology blood sampling. There were no significant macroscopic or microscopic findings noted in these 2 animals.

Clinical signs: (Cage side - twice daily; Detailed observations – predosing, Day 1, and once weekly thereafter)

Decreased feces (10/10 M; 10/10 F), thin (1/10 M; 9/10 F), and rough haircoat (4/10 M; 3/10 F) were noted in high dose animals and decreased feces (2/10 F) was noted at the mid dose.

Body weights: (Pre-dosing and before each dose administration)

Decreased body weight gain was noted in all test article-treated groups.

Dose (mg/kg)	0		1.6		4.9		16.3	
Sex	M	F	M	F	M	F	M	F
Day 1 (g)	244	181	244	181	243	183	244	179
Day 92 (g)	614	313	519*	282*	516*	287*	485*	271*
Weight gain (g)	370	132	275*	101*	273*	104*	241*	92*
Final weight - diff from control (g)	-	-	-95	-31	-98	-26	-129	-42
Final weight - % diff from control	-	-	↓15%	↓10%	↓16%	↓8%	↓21%	↓13%

* $p \leq 0.05$ (ANOVA)

Figure 1: Mean body weight data - males.

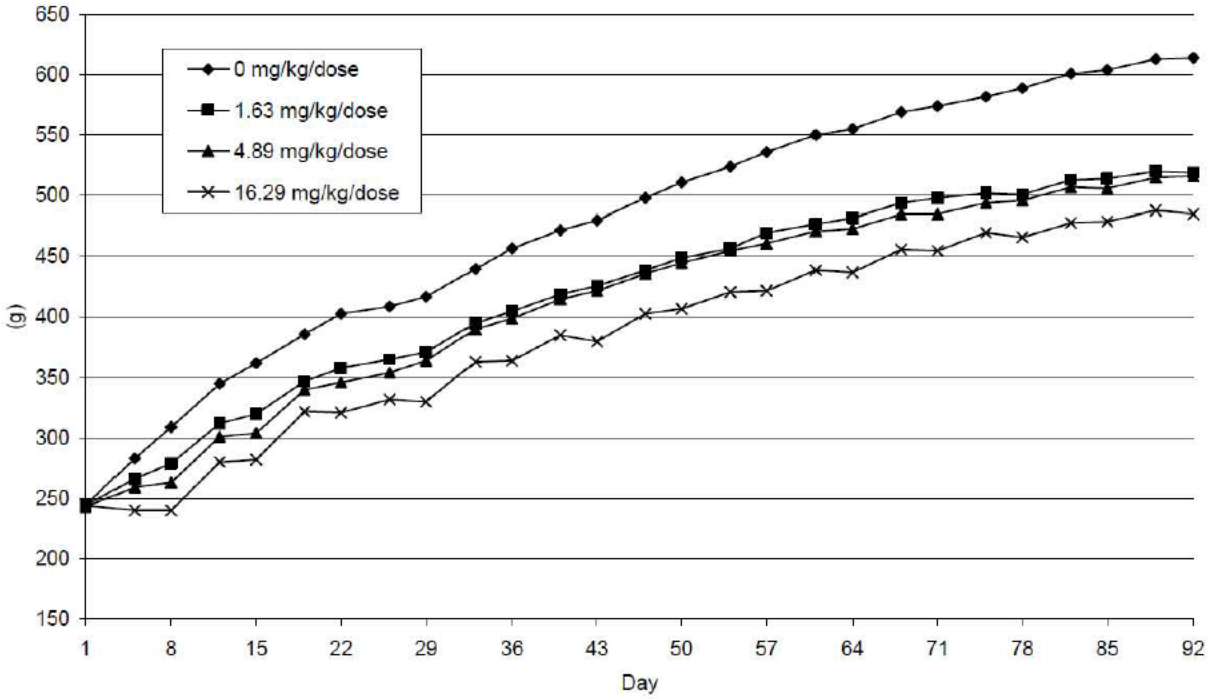
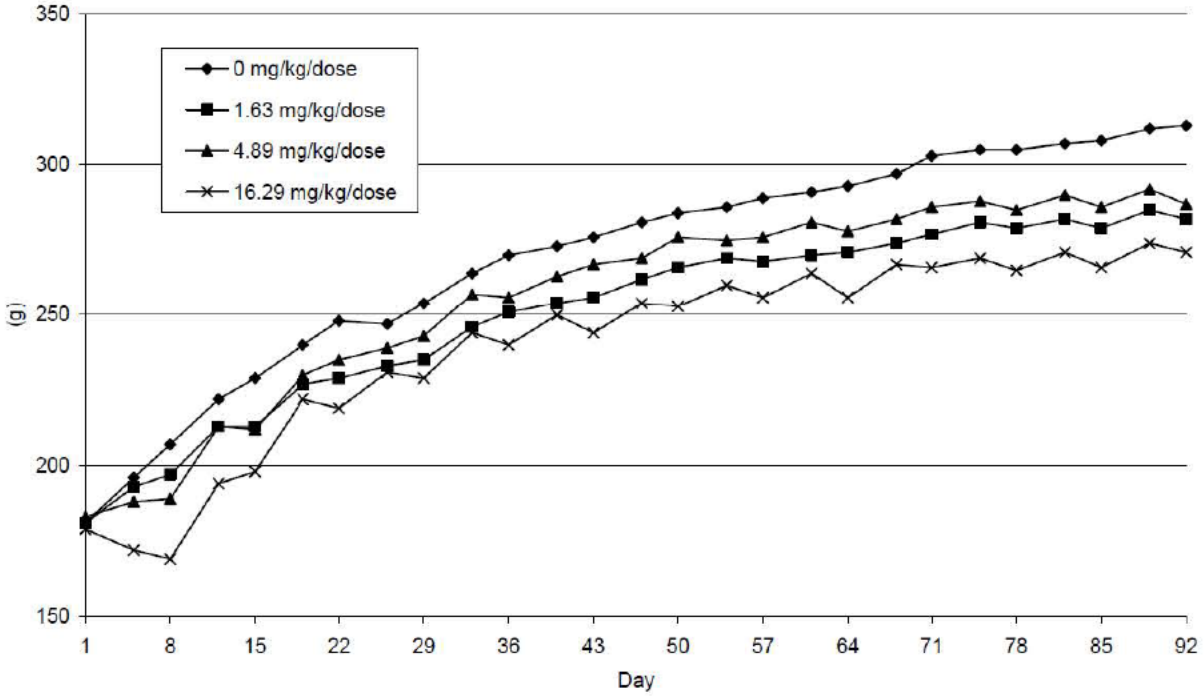


Figure 2: Mean body weight data - females.



Food consumption: (Twice weekly)
Decreased food consumption was observed in all test article-treated groups.

	% Change from Control					
	Males			Females		
mg LY2189265/kg/dose	1.63	4.89	16.29	1.63	4.89	16.29
Days 1-4 (4-day interval)	↓23*	↓30*	↓49*	↓21*	↓33*	↓52*
Days 5-7 (4-day interval)	↓25*	↓37*	↓51*	↓21*	↓32*	↓51*
Days 85-88 (3-day interval)	↓12*	↓12*	↓13*	NS	NS	NS
Days 89-91 (4-day interval)	↓18*	↓15*	↓17*	NS	NS	↓14*

Abbreviations: ↓ = decrease; NS = not significant.
* = $p \leq 0.05$; statistical significance based on actual data, not on percent change from control.

Ophthalmoscopy: (Pre-dose and once during the final week of dosing)
No treatment-related effects noted.

Hematology: (Assessed during Weeks 4 and 13)
Day 96 values

Dose (mg/kg)	0		1.6		4.9		16.3	
Sex	M	F	M	F	M	F	M	F
RBC (1×10^6/µL)	9.15	-	8.85	-	8.81	-	8.57* (↓6%)	-
Hgb (g/dL)	16.6	-	16.1	-	16.1	-	15.9 (↓4%)	-
Hct (%)	49.8	-	47.7	-	48.2	-	47.3 (↓5%)	-
Reticulocytes (1×10^3/µL)	229.3	196.8	180.7* (↓21%)	190.9 (↓3%)	183.3* (↓20%)	182.9 (↓7%)	161.6* (↓30%)	169.7 (↓14%)
Reticulocytes (%)	2.5	2.4	2.0* (↓20%)	2.3 (↓4%)	2.1* (↓16%)	2.2 (↓8%)	1.9* (↓24%)	2.0 (↓17%)

*$p \leq 0.05$ (ANOVA)

Clinical chemistry: (Assessed during Weeks 4 and 13)
Day 96 values

Dose (mg/kg)	0		1.6		4.9		16.3	
Sex	M	F	M	F	M	F	M	F
Glucose (mg/dL)	108	102	90* (↓17%)	93 (↓9%)	93* (↓14%)	89 (↓13%)	88* (↓19%)	93 (↓9%)
Total Protein (g/dL)	7.3	8.1	6.8* (↓7%)	7.5* (↓7%)	6.9 (↓5%)	7.4* (↓9%)	6.9 (↓5%)	7.1* (↓12%)
Cholesterol (mg/dL)	88	88	59* (↓33%)	61* (↓31%)	62* (↓30%)	55* (↓38%)	66* (↓25%)	54* (↓39%)
Triglycerides (mg/dL)	62	-	47 (↓24%)	-	37* (↓40%)	-	39 (↓37%)	-
Phosphorus (mg/dL)	-	6.2	-	6.6	-	7.1	-	7.4 (↑19%)
Potassium (mmol/L)	-	5.3	-	5.4	-	5.5	-	5.7 (↑8%)

Urinalysis: (Assessed during Weeks 4 and 13)
No treatment-related urinalysis findings were observed.

Gross pathology: (Conducted on all main study animal)
No treatment-related macroscopic findings were noted at necropsy.

Organ weights: (Conducted on all main study animals)
There were dose-dependent decreases in weights of several tissues due to treatment-related decreases in body weight. A slight decrease in testis weight may have occurred independent of the effects on body weight.

Dose (mg/kg)	0		1.6		4.9		16.3	
Sex	M	F	M	F	M	F	M	F
Testes (g)	3.647	NA	3.575	NA	3.679	NA	3.452 (↓5%)	NA
Testes (%) (relative to brain)	166.71	NA	165.47	NA	169.31	NA	159.64 (↓4%)	NA

NA = not applicable

Histopathology: (Conducted on all main study animals)

Adequate Battery: Yes - typical organs not evaluated included: lachrymal gland and rectum.

Peer review: Yes - all tissues from the 1st 3 animals of both sexes from control and high dose groups.

Dose (mg/kg)	0		1.6		4.9		16.3	
Sex	M	F	M	F	M	F	M	F
Testes (Number of animals affected)	0	NA	0	NA	0	NA	2	NA
Degeneration, seminiferous tubules – minimal to slight	0	NA	0	NA	0	NA	2	NA
Epididymis (Number of animals affected)	0	NA	0	NA	0	NA	2	NA
Inflammation - slight	0	NA	0	NA	0	NA	1	NA
Cellular debris, lumen - slight	0	NA	0	NA	0	NA	1	NA
Hypospermia - minimal	0	NA	0	NA	0	NA	1	NA

NA = not applicable.

Antibody production:
Anti-LY2189265 antibodies were not measured. Expected pharmacodynamic effects of decreased body weight gain and decreased food consumption were noted throughout the study. TK data show a slight increase in mean exposure on Day 92 compared to Day 1 at the low and high dose levels. This apparent slight accumulation was likely due to the long half-life of the drug. Overall, the data suggest that even if antibodies were produced, they did not have a significant effect on exposure or a neutralizing effect.

Toxicokinetics:

Group (Dose)	Study Day	Sex	C_{max} (mcg/mL)	T_{max} (h)	$AUC_{0\text{-last}}$ (mcg*h/mL)
6 (1.63 mg/kg)	1	M	1.89	24.0	77.20
		F	1.95	24.0	88.84
		M + F	1.92	24.0	83.00
	92	M	2.92	24.0	114.28
		F	2.41	24.0	134.00
		M + F	2.66	24.0	124.06
7 (4.89 mg/kg)	1	M	6.45	12.0	234.66
		F	11.60	12.0	323.30
		M + F	9.03	12.0	279.36
	92	M	8.10	24.0	299.72
		F	7.77	24.0	254.54
		M + F	7.94	24.0	277.46
8 (16.29 mg/kg)	1	M	45.90	12.0	943.38
		F	21.58	12.0	721.24
		M + F	33.74	12.0	832.16
	92	M	22.32	12.0	1042.34
		F	30.87	24.0	1318.94
		M + F	26.56	24.0	1180.84

Abbreviations: $AUC_{0\text{-last}}$ = area under the plasma concentration curve from 0 to last quantifiable time point, C_{max} = maximum observed plasma concentration, F = Female, M = Male, M + F = Males and Females combined, T_{max} = time to achieve C_{max}.

Study title: A repeat-dose toxicology study in Cynomolgus monkeys given LY2189265 twice weekly by subcutaneous injection for a total of 27 doses

Key study findings:

- Treatment resulted in a dose-dependent increase in the incidence of vomiting in both males and females, especially during the first two weeks of dosing; the incidence decreased once the animals acclimated to treatment. Over the course of the study, two HD females required supplemental treatment and/or a dosing holiday due to dehydration and anorexia.
- Dose-dependent decreases in mean body weight and food consumption occurred for all treated groups during the first 2 weeks of dosing followed by decreased body weight gain. At the end of the dosing phase, the mean body weight for males given ≥1.63 mg/kg was 17% lower than controls and females given 8.15 mg/kg was 15% lower than controls. Body weights rebounded significantly for all treated groups during the post-treatment period, indicating that pharmacodynamic activity was present throughout the dosing period.
- An increase in mean heart rate (up to 45%) was noted for HD groups on Day 5 and Day 89 compared with baseline and control values. Some increases in mean QTc values were noted for MD and HD groups on Day 5 and/or Day 89 compared with control values. However, the values for these groups were not much higher than at baseline and baseline values were also slightly higher than control values. Therefore, these findings do not appear to be biologically relevant.
- Absolute and relative thymus weights were significantly decreased for MD and HD males and were considered compound-related but not adverse. Decreased thymus weights correlated with an increased incidence and/or severity of thymic involution in some males or females given 1.63 or 8.15 mg/kg and some females given 0.41 mg/kg. As noted in monkeys treated with other GLP-1 receptor agonists, increased mean testicular weights were noted at ≥1.63 mg/kg. Similarly, increased mean weights were noted for epididymis, prostate, and seminal vesicles. There were no correlative histopathology findings for male reproductive organs.
- No anti-LY2189265 antibodies were detected in animals at predose, Day 96 of the dosing period, or at the end of the recovery phase (Day 120). Based on body weight gain during the recovery period for treated groups, there was no indication of neutralizing activity.
- Based on the need for the treatment of dehydration for one female at 8.15 mg/kg dose, initial body weight loss, and increased incidence of no supplement consumption, the NOAEL for LY2189265 when administered twice weekly by subcutaneous injection to Cynomolgus monkeys for 3 months was 1.63 mg/kg. On Day 92, the 1.63 mg/kg dose level resulted in a mean C_{max} of 3.47 µg/mL and a mean $AUC_{0\text{-}672hr}$ of 1047 µg•h/mL.

Reference ID: 3527260

Study number: 7608-192, Amendment 2
Report location: Module 4.2.3.2
Conducting laboratory: [redacted] (b) (4)
Date of study initiation: 11 May 2006
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: LY2189265, [redacted] (b) (4) C1301-78, Main HPLC peak = [redacted] (b) (4)
Main HPLC (reduced) peak = [redacted] (b) (4);
Main SDS-PAGE band = [redacted] (b) (4)
Formulation: [redacted] (b) (4) citrate [redacted] (b) (4) (pH [redacted] (b) (4) [redacted] (b) (4) (w/v) polysorbate-80, [redacted] (b) (4) sodium chloride in sterile water for injection, USP

Methods

Species/strain: Monkey / Cynomolgus
Study Design: (sponsor-generated table)

	No. of Animals[a]		Dose Level (mg LY2189265/kg/dose)[b]		Dose Concentration (mg LY2189265/mL)[b]	
Group	Male	Female	Nominal	Actual	Nominal	Actual
1 (Control)[c]	6	6	0	0	0	0
2 (Low)	6	6	0.5	0.41	0.5	0.41
3 (Mid)	6	6	2	1.63	2	1.63
4 (High)	6	6	10	8.15	10	8.15

a The last three animals/sex/group (based on survival) were designated as posttreatment animals and were dosed with test article or control article for 27 doses, after which dosing was discontinued and immunogenicity was assessed after a 4-week post-treatment period.
b The dose volume was 1 mL/kg.
c Animals in Group 1 received the control article only.

Administration route: Subcutaneous
Dosing regimen: Twice weekly
TK sampling times: Predose (Day 92) and 4, 12, 24, 48, and 96 hours post dose on Day 1 and Day 96. Samples also collected at 168, 240, 336, and 672 hours after the last dose for post-treatment (Ab assessment) animals.
Ab sampling times: Predose, 96 hours after the final dose, and at the end of the post-treatment phase.
Age: 2 to 4 years
Weight: 2 to 3.2 kg (males) and 2 to 2.8 kg (females)
Recovery group: Recovery animals did not undergo necropsy. Recovery animals were primarily used for Ab analysis.
Protocol deviations: No GLP or protocol deviations occurred that impacted the validity or interpretability of the study data. After the study report was finalized, it was determined that the extinction coefficient of the test article was different than previously calculated resulting in lower administered dose levels than planned. The actual dose levels were 0, 0.41, 1.63, and 8.15 mg/kg rather than 0.5, 2, and 10 mg/kg as planned.

Results:

Mortality: (assessed twice daily)
No unscheduled deaths occurred.

Clinical signs: (assessed twice daily and detailed exams once per week)
On Day 2, one female from the 8.15 mg/kg dose group presented with signs of anorexia and dehydration. This animal was treated with fluids and fruit/treat supplements and continued to be given fruit and high caloric supplements throughout the study. On Day 85, another HD female received a dosing holiday because of low body weight. This animal was administered drug at the next dosing interval and remained on study as scheduled.

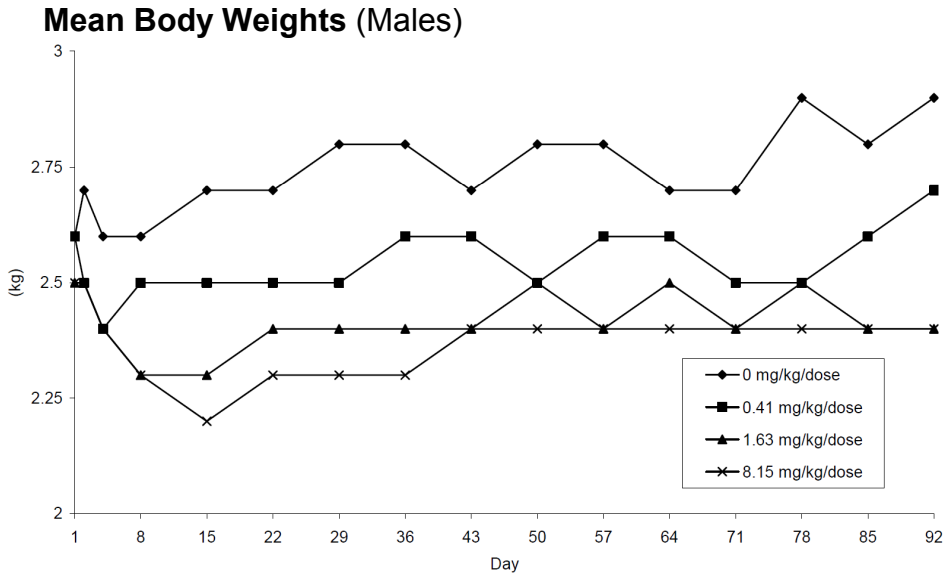
	Dose Level (mg LY2189265/kg/dose)							
Sex	Males (N=6)				Females (N=6)			
Dosing Phase Clinical Signs	0[a]	0.41	1.63	8.15	0[a]	0.41	1.63	8.15
Vomitus								
Incidence on Days 2-6 (animals observed)	0 (0)	0 (0)	1 (1)	3 (3)	0 (0)	0 (0)	1 (1)	4 (4)
Qualitative Food Consumption Incidence (animals observed)								
Low	243 (6)	450 (6)	440 (6)	472 (6)	442 (6)	367 (6)	458 (6)	471 (6)
None	7 (4)	6 (4)	1 (1)	9 (4)	5 (3)	2 (2)	0 (0)	10 (5)
Qualitative Supplement Consumption Incidence (animals observed)								
All	559 (6)	530 (6)	549 (6)	510 (6)	519 (6)	554 (6)	552 (6)	489 (6)
Some	9 (4)	40 (0)	19 (5)	52 (6)	47 (4)	14 (4)	16 (6)	67 (6)
No supplement	0 (0)	0 (0)	2 (2)	5 (2)	4 (2)	1 (1)	2 (1)	13 (4)
Thin Incidence (animals observed)	0 (0)	0 (0)	32 (3)	83 (4)	32 (3)	82 (4)	99 (6)	210 (6)

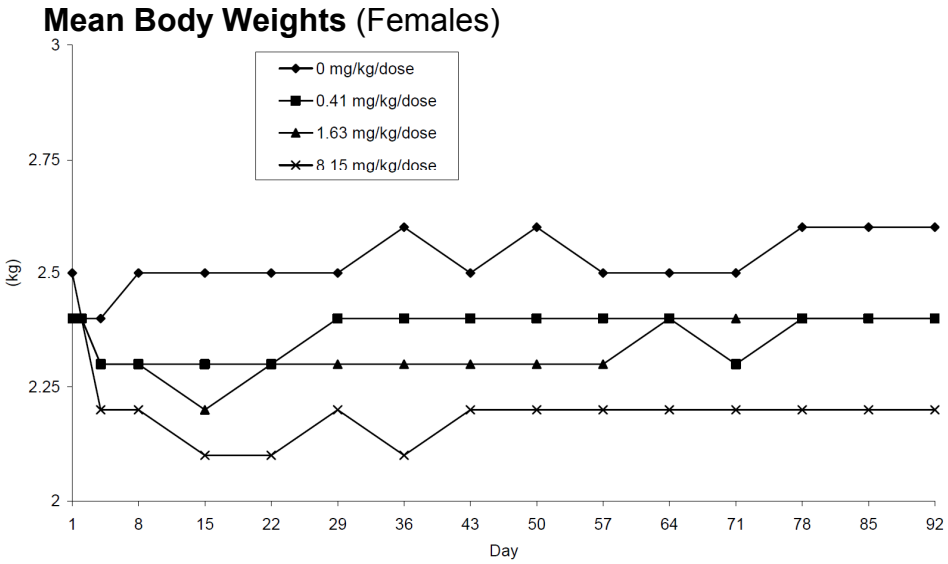
a Control article.

Body weights: (assessed predose, Days 1, 2, 4, and 8 during dosing, and weekly thereafter)
Treatment resulted in initial body weight losses followed by decreased body weight gain. Mean body weights were statistically significantly higher for all treated groups during the recovery period.

Dose (mg/kg)	0		0.41		1.63		8.15	
Sex	M	F	M	F	M	F	M	F
Day 1	2.6	2.5	2.6	2.4	2.5	2.4	2.5	2.4
Day 92	2.9	2.6	2.7	2.4	2.4*	2.4	2.4*	2.2
Weight gain (kg)	0.3	0.1	0.1	0	-0.1	0	-0.1	-0.2
% Diff. from baseline	↑12%	↑4%	↑4%	0%	↓4%	0%	↓4%	↓8%
Diff. from control (kg)	-	-	-0.2	-0.3	-0.4	-0.3	-0.4	-0.5
% Diff. from control	-	-	↓67%	↓100%	↓133%	↓100%	↓133%	↓167%

*p < 0.05 (ANOVA)





Food Consumption: (assessed qualitatively once daily)
Decreased food and supplement consumption was observed in LY2189265-treated animals, especially during the first 2 weeks of dosing. However, there was also a high incidence of low food consumption in the control group (see clinical signs table above), making it difficult to definitively detect a treatment-related effect on qualitative food consumption over the course of the study.

Ophthalmoscopy: (assessed once during predose and Week 13)
No treatment-related findings were observed.

Neurobehavioral Observations: (assessed once during predose, Week 1, and Week 13 approximately 24 hours after dosing)
No abnormalities in neurobehavioral function were observed.

EKG, Blood Pressure, Heart Rate, and Respiration Rate: (assessed predose and approximately 22 hours after dosing on Days 5, 29, and 89)

Some increases in mean QTc (Bazett method) and heart rate were observed, but mean pre-dose values were also often greater than control values. Additionally, increases during the dosing period were not always dose dependent and increases were not consistent across all time points. The reviewing cardiologist concluded that the values were within the range of normal variation for Cynomolgus monkeys, and therefore, not clinically meaningful or compound related.

Dose (mg/kg)	0			0.41			1.63			8.15		
Sex	M	F	M+F	M	F	M+F	M	F	M+F	M	F	M+F
QTc (msec)												
Baseline	341	351	345	350	349	350	370 ↑9%	347	359 ↑4%	354	365	360 ↑4%
Day 5 (22 h post dose)	331	337	334	351	355	353	363	357	360* ↑8%	360	376	368* ↑10%
Day 89 (predose)	327	334	331	353	352	353	370	349	360* ↑9%	367	362	365* ↑10%
Heart Rate (beats/min)												
Baseline	104	110	106	117	131	124	127	152	140	114	143	129
Day 5 (22 h post dose)	104	116	110	126	145	135	133	158	146	133	187	160 ↑45%
Day 89 (predose)	115	133	124 ↑11%	169	160	165 ↑33%	131	154	142 ↑1%	159	192	176* ↑42%

*statistically positive trend at $p \leq 0.05$

Hematology: (assessed predose, Week 4, and Week 13)
No toxicologically significant effects on hematology or coagulation parameters were observed.

Clinical chemistry: (assessed predose, Week 4, and Week 13)
There were no toxicologically meaningful effects on clinical chemistry parameters.

Clinical Chemistry: Day 96

Dose (mg/kg)	0		0.41		1.63		8.15	
Sex	M	F	M	F	Sex	M	F	M
Glucose (mg/dL)	75	63	60	77	66	64	59 (↓21%)	55 (↓13%)
Potassium (mmol/L)	5.1	4.5	5.0	4.8 (↑7%)	5.1	4.9 (↑9%)	5.5 (↑8%)	5.6 (↑24%)

Urinalysis: (assessed predose and Week 13)
On Day 96, mean control values for urine volume were much higher than baseline values (94.3 and 77.2 mL for males and females, respectively), which increased the apparent degree of treatment-related decreased urine volume. In fact, the only group that consistently had individual animal decreases from baseline values was the HD female group. Thus, urine volume differences from control do not appear to be biologically relevant.

Urinalysis: Day 96

Dose (mg/kg)	0		0.41		1.63		8.15	
Sex	M	F	M	F	Sex	M	F	M
Urine Volume (mL)	171	140	83.7 (↓51%)	44.3 (↓68%)	76.7 (↓55%)	83.3 (↓41%)	49.7 (↓71%)	46.0 (↓67%)

Gross pathology: (conducted on all animals, excluding post-treatment animals)
No treatment-related macroscopic findings were observed.

Organ weights: (conducted on all animals, excluding post-treatment animals)

Dose (mg/kg)	0		0.41		1.63		8.15	
Sex	M	F	M	F	M	F	M	F
Thymus absolute (g)	3.790	2.767	1.945 (↓49%)	1.154 (↓58%)	1.681* (↓56%)	1.362 (↓51%)	1.066* (↓72%)	0.860 (↓69%)
BW ratio	0.132	0.111	0.082 (↓38%)	0.052 (↓53%)	0.077 (↓42%)	0.057 (↓49%)	0.048 (↓64%)	0.045 (↓59%)
Brain ratio	5.653	4.893	3.420 (↓40%)	1.877 (↓62%)	2.592 (↓54%)	2.198 (↓55%)	1.703* (↓70%)	1.446 (↓70%)
Pituitary absolute (g)	-	0.35	-	0.43	-	0.56 (↑60%)	-	0.50 (↑43%)
BW ratio	-	0.001	-	0.002	-	0.002 (↑100%)	-	0.003 (↑200%)
Brain ratio	-	0.062	-	0.072	-	0.091 (↑47%)	-	0.085 (↑37%)
Epididymis absolute	0.986	NA	1.078 (↑9%)	NA	1.256 (↑27%)	NA	1.540 (↑43%)	NA
BW ratio	0.036	NA	0.042 (↑17%)	NA	0.057 (↑58%)	NA	0.069* (↑92%)	NA
Brain ratio	1.510	NA	1.799 (↑19%)	NA	1.940 (↑28%)	NA	2.435 (↑61%)	NA
Prostate absolute (g)	0.434	NA	0.448 (↑3%)	NA	0.652 (↑50%)	NA	0.979 (↑126%)	NA
BW ratio	0.015	NA	0.018 (↑20%)	NA	0.030 (↑100%)	NA	0.044* (↑193%)	NA
Brain ratio	0.647	NA	0.758 (↑17%)	NA	1.002 (↑55%)	NA	1.548* (↑139%)	NA

Dose (mg/kg)	0		0.41		1.63		8.15	
Sex	M	F	M	F	M	F	M	F
Seminal Vesicles absolute (g)	0.702	NA	0.851 (↑21%)	NA	0.939 (↑34%)	NA	1.484* (↑111%)	NA
BW ratio	0.025	NA	0.034 (↑%)	NA	0.043 (↑%)	NA	0.066* (↑%)	NA
Brain ratio	1.070	NA	1.427 (↑33%)	NA	1.449 (↑35%)	NA	2.344* (↑119%)	NA
Testes absolute (g)	1.845	NA	2.008 (↑9%)	NA	3.078 (↑67%)	NA	4.130 (↑124%)	NA
BW ratio	0.068	NA	0.078 (↑15%)	NA	0.139 (↑104%)	NA	0.18 (↑165%)	NA
Brain ratio	2.863	NA	3.147 (↑10%)	NA	4.763 (↑66%)	NA	6.431 (↑125%)	NA

*p ≤ 0.05; abs = absolute weight; BW = body weight; NA = not applicable.

Histopathology: (conducted for all main group animals)

Adequate Battery: Yes

Peer review: Yes; First animal from control group and first 2 animals from the HD group only.

There were no apparent adverse microscopic findings. A slight increase in the incidence of minimal to slight thymic involution was noted for most treated groups (see sponsor-generated table below). This effect was likely secondary to the treatment-related effects on body weight and correlated with decreased mean thymus weights.

Incidence and Severity of Thymus Involution

Sex	Males				Females			
Dose Level (mg/kg)	0	0.41	1.63	8.15	0	0.41	1.63	8.15
No. Examined	3	3	3	3	3	3	3	3
Thymus Involution								
Not Noted	3	3	2	1	2	1	1	1
Minimal	0	0	1	2	1	1	1	1
Slight	0	0	0	0	0	1	1	1

Toxicokinetics: (sponsor-generated table)

Study Day	Parameter	Units	Dose 0.41 mg/kg Mean	SD	n	1.63 mg/kg Mean	SD	n	8.15 mg/kg Mean	SD	N
1	C_{max}	mcg/mL	1.10	0.21	12	3.68	0.63	12	34.60	11.31	12
	T_{max}	h	19.00	6.18	12	19.00	13.97	12	11.67	4.96	12
	$AUC_{0\text{-}96hr}$	mcg*h/mL	67.52	11.72	12	220.01	40.98	12	1665.88	350.88	12
92	C_{max}	mcg/mL	2.82	0.47	12	11.02	2.45	12	51.61	12.82	12
	T_{max}	h	9.67	6.02	12	15.00	5.43	12	14.00	4.67	12
	$AUC_{0\text{-}96hr}$	mcg*h/mL	154.83	41.32	12	681.67	212.87	12	3034.11	605.05	12
	$AUC_{0\text{-}672hr}$	mcg*h/mL	248.10	88.22	6	1046.80	307.84	6	4104.79	793.91	6
	C_L/F	mL/h/kg	1.74	0.42	6	1.66	0.55	6	2.04	0.45	6
	V_d/F	mL/kg	391.44	70.64	6	337.09	86.49	6	374.30	49.07	6
	$t_{1/2}$	h	159.75	23.00	6	146.42	33.29	6	130.78	24.94	6

Abbreviations: $AUC_{0\text{-}xh}$ = area under the plasma concentration curve from 0 to "x" hours, C_L/F = total body clearance as a function of bioavailability, C_{max} = maximum plasma concentration, SD = standard deviation, T_{max} = time of C_{max}, $t_{1/2}$ = elimination half-life, V_d/F = volume of distribution as a function of bioavailability.

Immunogenicity

Samples were assessed for anti-LY2189265 antibodies by using a validated bridging ELISA. Samples were stored at -20°C for a total of 210 days. However, long-term stability was not assessed per the sponsor. It is also not clear what the limit of drug interference was for the assay (method validation report 06-077 wasn't submitted), although this shouldn't be an issue since samples were also assessed after a 1-month recovery period.

No anti-LY2189265 antibodies were detected in serum samples taken predose, at Day 92, or Day 120 (recovery).

Dose Formulation Analysis:

LY2189265 was shown to be stable throughout the dosing phase. The concentration of each sampled dosing formulation did not deviate substantially from its theoretical value (98 to 104% of nominal).

Study title: A repeat-dose toxicology and toxicokinetic study in rats given LY2189265 twice weekly by subcutaneous injection for 6 months with a 1 month recovery

Key study findings:

- One mid-dose female was sacrificed moribund on Day 78. Pathology findings indicated that the moribund condition was due to urinary inflammation/calculi and renal pyelonephritis, which was considered an incidental finding.
- Abnormal clinical signs were limited to few feces, thin, and rough haircoat, which were more pronounced at the mid- and high-dose levels.
- Body weights were statistically significantly decreased from control values at all dose levels for both males (↓7% to 18%) and females (↓16% to 19%). The effects on body weight generally correlated with decreased food consumption. Decreases in body weight and food consumption are expected pharmacological effects of GLP-1 receptor agonists.
- Slight decreases in mean reticulocyte counts were noted for all male dose groups, high dose females, and the high-dose female recovery group.
- Slight decreases in mean glucose (high-dose males), cholesterol (high-dose females), and triglycerides (high-dose males and females) were noted.
- Dose-dependent decreases in organ weights were considered a secondary effect of the decreases in body weights.
- Microscopic findings noted at the high dose included a slight increase in focal congestion/hemorrhage of the stomach (2/15 males, 1/15 females) and dilatation of the uterus (6/15). An increase in the number of animals with bile duct proliferation was noted for the high-dose recovery males (2/10).
- Anti-drug Ab production was not assessed; however, signs of pharmacological activity (decreased mean body weights and decreased mean food consumption) were noted during the last week of the study indicating that sufficient drug exposure was maintained throughout the study.
- Because decreased body weight and food consumption are expected pharmacological effects and did not produce adverse outcomes with regard to overall animal health, the NOAEL of this study was considered to be 16.3 mg/kg. At the NOAEL, the mean C_{max} and $AUC_{0\text{-last}}$ values at Day 183 were 43.7 µg/mL and 1590 µg·h/mL, respectively.

Study number:	7608-236, Amendment 2
Report location:	Module 4.2.3.2
Conducting laboratory:	(b) (4)
Date of study initiation:	14 August 2006
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, % purity, and:	LY2189265, Lot # (b) (4) C1301-78, 65.9%
% unmodified peptide	(b) (4) %

Reference ID: 3527260

Formulation/vehicle: Diluent A: (b) (4) citrate (b) (4) and (b) (4) (w/v) polysorbate-80 with (b) (4) sodium chloride in sterile water for injection, USP.
Control article (diluent B): (b) (4) citrate (b) (4) (pH (b) (4)) and (b) (4) (w/v) polysorbate-80 with (b) (4) sodium chloride in sterile water for injection, USP.

Methods

Species/strain: Rat / Sprague Dawley
Study design: (sponsor-generated table)

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg LY2189265/kg/dose)[a] Nominal	Dose Level (mg LY2189265/kg/dose)[a] Actual	Dose Concentration (mg LY2189265/mL)[a] Nominal	Dose Concentration (mg LY2189265/mL)[a] Actual
Toxicity Animals						
1 (Control)[b,c]	25	25	0	0	0	0
2 (Low)	15	15	2	1.63	2	1.63
3 (Mid)	15	15	6	4.89	6	4.89
4 (High)[c]	25	25	20	16.29	20	16.29
Toxicokinetic Animals[d]						
5 (Control)[b]	6	6	0	0	0	0
6 (Low)	12	12	2	1.63	2	1.63
7 (Mid)	12	12	6	4.89	6	4.89
8 (High)	12	12	20	16.29	20	16.29

a The dose volume was 1 mL/kg.
b Animals in Groups 1 and 5 received the control article only.
c The last 10 toxicity animals/sex in the control and high-dose groups (based on survival) were designated as recovery animals and were dosed with test article or control article for at least 26 weeks, after which dosing was discontinued, and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 4 weeks posttreatment.
d Toxicokinetic animals were included solely for the purpose of blood sample collections. Three animals/sex/group served as replacement animals, if needed.

Route: Subcutaneous
Frequency of dosing: Twice weekly
Age: 6 to 8 weeks
Weight: 179 to 268 g (males); 153 to 221 g (females)
TK sampling times: Control - Days 1 and 183 – predose (Day 183 only) and ~24 hours postdose
Test article – Days 1 and 183 - predose (Day 183 only) and 4, 12, 24, 48, and 96 hours postdose
Ab sampling times: Samples collected on recovery phase sacrifice day only; samples discarded without analysis
Protocol deviations: No GLP or protocol deviations occurred that impacted the validity or interpretability of the study data. After the study report was finalized, it was determined that the extinction coefficient of the test article was different than previously calculated resulting in lower

administered dose levels than planned. The actual dose levels were 0, 1.63, 4.89, and 16.3 mg/kg rather than 2, 6, and 20 mg/kg as planned.

Results:

Mortality:

One mid-dose female was sacrificed moribund on Day 78. Prior to sacrifice, the animal was debilitated (i.e., thin, recumbent, genitals that were red in color, and cold to touch). Pathology results indicated that the moribund condition was due to urinary inflammation/calculi and renal pyelonephritis, which was considered an incidental finding. One low-dose TK male animal was found dead on Day 96; the cause of death was not determined. Neither event was considered test article-related.

Clinical signs: (twice daily; detailed observations on Day 1 and once weekly thereafter)

Treatment-related clinical signs are shown in the sponsor-generated table below.

	Few Feces		Thin		Rough Haircoat	
mg LY2189265/kg/dose	Male	Female	Male	Female	Male	Female
0	(0) 2	(0) 4	(0) 0	(0) 0	(0) 0	(0) 15
1.63	(0) 0	(0) 0	(0) 0	(0) 0	(0) 7	(0) 16
4.89	(0) 0	(11) 11	(0) 0	(5) 7	(2) 15	(0) 29
16.29	(39) 39	(35) 45	(6) 6	(20) 30	(14) 33	(0) 27

Parentheses indicate occurrence within the first 8 weeks of the study.

Body weights: (pre-dosing and before each dose administration)

Decreased body weights were noted in all test article-treated groups. As shown in the sponsor-generated mean body weight figures, high-dose recovery animals gained more weight than controls during the recovery period.

Dose (mg/kg)	0		1.6		4.9		16.9	
Sex	M	F	M	F	M	F	M	F
Day 1 (g)	224	184	223	181	222	182	222	180
Day 183 (g)	642	358	597*	299*	555*	299*	527*	290*
Weight gain (g)	418	174	374*	118*	333*	117*	305*	110*
Final weight - diff from control (g)	-	-	-45	-59	-87	-59	-115	-68
Final weight - % diff from control	-	-	↓7%	↓16%	↓14%	↓16%	↓18%	↓19%

* $p \leq 0.05$ (ANOVA)

(Sponsor-generated figures)

Figure 1: Mean body weight data - males.

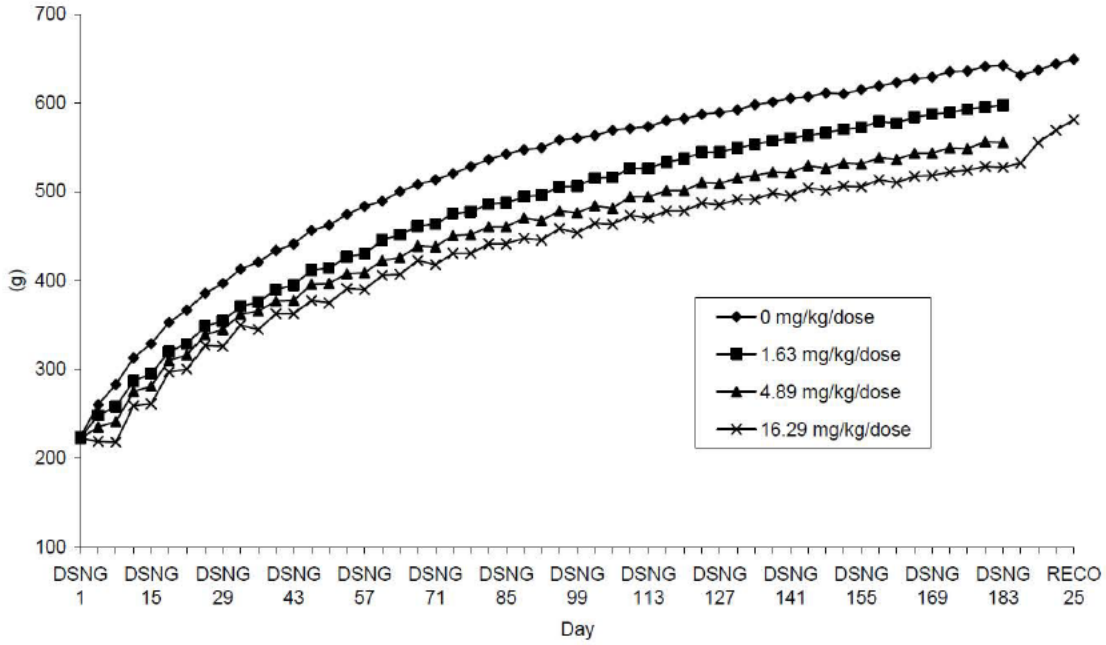
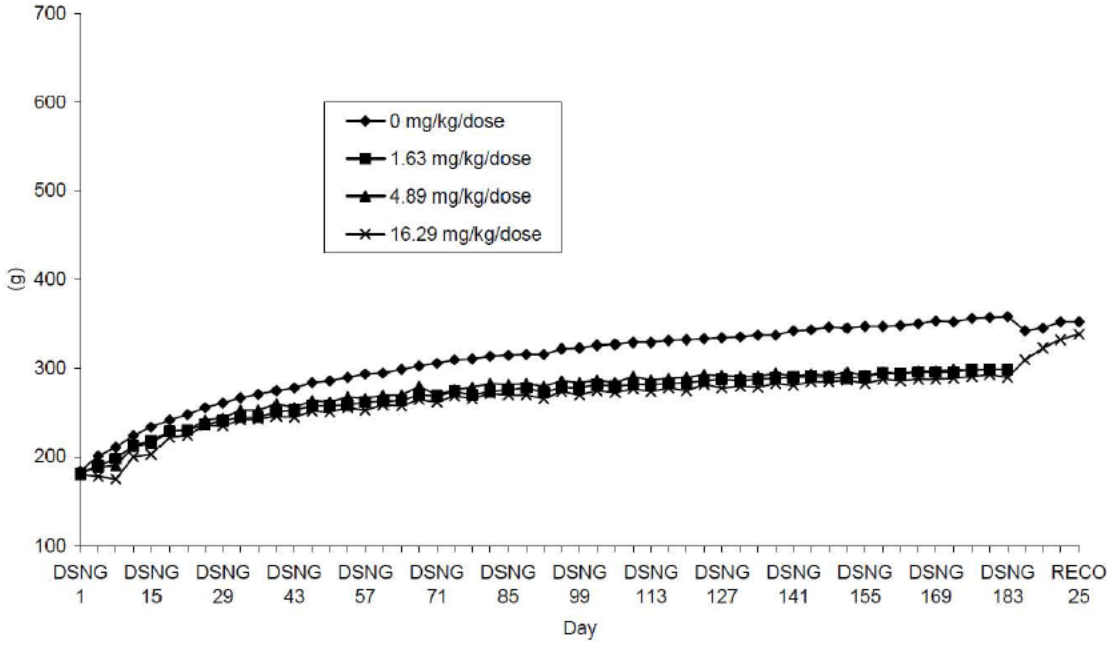


Figure 2: Mean body weight data - females.



<u>Food consumption</u>: (twice weekly [quantitatively])
Decreased food consumption was observed in all test article-treated groups.

	% Change from Control					
	Males			Females		
mg LY2189265/kg/dose	1.63	4.89	16.29	1.63	4.89	16.29
Days 1-3 (3-day interval)	↓18*	↓46*	↓70*	↓29*	↓50*	↓66*
Days 4-7 (4-day interval)	↓14*	↓21*	↓34*	↓17*	↓20*	↓34*
Days 85-87 (3-day interval)	↓13*	↓16*	↓26*	↓17*	↓25*	↓34*
Days 88-91 (4-day interval)	NS	↓9*	↓11*	↓9*	NS	NS
Days 183-185 (3-day interval)	↓9*	↓12*	↓19*	↓15*	↓11*	↓14*
Days 7-10 (recovery phase)	NA	NA	↑17*	NA	NA	↑25*
Days 25-27 (recovery phase)	NA	NA	NS	NA	NA	NS

Abbreviations: ↓ = decrease; ↑ = increase; NS = not significant; NA = not applicable.
* = $p \leq 0.05$; statistical significance based on actual data, not on percent change from control.

Ophthalmoscopy: (pre-dose and once during the final week of dosing)
No treatment-related effects were noted.

Hematology: (collected on the day of main and recovery group sacrifices)

Dose (mg/kg)	0		1.6		4.9		16.9	
Sex	M	F	M	F	M	F	M	F
Reticulocytes ($1 \times 10^3/\mu L$)	180.4	133.0	146.9*	119.2	144.2*	119.9	151.4* (↓16%)	142.6 (↓7%)
Reticulocytes (%)	2.0	1.6	1.6*	1.5	1.6*	1.5	1.7	1.8
Recovery								
Reticulocytes ($1 \times 10^3/\mu L$)	194.1	144.3					175.3	112.7* (↓22%)
Reticulocytes (%)	2.1	1.8					2.0	1.3* (↓28%)

*$p \leq 0.05$ (ANOVA)

Clinical chemistry: (collected on the day of main and recovery group sacrifices)

Dose (mg/kg)	0		1.6		4.9		16.9	
Sex	M	F	M	F	M	F	M	F
Glucose (mg/dL)	100	102	98	96	94	91*	87* (↓13%)	95
Total Protein (g/dL)	7.1	7.8	7.0	7.5	6.8*	7.5	6.7* (↓6%)	7.3
Cholesterol (mg/dL)	-	87	-	66	-	63	-	57* (↓33%)
Triglycerides (mg/dL)	74	43	49*	31*	43*	28*	42* (↓43%)	29* (↓33%)
ALP (U/L)	-	29	-	34	-	32	-	39* (↑34%)

"-" = no change from control value; ALP = alkaline phosphatase.

Urinalysis: (collected on the day of main and recovery group sacrifices)
No treatment-related urinalysis findings were observed.

Gross pathology: (all main study and recovery group animals)
No treatment-related macroscopic findings were noted at necropsy.

Organ weights: (all main study and recovery group animals)
There were no organ weight changes directly attributed to test article. There were dose-dependent decreases in weights of several tissues due to treatment-related decreases in body weight.

Histopathology: Conducted on all main study and recovery group animals - see histopathology inventory list at end of section.
Adequate Battery: Yes
Peer review: Yes, performed by sponsor - all tissues from the 1st 3 animals of both sexes from control and high dose groups. Additionally, all tissues from the Group 3 female that was sacrificed moribund were examined.

Histopathology:

Dose (mg/kg)	**0**		**1.6**		**4.9**		**16.9**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Stomach - focal congestion/ hemorrhage -minimal	0	0	0	0	0	0	2/15	1/15
Uterus - dilatation	NA		NA		NA	0	NA	
-minimal		-		-				1/15
-slight		1/15		-				2/15
-moderate		-		-				3/15
-marked		-		1/15				-
Liver - bile duct hyperplasia	0	0	0	0	0	0	0	0
Recovery								
Liver - bile duct hyperplasia	0	0	NA	NA	NA	NA		0
-minimal							1/10	
-slight							1/10	

NA = not applicable.

Toxicokinetics: There were no apparent differences in PK data between genders. Slight accumulation was noted at the mid and high doses.

Table 2: Mean Plasma Toxicokinetic Data Summary of LY2189265 in Rats Following Twice Weekly Subcutaneous Injections of 1.63, 4.89, or 16.29 mg/kg (Groups 6 through 8, respectively) LY2189265 on Study Days 1 and 183

Group (Dose)	Study Day	Sex	C_{max} (µg/mL)	T_{max} (h)	$AUC_{0\text{-last}}$ (µg*h/mL)
6 (1.63 mg/kg)	1	M	2.72	12.0	86.42
		F	3.42	12.0	114.33
		M + F	3.07	12.0	100.57
	183	M	3.58	24.0	136.90
		F	2.46	12.0	104.68
		M + F	3.02*	24.0/12.0	120.90
7 (4.89 mg/kg)	1	M	6.60	12.0	199.20
		F	6.89	12.0	253.37
		M + F	6.74	12.0	226.22
	183	M	9.76	24.0	301.08
		F	13.15	12.0	293.70
		M + F	11.46*	24.0/12.0	297.44
8 (16.29 mg/kg)	1	M	14.25	12.0	451.74
		F	20.57	12.0	646.16
		M + F	17.41	12.0	548.95
	183	M	37.55	24.0	1203.26
		F	33.56	24.0	1386.72
		M + F	35.55	24.0	1295.24

Abbreviations: $AUC_{0\text{-last}}$ = area under the plasma concentration curve from 0 to last quantifiable time point, C_{max} = maximum observed plasma concentration, F = Female, M = Male, M + F = Males and Females combined, T_{max} = time to achieve C_{max}.

*Replaced Watson generated values which were generated by using the highest overall (Male+Female) mean plasma concentrations of both sexes instead of the mean Cmax values at the actual tmax for each sex.

Antibody measurements:

Anti-LY2189265 antibody levels were not measured. Although antibody production was not assessed in this study, signs of pharmacological activity (decreased mean body weights and decreased mean food consumption) were noted during the last week of the study indicating that sufficient drug exposure was maintained throughout the study. Additionally, the high-dose group gained considerably more weight during the recovery period compared with controls indicating that treatment-related weight suppression was maintained throughout the dosing period.

Study title: A repeat-dose toxicity, toxicokinetic, and immunotoxicology study in Cynomolgus monkeys given LY2189265 twice weekly by subcutaneous injection for 9 months with a 2 month recovery

Study no.:	7608-235
Study report location:	Module 4.2.3.2
Conducting laboratory:	(b) (4)
Date of study initiation:	21 July 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and conc.:	LY2189265, (b) (4) C1301-78, 47.0 mg/mL
Formulation/Vehicle:	(b) (4) citrate (b) (4) (pH (b) (4) (w/v) polysorbate-80, (b) (4) sodium chloride in sterile water for injection, USP

Key Study Findings

- One HD monkey was sacrificed moribund on Day 17 after receiving only a single dose on Day 1. This monkey was observed to have hunched posture, dehydration with few feces, low food consumption, and rough haircoat. Fruit/treat supplements and IV fluids were administered and further dosing was withheld. The monkey's condition did not improve sufficiently and the animal was removed from the study.
- A dose-related increase in the incidence of decreased food and supplement consumption was observed during the first 2 weeks of treatment. Decreased fecal output was also noted at all dose levels, correlating with low food consumption. After the initial treatment period, food consumption and fecal output gradually returned to near control levels.
- During Week 25, one LD female was treated for dehydration and poor food consumption with subcutaneous fluids and extra high caloric supplements. Because of the late onset with relation to the first dose administration, no correlation to dose, and complete recovery while dosing continued, the sponsor felt that the clinical signs were not adverse and were of uncertain relationship to the test article.
- Decreases in mean body weight were noted at all dose levels during the first week of treatment, after which time body weight gains were similar to controls. At the end of the study, final mean body weights were slightly decreased at all dose levels for females and for HD males. During the recovery period, body weights for HD animals rebounded and approached or exceeded the control weights. Effects on body weight during the first 2 weeks of treatment correlated with decreased food consumption.
- There were no toxicologically meaningful effects on neurobehavior, ophthalmology, heart rate, ECG, or clinical pathology endpoints.
- Increased mean absolute and relative prostate (up to 2-fold) and testis weights (up to 2.5-fold) were noted for all male treatment groups. Decreased mean ovary weights were noted for all female treatment groups (up to 39% for absolute weight and 31% for relative weight). At the end of the recovery period, increased testis weights and decreased ovary weights were still noted for the HD groups.

The effects on reproductive organs correlated with the observation of fewer treated males having immature testes and a greater number of treated females having immature ovaries.

- No treatment-related adverse microscopic findings were observed. No microscopic findings were noted in the thyroid or pancreas for any LY2189265-treated animal.
- The NOAEL was considered to be 1.63 mg/kg based on the adverse effects on food and water intake leading to the moribund sacrifice of a single HD female. At the NOAEL, mean C_{max} and $AUC_{0\text{-}96h}$ values were 12.37 µg/mL and 718.93 µg•h/mL on Day 274, respectively.

Methods

Species/Strain: Monkey/Cynomolgus

Study Design: (sponsor-generated table)

	No. of Animals[a]		Dose Level (mg LY2189265/kg/dose)[b]		Dose Concentration (mg LY2189265/mL)[b]	
Group	Male	Female	Nominal	Actual[c]	Nominal	Actual[c]
1 (Control)[d]	6	6	0	0	0	0
2 (Low)	4	4	0.5	0.41	0.5	0.41
3 (Mid)	4	4	2	1.63	2	1.63
4 (High)	6	6	10	8.15	10	8.15

a The last two animals/sex in the Groups 1 and 4 (based on survival) were designated as recovery animals and were dosed with LY2189265 or control article for at least 39 weeks, after which dosing was discontinued and the animals were observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 8 weeks recovery.
b The dose volume was 1 mL/kg.
c Actual concentrations calculated to correspond with nominal concentrations.
d Animals in Group 1 received the control article only.

Frequency of dosing: Twice weekly

Route of administration: Subcutaneous

Age: 3 to 5 years

Weight: 2.8 to 3.6 kg (males); 2.2 to 2.6 kg (females)

Unique study design: Anti-Keyhole Limpet Hemacyanin (KLH) IgM and IgG analysis and peripheral blood immunophenotyping

Protocol deviations: No GLP or protocol deviations occurred that impacted the validity or interpretability of the study data. After the study report was finalized, it was determined that the extinction coefficient of the test article was different than previously calculated resulting in lower administered dose levels than planned. The actual dose levels were 0, 0.41, 1.63, and 8.15 mg/kg rather than 0.5, 2, and 10 mg/kg as planned.

Observations and Results

Mortality: (assessed twice daily)

One HD female was sacrificed in moribund condition on Day 17. On Day 4, after receiving only a single dose on Day 1, this female was observed as being hunched, appearing dehydrated with few feces, and having low food consumption and a rough

haircoat. The animal was noted as being thin prior to the initiation of dosing. Subcutaneous fluids were administered and fruit/treat supplements were provided. Further dose administrations were withheld. No food consumption was observed on Days 9, 10, or 11.

Clinical Signs: (assessed twice daily)
A dose-related increase in the incidence of decreased food and supplement consumption was observed during the first 2 weeks of treatment. Decreased fecal output was also noted, correlating with low food consumption. After the initial treatment period, food consumption and fecal output gradually returned to near control levels. During Week 25, one LD female was treated for dehydration and poor food consumption with subcutaneous fluids and extra high caloric supplements. Because of the late onset with relation to the first dose administration, no correlation to dose, and complete recovery while dosing continued, the sponsor felt that the clinical signs were not adverse and were of uncertain relationship to the test article. For an unknown reason, this incidence of dehydration was not included in the clinical signs summary table in the study report.

Decreased fecal output secondary to decreased food consumption was observed at all dose levels. A total of 18 and 12 incidences of no feces was noted for treated males and females, respectively, compared with none for controls. The majority of these observations occurred within the first 2 weeks of the dosing phase. The observation of few feces also occurred in a dose-related manner, with 113 observations for treated animals compared with 4 for untreated animals.

Dose (mg/kg)	0		0.41		1.63		8.15	
Sex	M	F	M	F	M	F	M	F
N	6	6	4	4	4	4	6	6
Thin	0	2	0	3	0	2	2	3
Hypoactive	0	0	0	0	1	0	0	0
Dehydrated	0	1	0	0	0	1	0	1
Hunched posture	0	0	0	0	0	0	0	1
No feces	0	0	1	2	4	4	4	3
Few feces	0	2	4	3	4	4	6	6

(number of animals/group affected)

Body Weights: (assessed in the pre-dose phase and on Days 1, 3, 5, and 8, and once weekly thereafter)

Body weights:

Dose (mg/kg/d)	0		0.41		1.63		8.15	
Sex	M	F	M	F	M	F	M	F
Day 274	4.1	3.0	4.6	2.8	4.0	2.7*	3.8	2.8
Diff from control (kg)			0.5	-0.2	-0.1	-0.3	-0.3	-0.2
% diff from control			↑12%	↓7%	↓2%	↓10%	↓7%	↓7%

*p < 0.05 (ANOVA)

Figure 1: Mean Body Weight Data - Males

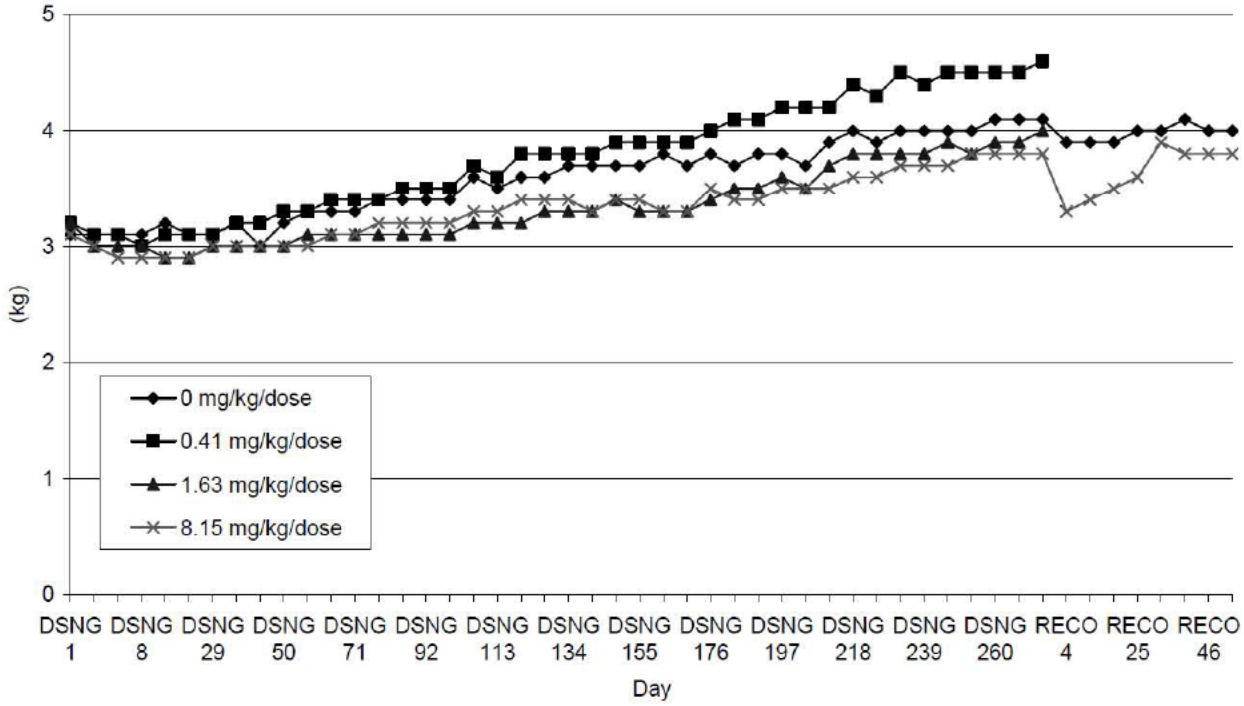
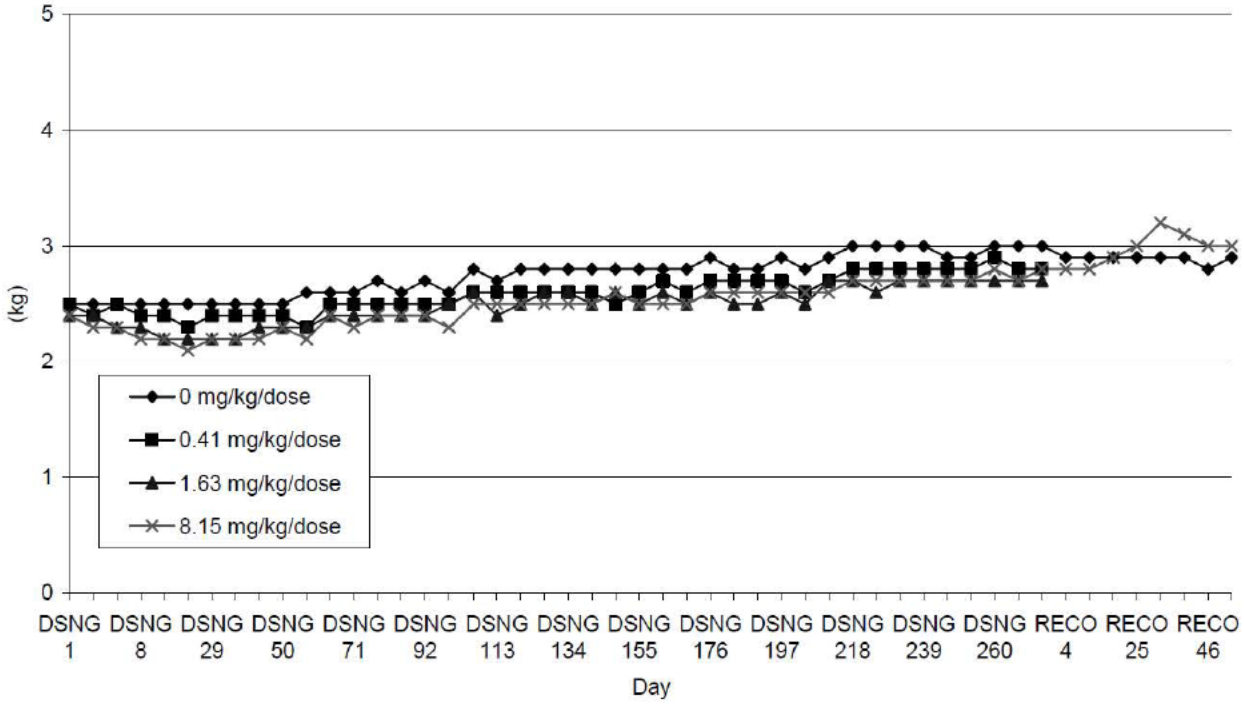


Figure 2: Mean Body Weight Data - Females



Feed Consumption: (assessed qualitatively once daily)
A transient compound-related reduction in food consumption was noted in treated animals during the first 2 weeks of dosing and then gradually returned to near control levels. Over the course of the study, HD males had over five times more observations of low food consumption compared with control males. There were a total of 48 and 39 incidences of no food consumption for MD and HD males compared with only 2 observations for control males. This observation is consistent with the pharmacodynamic activity of the test article.

Food Consumption

Dose (mg/kg)	0		0.41		1.63		8.15	
Sex	M	F	M	F	M	F	M	F
N	6	6	4	4	4	4	6	6
Qualitative Food Consumption								
Low	6	6	4	4	4	4	6	6
None	1	3	2	4	4	2	5	5
Dietary Supplement Consumption								
None	1	1	0	2	1	2	1	5
Some	2	5	3	4	4	3	6	6
All	6	6	4	4	4	4	6	6

(number of animals/group affected)

Neurobehavioral Observations: (assessed during Weeks -1, 1, and 39)
No abnormal neurobehavioral observations were noted.

Ophthalmoscopy assessed during Week -1 and Week 39)
No ophthalmic effects were observed.

ECG and Heart Rate (recorded twice during the predose phase and before dosing and 24 hours after dosing on Days 5, 29, 92, 180, and 274, and during the last week of the recovery phase)

There were no toxicologically meaningful changes in EKG or heart rate for treated animals. There were some slight increases in mean heart rate for the 1.63 and 8.15 mg/kg groups compared with baseline and control values, however the observed changes were often not dose related and the degree of change was not consistent across the dosing period.

Hematology: (assessed during the pre-dose phase, and during Weeks 4, 13, 26, and 39, and at the end of recovery)
No toxicologically meaningful changes in hematology parameters were noted.

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Clinical Chemistry: (assessed during the pre-dose phase, and during Weeks 4, 13, 26, and 39, and at the end of recovery)
No toxicologically meaningful changes in clinical chemistry parameters were noted.

Urinalysis: (assessed during the pre-dose phase, Week 39, and at the end of recovery)
No effects on urinalysis parameters were noted.

Peripheral Blood Immunophenotyping:
LY2189265 administration produced mildly to moderately decreases in total lymphocytes, absolute total T cells, absolute helper T cells, absolute and relative B cells in males given ≥0.41 mg/kg, and in females given 0.41 or 1.63 mg/kg/ dose. A slight decrease in absolute natural killer cell counts was observed in males given ≥0.41 mg/kg. However, relative percentages of cytotoxic T cells were slightly increased at 1.63 and 8.15 mg/kg when compared with controls. Females receiving ≥0.41 mg/kg showed mildly to moderately increased values for relative and absolute natural killer cell counts when compared with control. All lymphocyte subset changes were generally not related to dose or sex. The subset changes were recovering or had recovered by Day 57 of the recovery phase for males given 8.15 mg/kg but not for females. The sponsor stated that, "Other statistically significant values noted in the dataset were considered incidental because they were inconsistent within sex, occurred once, and/or lacked a concurrent change for both sexes. It has been reported that lymphoid cells have inducible receptors to glucagon (Bhathena et al, 1982) and are potentially responsive to glucagon and glucagon-like peptide 1 (GLP-1) (Baggio et al, 2006). The lymphoid effects present in this study were minimal with no evidence of adverse effects on the immune system overall." The reviewer agrees with sponsor's assessment.

Gross Pathology: (conducted on all animals)
No treatment-related macroscopic findings were noted.

Organ Weights: (conducted on all animals - see histopathology inventory table)

Dose (mg/kg/d)	0		0.41		1.63		8.15	
Sex	M	F	M	F	M	F	M	F
Prostate (g)	1.071		1.630		1.489		2.218 (↑107%)	
Rel to BW (%)	0.027		0.036		0.039		0.057* (↑111%)	
Testes (g)	6.577		15.642 (↑138%)		16.234 (↑147%)		14.578 (↑122%)	
Rel to BW (%)	0.162		0.327 (↑102%)		0.431 (↑166%)		0.359 (↑122%)	
Ovary (g)		0.362		0.249		0.237		0.220 (↓39%)
Rel to BW (%)		0.013		0.010		0.010		0.009 (↓31%)

*p<0.05. BW = body weight.

After recovery, mean absolute and relative testis weights for HD males were approximately 3-fold greater than controls. Mean absolute and relative ovary weights for HD females were approximately 2-fold less than controls. Mean prostate weight was similar to controls.

Histopathology: (conducted on all animals - see histopathology inventory table)

Adequate Battery: Yes

Peer Review: Conducted by sponsor representative. All tissues from a single animal/sex in the control group and all tissues from 2 animals/sex for the HD group.

Histological Findings

Dose (mg/kg/d)	0		0.41		1.63		8.15	
Sex	M	F	M	F	M	F	M	F
N	4	4	4	4	4	4	4	3
Liver								
-Fibrosis, periportal, surface associated,								
-minimal	1	1	1	-	-	-	1	-
-slight	-	-	-	-	-	-	2	-
Pancreas*								
-Infiltrate, lymphocytes/ macrophages, minimal	1	-	-	-	-	-	-	-
Testes		NA		NA		NA		NA
-Dilation, tubular (with degeneration)								
-minimal	-		1		-		1	
-slight	-		-		1		-	
-Immature	3		2		1		2	
Ovary	NA		NA		NA		NA	
-Immature		1		3		3		3

*This was the only microscopic finding noted in the pancreas for any monkey. No findings were noted for the thyroid. F = female; M = male; NA = not applicable.

Anti-Keyhole Limpet Hemacyanin Assay: (assessed in 4 animals/sex/group prior to dosing on Days 180, 264, 271, and 278, which correlates with pre-KLH administration and 10, 17, and 24 days after KLH administration, respectively)

Treatment did not result in any differences in interpolated IgM or IgG titers after immunization with KLH during either the dosing phase or the recovery phase.

Toxicokinetics: (assessed on Days 1 and 274 at predose (Day 274 only) and 4, 12, 24, 48, and 96 hours following dose administration; recovery group assessed at 168, 240, 336, and 672 hours after the last dose on Day 274 and on Days 4, 7, 11, and 25 of the recovery phase)

(sponsor-generated table)

				Dose		
Sex	Study Day	Parameter	Units	0.41 mg/kg	1.63 mg/kg	8.15 mg/kg
Overall	1	C_{max}	µg/mL	1.52	5.61	26.51
		T_{max}	h	11.00	12.50	14.33
		$AUC_{0\text{-}96hr}$	µg*h/mL	76.38	326.81	1418.95
	274	C_{max}	µg/mL	3.81	12.37	73.46
		T_{max}	h	15.50	22.50	22.55
		$AUC_{0\text{-}96hr}$	µg*h/mL	205.49	718.93	3792.38
		$AUC_{0\text{-}672hr}$	µg*h/mL			5420.30
		$C_{L/F}$	mL/h/kg			1.69
		$V_{d/F}$	mL/kg			421.94
		$t_{1/2}$	h			173.59

Abbreviations: $AUC_{0\text{-}x\ hr}$ = area under the plasma concentration curve from 0 to "x" hours, $C_{L/F}$ = total body clearance as a function of bioavailability, C_{max} = maximum plasma concentration, T_{max} = time of C_{max}, $t_{1/2}$ = elimination half-life, $V_{d/F}$ = volume of distribution as a function of bioavailability.

Immunogenicity: (assessed in the pre-dosing phase, on Day 25 of recovery, and at the end of recovery)
Anti-LY2189265 antibodies were detected in only 2 animals, both from the control group. A cause for the positive antibody response in these animals is unknown. Neither the bioanalytical data nor the clinical signs support misdosing of the control group.

Dosing Solution Analysis:
All dosing preparations were 98% to 104% of the nominal values.

Histopathology Inventory for BLA 125469

Study Number	7608-191	7608-236	7608-192	7608-235
Study Duration/Species	**3-Month Rat**	**6-Month Rat**	**3-Month Monkey**	**9-Month Monkey**
Adrenals	X*	X*	X*	X*
Aorta	X	X	X	X
Bone Marrow smear	X		X	
Bone (femur) with marrow	X	X	X	X
Brain	X*	X*	X*	X*
Cecum	X	X	X	X
Cervix	X	X	X	X
Colon	X	X	X	X
Duodenum	X	X	X	X
Epididymis	X	X	X*	X
Esophagus	X	X	X	X
Eye	X	X	X	X
Gall bladder[b]			X*	X
Gross lesions	X	X	X	X
Harderian gland	X			
Heart	X*	X*	X*	X*
Ileum	X	X	X	X
Injection site	X	X	X	X
Jejunum	X	X	X	X
Kidneys	X*	X*	X*	X*
Lachrymal gland			X	X
Larynx				
Liver	X*	X*	X*	X*
Lungs	X	X	X*	X
Lymph nodes mandibular	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	X	X	X
Nasal cavity				
Optic nerves	X	X	X	X
Ovaries	X*	X*	X*	X*
Pancreas	X	X	X	X
Pharynx				
Pituitary	X*	X*	X*	X*
Prostate	X*	X*	X*	X*
Rectum			X	
Salivary gland	X	X	X*	X
Sciatic nerve	X	X	X	X
Seminal vesicles	X	X	X*	X
Skeletal muscle	X	X	X	X
Skin	X	X	X	X
Spinal cord	X	X	X	X
Spleen	X	X*	X	X*
Sternum	X	X	X	X
Stomach	X	X	X	X
Testes	X*	X*	X*	X*
Thymus	X*	X*	X*	X*
Thyroid w/ parathyroid	X*	X*	X*	X*
Tongue	X	X	X	X
Trachea	X	X	X	X
Urinary bladder	X	X	X	X
Uterus	X*	X*	X*	X*
Vagina	X	X	X	X
Zymbal gland				

X, histopathology performed; *, organ weight obtained; [a], weighed with thyroid; [b], weighed with liver

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7 Genetic Toxicology

Genetic toxicology studies were not conducted per the guidance presented in ICH S6 for biotechnology-derived products. The linker that connects the peptide with the IgG molecule is a small peptide linker of natural amino acids, and therefore is not an organic linker of genotoxic concern.

8 Carcinogenicity

Study title: A carcinogenicity and toxicokinetic study in 001178-T (hemizygous) mice given LY2189265 twice weekly by subcutaneous injection for 26 weeks

Note: The primary reviewer for this study report was Dr. Dan Minck.

Study Number:	8224144, Amendment 1
Study report location:	Module 4.2.3.4
Laboratory name:	(b) (4)
Date of study initiation:	30 March 2011
GLP compliance statement:	Yes
GLP issues identified:	No
QA statement:	Yes
Drug, lot #, and % purity:	LY2189265, lot 17804-1, 99.3% purity
CAC concurrence:	Yes

Key Study Findings

- There were no LY2189265-related mortalities or adverse clinical signs.
- Reduced body weight gains in the 3-day dosing intervals and increased weight gains in the 4-day dosing intervals occurred in all drug-treated groups of both sexes. In males, dose-related reductions in body weight gain of 42% to 54% led to 14% to 20% reductions in mean body weight by the end of the study. In contrast, inverse dose-related increases of 8% to 30% in weight gain led to body weights that were 4% to 7% higher than control in all drug-treated female groups by the end of the study. Effects on feed consumption followed a similar pattern.
- There were no statistically significant increases in the incidence of neoplastic lesions for groups treated with LY2189265.
- Non-neoplastic lesions included an increased incidence of extramedullary hematopoiesis in females at all dose levels. This was associated with increased spleen weights and likely reflects a regenerative response to the decreases in erythrocyte parameters (red blood cell count, hemoglobin, and hematocrit), an increase in cytoplasmic volume of thyroid C-cells stained with calcitonin (increased C-cell hyperplasia or neoplasia was not observed) at all dose levels, and a slight increase in incidence and severity of injection site inflammation in HD males and females. There were no compound-related effects observed in histologic sections of the pancreas.

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- The NOEL for carcinogenicity was the highest dose administered, 3 mg/kg. At this dose level, the mean AUC_{0-96} was 37 µg•hr/mL, which is approximately 5-fold higher than the anticipated mean weekly clinical exposure (AUC_{0-168h}).
- The positive control MNU induced neoplastic lesions in multiple tissues, with the lesions being responsible for much of the mortality observed.

Adequacy of Carcinogenicity Study
The carcinogenicity study was adequately designed and conducted.

Appropriateness of Test Models
The test model was appropriate to assess the carcinogenic potential of LY2189265.

Evaluation of Tumor Findings
The administration of LY2189265 by twice weekly subcutaneous injection to hemizygous CByB6F1-Tg(HRAS)2Jic mice for 26 weeks had no effect on survival nor was there an indication of an increase in tumor induction. Drug-related effects of note were limited to microscopic effects in the thyroid gland and an increased incidence of extramedullary hematopoiesis in females at all dose levels. The NOEL for carcinogenicity in this study was the high dose of 3 mg/kg.

The administration of the positive control substance N-methyl-N-nitrosourea produced increased incidences of tumors, particularly in the stomach and skin. The positive control showed higher incidences of non-glandular stomach papilloma and/or carcinoma, skin/subcutis squamous cell lesions (papilloma and carcinoma), and higher incidences of body, whole/cavity lymphosarcoma in both males and females.

Methods

Species/Strain:	Carcinogenicity Animals: 001178-T (hemizygous), CByB6F1-Tg(HRAS)2Jic Toxicokinetic Animals: 001178-W (wild type), CByB6F1-Tg(HRAS)2Jic
Dose Levels:	0 (vehicle), 0.3, 1, and 3 mg/kg
Frequency of dosing:	Twice weekly
Dose volume:	3 mL/kg
Route of administration:	Subcutaneous injection
Positive control:	Single i.p. injection of 75 mg/kg MNU (10 mL/kg)
Formulation/Vehicle:	(b) (4) Na citrate (b) (4) (b) (4) mannitol, and (b) (4) polysorbate 80 in sterile water for injection. Saline for MNU.
Basis of dose selection:	2-week study in CD-1 mice; 4-week study in 001178-T wild type mice
Number/Sex/Group:	25 for main group
Age at initiation:	8 – 9 weeks
Animal housing:	Individually in wire cages

Paradigm for dietary restriction: NA
Dual control employed: Yes
Interim sacrifice: No
TK Satellite groups: 12/sex/group (control) + 42/sex/group (treated)
Protocol Deviations: Multiple, but none were considered to affect study integrity or overall study interpretation

Note: The following abbreviations are used in reference to the dose levels: C = 0 (control); LD = 0.3 mg/kg; MD = 1 mg/kg; HD = 3 mg/kg; MNU = N-methyl-N-nitrosourea

Observations and Results

Mortality: There was no adverse effect on survival. The numbers of animals found dead (FD) or electively euthanized (EE) at unscheduled intervals are summarized in the sponsor table below:

Text Table 3
Unscheduled Mortalities (Control and LY2189265-Treated Animals)

Sex	Male				Female			
Dose Level (mg/kg)	0	0.3	1.0	3.0	0	0.3	1.0	3.0
Carcinogenicity Animals	5	2	2	1	1	0	1	2
Toxicokinetic Animals	0	1	0	0	0	0	1	0

Clinical signs in FD/EE animals included general signs of poor condition, such as rough hair coat, few feces, hunched posture, irregular breathing. Additional reasons for euthanizing animals included a single MD female found out of its cage and 2 females (1 C and 1 HD) with limited use of hind limbs. The main cause of death for control and drug-treated animals were non-treatment-related neoplasms, including squamous cell carcinoma, hemangiosarcoma, bronchiolar-alveolar carcinoma, and lymphosarcoma. Non-neoplastic causes of death included inflammation and hemmorhage at various sites.

A high incidence of unscheduled mortality occurred in the MNU groups. There were 11/25 males and 15/25 females found dead or euthanized early (statistically significant). Masses were observed on the backs, scrotum, or perivaginal areas of these animals.

Clinical Signs: There were no compound-related clinical signs, although some observations occurred more frequently in some of the drug-treated groups. Observations included increased incidences of missing parts of the ear in HD males and females, hunched posture in HD females, few feces in HD males/females, and a slightly higher incidence of rough hair coat in all drug treated male groups. Many of these signs were noted in the animals found dead or electively euthanized.

For males and females administered MNU, treatment-related clinical signs included hunched posture, few feces, masses, and rough hair coat; irregular and labored breathing was also noted in MNU females. As with the drug-treated groups, these signs were most often associated with animals that were later found dead or euthanized early.

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Reviewer comment: The relevance of some of these observations is unclear. For instance, missing/partial ears were observed on days 8 and/or 15 yet not reported at later time points.

Body Weights: Effects on body weight followed a pattern of reduced weight gain or weight loss during the intervals with 3 days between doses and increases in weight gain during intervals with 4 days between doses. This pattern was observed in both males and females. However, the overall effect on weight gain was different for the two sexes. For males, over the course of the entire study (Days 1 – 183) there was a statistically significant dose-related reduction in weight gain leading to statistically significant reductions in absolute body weights in all drug-treated groups (14% to 20% lower) by the end of the study (Day 183). In contrast, all groups of females gained weight over the course of the treatment period (Days 1 – 183; statistically significant) leading to slightly higher body weights (4% to 7% higher; statistically significant) at the end of the study (Day 183). Selected data are summarized in the reviewer created tables and sponsor figures below:

Males - Mean Body Weight/Body Weight Gain (% difference from controls)

	Dosage (mg/kg)				
Day or Interval	**0**	**0.3**	**1**	**3**	**MNU[1]**
1	23.4	23.0	23.0	22.6	23.3
4	23.9	23.1	22.6* (-5)	21.9* (-8)	22.8
92	29.8	27.6	26.4* (-11)	26.3* (-12)	28.1
183	33.7	29.0* (-14)	28.7* (-15)	27.4* (-20)	30.2
1 – 92[2]	6.4	4.6 (-28)	3.4 (-47)	3.7 (-42)	4.8
92 – 183[2]	3.9	1.4 (-64)	2.3 (-40)	1.1 (-72)	2.1
1 - 183	10.3	6.0* (-42)	5.8* (-44)	4.7* (-54)	7.2

[1] MNU group not statistically evaluated
[2] Calculated by reviewer; not statistically evaluated
* $p \leq 0.05\%$

Females - Mean Body Weight/Body Weight Gain (% difference from controls[1])

	Dosage (mg/kg)				
Day or Interval	**0**	**0.3**	**1**	**3**	**MNU[1]**
1	17.4	17.5	17.6	17.8	18.0
4	17.9	17.9	17.3	16.9* (-6)	17.7
92	21.8	23.1* (+6)	22.8* (+5)	22.3 (+2)	21.8
183	22.4	24.0* (+7)	24.0* (+7)	23.4* (+4)	23.0
1 – 92[2]	4.4	5.6 (+27)	5.2 (+18)	4.5 (+2)	3.8
92 – 183[2]	0.6	0.9 (+50)	1.2 (+100)	1.1 (+83)	1.2
1 - 183	5.0	6.5* (+30)	6.4* (+28)	5.4* (+8)	5.2

[1] MNU group not statistically evaluated
[2] Calculated by reviewer; not statistically evaluated
* $p \leq 0.05\%$

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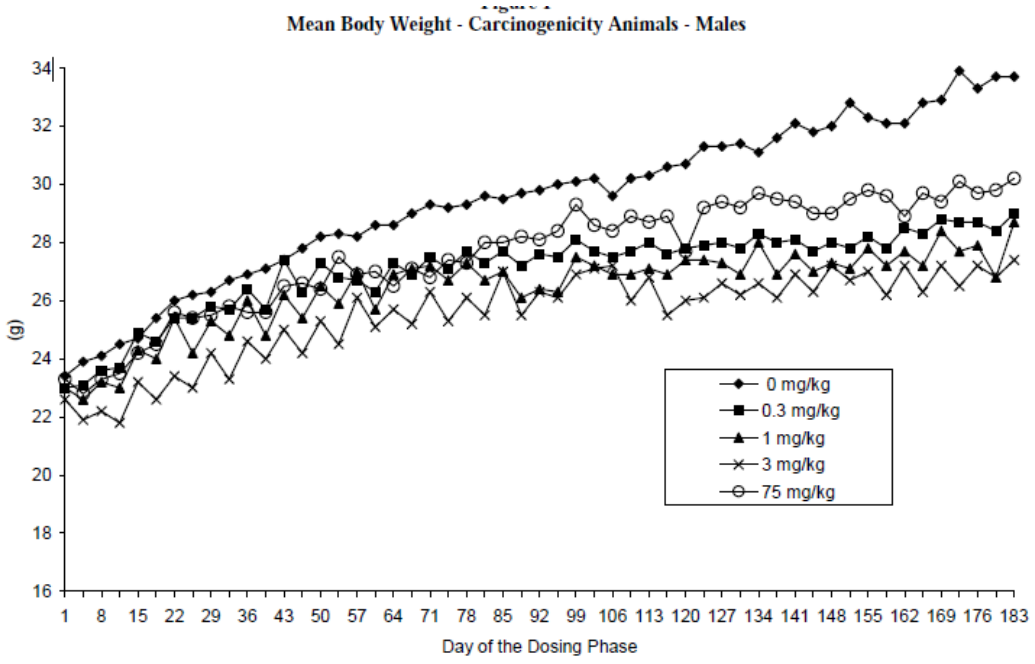
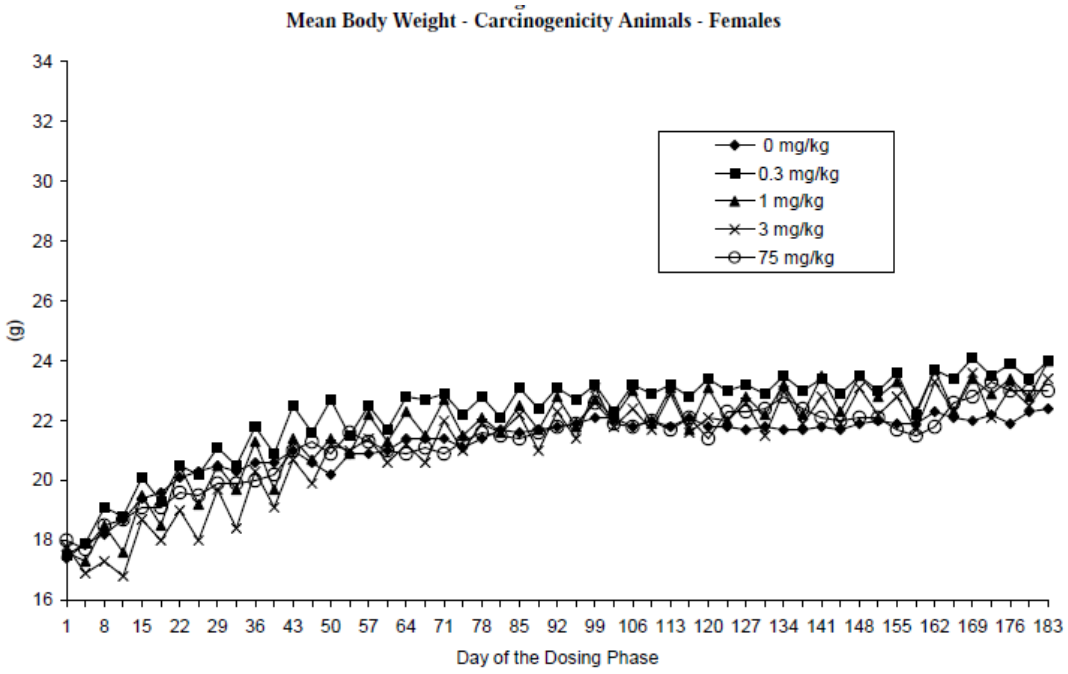
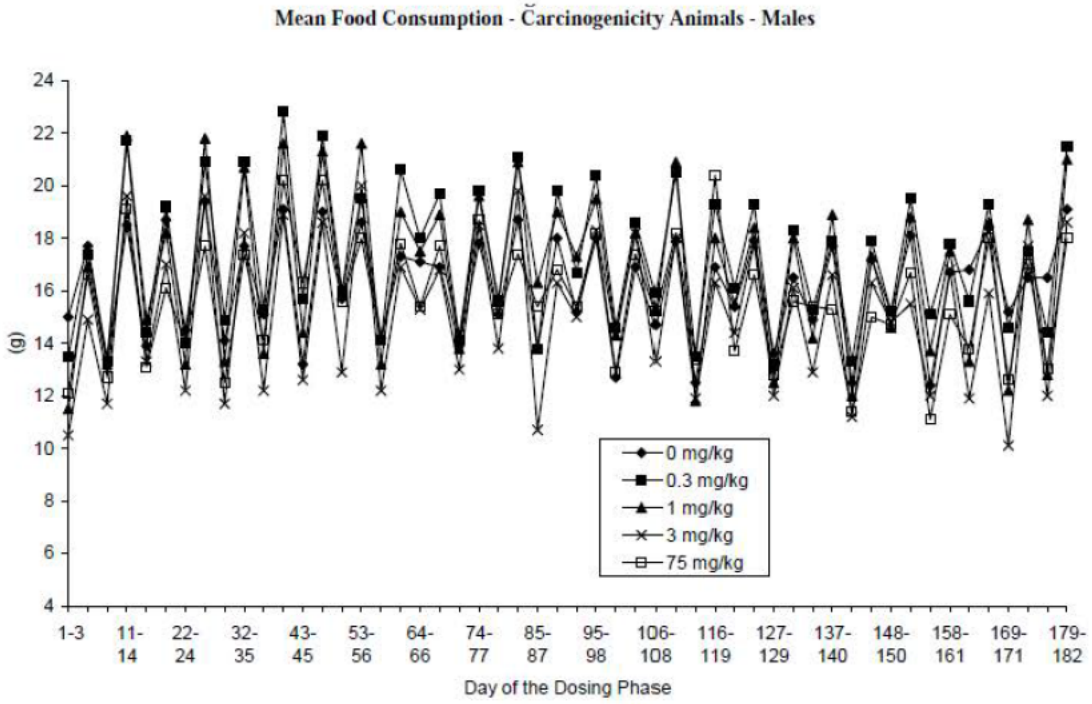


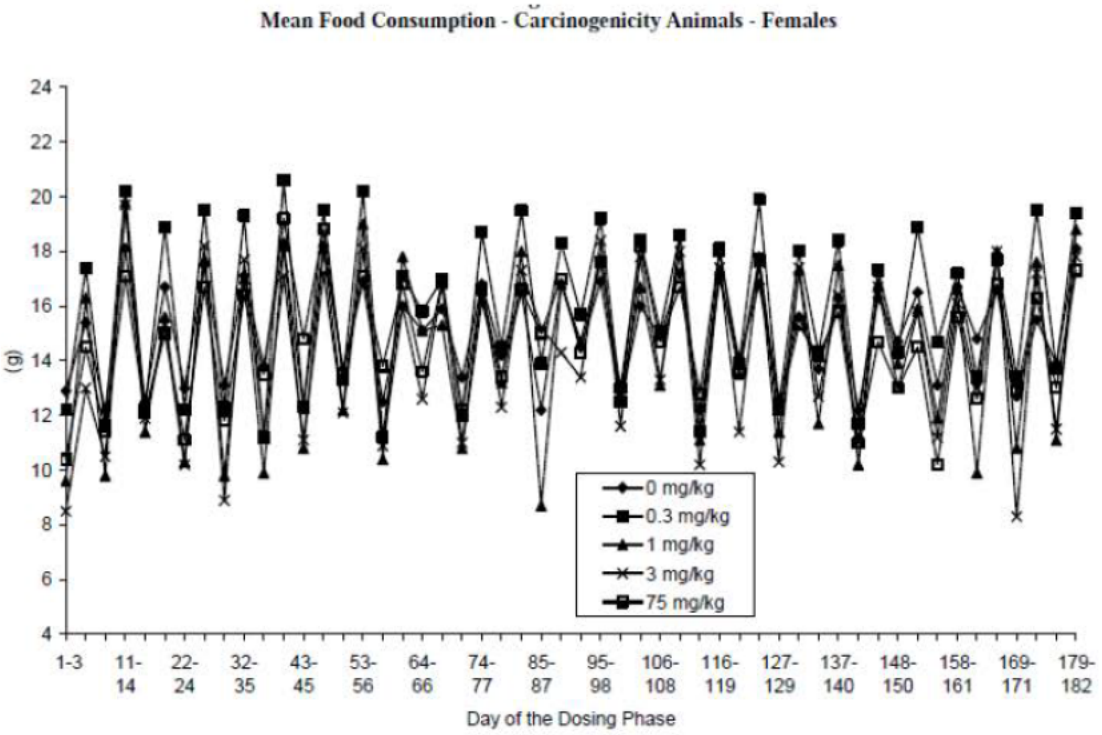


Although not summarized or shown, a similar pattern was seen with the TK groups administered LY2189265. For animals administered MNU, slight reductions in mean body weight or mean body weight gain were seen in males, but not females.

Feed Consumption: The pattern of feed consumption typically paralleled that seen for body weights. In MD and/or HD males, feed consumption was lower than controls during the intervals with 3 days between doses but occasionally higher than controls

during the intervals with 4 days between doses. A similar pattern was seen in females, but the differences from controls for any given interval were minimal. For both sexes, some of the differences for a given interval reached statistical significance. Feed consumption for the MNU group was similar to controls for both sexes. Data are shown in the sponsor figures below:





Ophthalmology: No effects noted.

Clinical Pathology: There were a number of minor variations that attained statistical significance noted in various clinical chemistry parameters.

Effects on hematologic parameters in males included decreases in red blood cells (<9%), hemoglobin (<8%), hematocrit (<6%), and mean cell hemoglobin concentration (<3%), and increases in mean cell volume (<3%) and reticulocytes (<22%) at all dose levels, with the effects attaining statistical significance for the majority of these alterations. In females, decreases in red blood cells (<8%), hemoglobin (<8%), and hematocrit (<6%) were evident at the MD and HD, and increases in reticulocytes (<43%) were observed at all dose levels. Although these effects may be treatment-related, they are considered minor changes. The decreased red cell mass may have contributed to the increased extramedullary hematopoiesis seen in the spleen of females (see microscopic observations below).

Statistically significant alterations in clinical chemistry parameters were limited to slight decreases in globulin (<15%) leading to slightly higher albumin/globulin ratios (<12%) at all dose levels in males. In females, slightly higher albumin levels (<9%) resulted in similar increases in the albumin/globulin ratios (<14%) at all dose levels. Minimally higher alkaline phosphatase levels (<39%) were also evident at all dose levels in males, but not in females.

In groups administered MNU, the same parameters affected by drug treatment were affected, but the magnitude of the changes were generally smaller than those occurring in the drug-treated groups.

Immunogenicity: Blood samples were collected from TK animals for possible immunogenicity analysis. However, as exposure to test article was seen through study termination and evidence of pharmacologic activity was seen throughout treatment, samples were not analyzed.

Gross Pathology: There were no treatment-related macroscopic observations in animals subjected to unscheduled sacrifices or to those euthanized at the end of treatment.

In MNU animals subjected to unscheduled exams, multiple macroscopic effects were noted. Organs/tissues affected included the spleen (large), thymus (mass), stomach (mass, raised area in glandular and/or non-glandular), various lymph nodes (large), skin/subcutis (mass), and uterus (cyst, mass, large). In MNU treated groups euthanized as scheduled, there was an increased incidence of masses and/or raised area observed in the glandular stomach of females and in the non-glandular stomach of both sexes, masses in the skin/subcutis of both sexes, and enlarged and/or cysts of the uterus.

Reference ID: 3527260

Organ Weights: There were no changes in mean organ weights for males that were attributed to treatment.

In all female groups receiving LY2189265, increases in mean absolute (5% - 28%; not dose-related and only statistically significant at MD) and relative (<35%; only statistically significant at the MD) spleen weights were observed. The increased weights were associated with a modest increase in extramedullary hematopoiesis at all dose levels. Similar effects did not occur in males.

Histopathology

Battery Considered Adequate? Yes
Peer Review Performed? Yes

The occurrence of non-neoplastic and neoplastic lesions was similar in incidence and severity between the various drug-treated groups and controls, with the exception of effects on the spleen, thyroid, and injection sites where non-neoplastic lesions were increased in incidence and/or severity in the drug-treated groups. For the positive control group, microscopic examinations were limited to the thymus and gross lesions.

Non-neoplastic observations

Non-neoplastic effects were evident in the spleen, thyroid, and at the injection sites.

In the spleen there was an increased incidence of minimal to mild extramedullary hematopoiesis in females at all dose levels. This may have been a regenerative response to the decrease in red cell mass (red blood cell count, hemoglobin, and hematocrit) noted in females and was also correlated with the increased spleen weight. A similar effect was not seen in males even though a similar decrease in red cell mass was noted. The incidence of other microscopic effects in the spleen was consistent between the groups. Data are summarized in the sponsor-generated table below:

In the thyroid there were no treatment-related effects based on evaluation of routine H and E stained sections. However, in sections stained with calcitonin to identify thyroid C-cells, an increase in cytoplasmic volume was detected in all LY2189265-treated groups. The severity grade was minimal in all cases. There was no apparent increase in the number of C-cells. Sponsor data from the immunohistochemistry exams are shown in the table below:

	LY2189265							
Sex	Males				Females			
Dose Level (mg/kg)	0	0.3	1	3	0	0.3	1	3
Thyroid (Immunohistochem-Calcitonin)								
Number Examined	20	23	23	23	24	25	24	23
C-cells, cytoplasmic hypertrophy/ increased calcitonin staining	1	6	10	16	0	3	11	10
Spleen								
Number Examined	25	25	25	25	25	25	25	25
Extramedullary Hematopoiesis, Increased	3	1	0	3	4	16	24	21

At the injection sites, inflammation (characterized by infiltrates of neutrophils and macrophages) was a common observation generally occurring at a higher incidence in the LY2189265 groups. It was typically minimal in severity though the severity was increased slightly at the HD. No microscopic evidence of degeneration or necrosis of subcutaneous tissues was noted. The data are shown in the sponsor table below:

Controls from group(s): 1	Animal sex:	-- Males --					-- Females --				
	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
Tissues With Diagnoses	No. in group:	25	25	25	25	25	25	25	25	25	25
Subcutan Site A .....Number examined:		25	25	25	25	0	25	25	25	25	0
Inflammation											
	->	14	11	7	5	0	17	8	5	4	0
	1>	11	13	17	15	0	8	16	18	14	0
	2>	0	1	1	5	0	0	1	2	7	0
.....Total Incidence of Finding Observed:		11	14	18	20	0	8	17	20	21	0
Subcutan Site B .....Number examined:		25	25	25	25	0	25	25	25	25	0
C-Vascular Neoplasm, see Body, Whole for type											
	->	25	25	25	24	0	25	25	25	25	0
	^>	0	0	0	1	0	0	0	0	0	0
.....Total Incidence of Finding Observed:		0	0	0	1	0	0	0	0	0	0
Inflammation											
	->	8	12	7	5	0	8	11	5	2	0
	1>	13	13	17	8	0	17	12	13	11	0
	2>	4	0	1	11	0	0	2	6	8	0
	3>	0	0	0	1	0	0	0	1	4	0
.....Total Incidence of Finding Observed:		17	13	18	20	0	17	14	20	23	0

Note: groups 2, 3, 4, and 5 refer to 0.3, 1, and 3 mg/kg of LY2189265 or the MNU group, respectively.

There were no drug-related effects observed upon microscopic examination of the pancreas. There was also no indication of a treatment-related increase in hyperplasia in any organ/tissues resulting from treatment with LY2189265. Additional effects observed occurred at an incidence comparable to that in control groups or did not follow a dose-response. Observations of hyperplasia are summarized in the sponsor-generated table included in the neoplastic observations section below.

Neoplastic observations

The incidence of neoplastic lesions was comparable between the LY2189265-treated groups and controls. There also was no indication of an increase in hyperplasia in any organ/tissues resulting from treatment with LY2189265 that may have progressed to neoplasia. In the various organs in which neoplastic lesions were observed, they typically occurred at comparable incidences between the drug-treated and control groups, did not necessarily follow a dose-response, were considered typical for the strain of mouse, and/or were in the range of published data.

In females, the highest incidences of vascular neoplasms were observed in the spleens (HD) and uterus (MD). The majority of these findings in both males and females were benign hemangiomas or hemangiosarcomas. These observations in females contributed to a statistically significant trend (p = 0.0196) for an increase in the incidence of 'body, whole cavity hemangioma' when evaluated across all organs/tissues (1, 0, 2, and 4 in the control, LD, MD, and HD groups, respectively), but the HD incidence was not statistically different from controls by pair-wise analysis. Additionally, no statistically significant effects were seen when hemangiomas and hemangiosarcomas were combined (see FDA statistical tables following incidence tables below). It should be

noted that the incidence of hemangiomas was in excess of the historical range (0% to 8%), but given there were no differences in the comparisons to controls, this observation is considered incidental (see sponsor-generated Table 1 below for historical control data).

In the positive control groups administered MNU (Group 5), there was a high incidence of neoplasms observed in multiple organs/tissues. The neoplastic lesions were the main cause of death in the MNU groups. For males, the positive control group showed increases in neoplasia of the nonglandular stomach (squamous cell papilloma, carcinoma, combined papilloma and carcinoma), skin/subcutis (squamous cell papilloma, combined papilloma and carcinoma), and a statistically significant increase in body, whole/cavity lymphosarcoma. A similar pattern was seen for females; the positive control group showed increases in neoplasia of the nonglandular stomach (squamous cell papilloma, combined papilloma and carcinoma), skin/subcutis (squamous cell papilloma; combined papilloma and carcinoma), uterus endometrial stromal polyp; and whole/cavity malignant lymphosarcoma. In addition to the organs/tissues summarized in the data display above, hematopoietic neoplasms were also observed in the eye (1F), axillary (2M, 2F) and mandibular (1F) lymph nodes, ovary (1F), and ureter (1M).

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males					Females				
Tissues With Diagnoses	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
	No. in group:	25	25	25	25	25	25	25	25	25	25
Body, Whole/Cav	Number examined:	25	25	25	25	25	25	25	25	25	25
	Unremarkable:	22	23	24	21	16	23	22	22	21	5
M-Leukemia, Erythrocytic		0	0	0	0	0	0	0	0	0	0
M-Hemangiosarcoma		3	2	1	4	0	1	3	1	0	3
M-Lymphosarcoma		0	1	0	0	9	0	0	0	0	19
B-Benign Hemangioma		0	0	0	0	0	1	0	2	4	2
Skin/Subcutis	Number examined:	25	25	25	25	14	25	25	25	25	15
	Unremarkable:	22	24	25	24	1	23	24	25	24	0
M-Carcinoma, Squamous Cell		1	0	0	0	0	0	0	0	0	3
B-Papilloma, Squamous Cell		1	0	0	0	6	0	0	0	0	9
B-Papilloma, Squamous Cell, Multiple		0	0	0	0	7	0	0	0	0	3
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	1
C-Vascular Neoplasm, see Body, Whole for type		1	0	0	0	0	1	0	0	0	0
Hemorrhage		0	1	0	1	0	0	0	0	0	0
Inflammation		0	0	0	1	0	1	0	0	1	0
Acanthosis		0	0	0	0	0	1	1	0	0	0
Acanthosis/Hyperkeratosis		0	0	0	0	0	0	0	0	0	2
Stomach, Nongl	Number examined:	24	25	25	25	22	25	25	25	25	21
	Unremarkable:	23	23	25	25	0	23	24	22	25	1
M-Carcinoma, Squamous Cell		0	0	0	0	5	0	0	0	0	1
B-Papilloma, Squamous Cell		0	0	0	0	10	0	0	0	0	10
B-Papilloma, Squamous Cell, Multiple		0	0	0	0	4	0	0	0	0	6
C-Hematopoietic Neoplasm, see Body, Whole for type		0	0	0	0	0	0	0	0	0	1
Hyperkeratosis		1	2	0	0	1	2	1	3	0	9
Hyperplasia, Squamous Cell		1	2	0	0	3	1	0	2	0	10
Inflammation		0	1	0	0	0	0	1	0	0	0
Uterus	Number examined:						25	25	25	25	13
	Unremarkable:						16	20	21	19	0

Note: Groups 2, 3, and 4 are the LD, MD, and HD LY2189265-treated groups, respectively. Group 5 is the positive control (MNU) group. The following codes are used for the neoplastic observations: B = primary benign neoplasm, C = multi-centric neoplasm, M = primary malignant neoplasm.

There were no statistically significant increases in tumor incidence following the combination of various tumor types, as shown in the FDA statistical reviewer tables below:

Table of reported tumors in Mouse Study
IND 70930
Animal carcinogenicity study
Male mice

Organ or tissue name	*Tumor name*	*Quantity*	*Control Size = 25*	*Low dose Size = 25*	*Mid dose Size = 25*	*High dose Size = 25*
Body, Whole/Cav	Hemangiosarcoma	P-value of test of trend or comparison	.2503	.8395	.9504	.5487
		Number of animals reported with tumor	3	2	1	4
Hemangiomas and hemangiosarcomas		P-value of test of trend or comparison	.2503	.8395	.9504	.5487
		Number of animals reported with tumor	3	2	1	4

Table of reported tumors in Mouse Study
IND 70930
Animal carcinogenicity study
Female mice

Organ or tissue name	*Tumor name*	*Quantity*	*Control Size = 25*	*Low dose Size = 25*	*Mid dose Size = 25*	*High dose Size = 25*
Body, Whole/Cav	Hemangioma	P-value of test of trend or comparison	.0196	1	.5000	.1605
		Number of animals reported with tumor	1	0	2	4
	Hemangiosarcoma	P-value of test of trend or comparison	.9075	.3046	.7449	1
		Number of animals reported with tumor	1	3	1	0
Hemangiomas and hemangiosarcomas		P-value of test of trend or comparison	.1882	.5000	.4800	.2933
		Number of animals reported with tumor	2	3	3	4

Table 1: [redacted] (b) (4) Historical Control Data: Incidence of Hemangiosarcoma and Hemangioma in 6-month studies of 001178-T(hemizygous), CByB6F1-Tg(HRAS)2Jic mice

Number of Studies = 6		Males Number Examined = 175		Females Number Examined = 175	
Observation	Tissue	Mean Incidence[a]	Range[b]	Mean Incidence[a]	Range[b]
Hemangiosarcoma	Body, Whole Cavity	8.0%	4.0% to 16.0%	8.6%	4.0% to 16.0%
Hemangioma	Body, Whole Cavity	1.1%	0.0% to 8.0%	2.3%	0.0% to 8.0%

Note: [redacted] (b) (4) 5-year Historical Control Search Conducted January 2014.

a Mean Incidence: Total number of animals recorded with observation/Total number of animals examined.

b Range: Range of control incidence across studies. For example, the incidence of hemangiosarcoma in males ranged from 4.0% in one study to 16.0% in a different study.

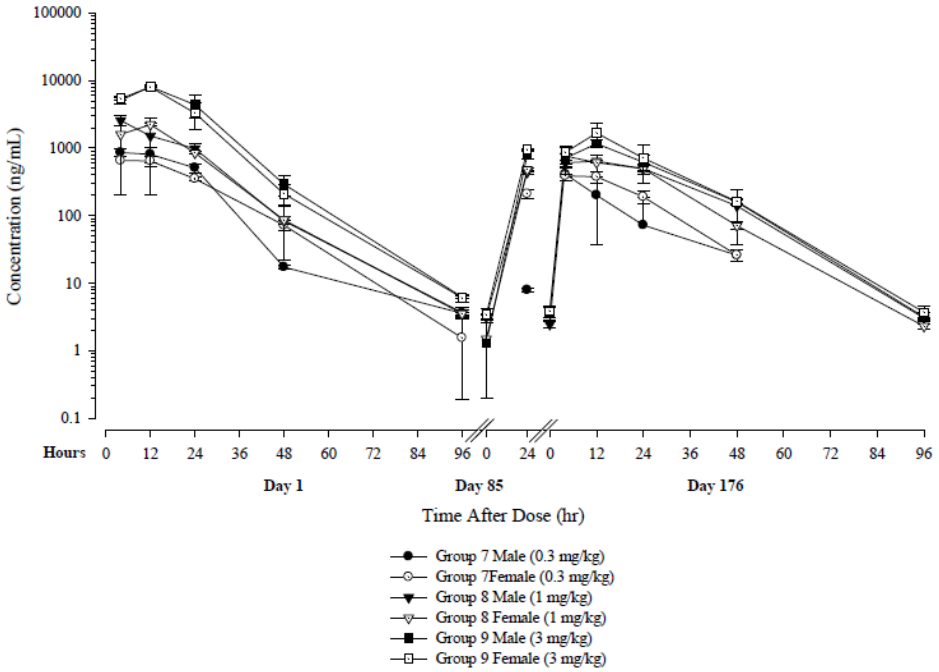
Toxicokinetics: Exposure to LY2189265 increased with dose, but not dose-proportionally. Values for both C_{max} and AUC were lower after multiple dosing on Day 176 as compared to that seen on Day 1 (plasma drug levels on Day 85 were also lower than those on Day 1). The t_{max} was similar on both Days 1 and 176, ranging from 4 to 12 hours. There was no apparent gender effect. Data are summarized in the sponsor table and figure below:

Table 1
Toxicokinetic Parameters of LY2189265 in Male and Female Mice Following Twice-Weekly Subcutaneous Administration of 0.3, 1, or 3 mg/kg LY2189265: Days 1 and 176, Study 8224144

Day	Group	Dose Level (mg/kg)	Sex	C_{max} (µg/mL)	SD C_{max}[a] (µg/mL)	T_{max} (hr)	AUC_{0-96hr} (µg·hr/mL)	SEM AUC_{0-96hr} (µg·hr/mL)
1	7	0.3	M	0.860	0.108	4.00	23.1	1.48
			F	0.653	NA	4.00	19.3	1.08
	8	1	M	2.56	0.457	4.00	51.2	7.98
			F	2.23	0.0742	12.0	50.3	4.86
	9	3	M	8.09	0.341	12.0	201	17.3
			F	8.00	0.630	12.0	179	15.5
176	7	0.3	M	0.414	0.0864	4.00	6.76	1.55
			F	0.391	0.0116	4.00	10.5	0.600
	8	1	M	0.645	0.0324	12.0	24.2	2.32
			F	0.769	0.239	4.00	22.4	1.59
	9	3	M	1.17	0.160	12.0	33.1	1.95
			F	1.69	0.627	12.0	40.9	5.65

NA Not applicable.
a SD for C_{max} is reported as the SD for the mean concentration at T_{max}.

Figure 1
Mean (±SD) Plasma Concentrations (ng/mL) of LY2189265 in Male and Female Mice Following Twice-Weekly Subcutaneous Administration of 0.3, 1, or 3 mg/kg LY2189265 for 26 weeks: Study 8224144



Dosing Formulation Analysis: Dose formulations were stable under the conditions of use. Dosing formulations ranged from 100% to 134% of claim, with the two highest values (112% and 134% occurring at the week 9 and 18 samplings from the LD formulations. All other formulations at this concentration as well as for the other groups ranged from 100% to 109% of claim. Although investigated, the causes for the above claim results were not determined. The higher than desired test-article formulations for the LD group were not considered to have affected study interpretations by the sponsor.

> *Reviewer comment: Given that half of the samples evaluated at the low concentration were above the desired claim range it is likely that dosing formulations prepared at this concentration for dosing during other weeks would also be higher than expected. As a result, it would not be surprising if the exposure data obtained at the LD are also slightly higher than that which would actually occur at the nominal dose. Regardless, this reviewer agrees with the sponsor interpretation that the overall effect on the study is minimal.*

Study title: 104-week subcutaneous injection carcinogenicity and toxicokinetic study with LY2189265 (Compound 2189265) in rats

Note: The primary reviewer for this study report was Dr. Dan Minck.

Study number:	8203405
Study report location:	Module 4.2.3.4
Conducting laboratory:	(b) (4)
Date of study initiation:	31 August 2009
GLP compliance:	Yes
GLP issues identified:	None that impacted the study results
QA statement:	Yes
Drug, lot #, and % purity:	LY2189265, lot 17804-1, 99.3% purity
CAC concurrence:	Yes

Key Study Findings

- Mortality in the control groups led to the termination of the study at 93 weeks. Enhanced survival of treated groups may have resulted from drug-related decreases in feed consumption and lower body weights.
- Reduced feed consumption and decreased body weight gain leading to lighter body weights was evident in the treated groups.
- No treatment-related increases in the incidence of non-neoplastic lesions were evident for combined scheduled- and unscheduled-sacrifice animals.
- A statistically significant increase in the incidence of thyroid C-cell adenomas was observed for males and females administered ≥0.5 mg/kg. Additionally, a non-statistically significant numerical increase in the incidence of C-cell carcinomas occurred in both sexes administered 5 mg/kg, resulting in a statistically significant trend for an increase in C-cell carcinomas. The increased incidence of adenomas at ≥0.5 mg/kg resulted in a statistically significant increase for the combination of adenomas and carcinomas in these same groups and by trend analysis.
- Hyperplasia of thyroid C-cells was also evident as an increased incidence of diffuse and focal (females only) thyroid C-cell hyperplasia was seen at ≥0.5 mg/kg. In males, there was a dose-related decrease in focal C-cell hyperplasia associated with a dose-related increase in C-cell adenomas, suggesting a progression from hyperplasia to adenoma in these groups.
- The NOEL for C-cell tumors in Sprague Dawley rats was the LD of 0.05 mg/kg/d, corresponding with a mean $AUC_{0\text{-}96h}$ of 4.06 µg•hr/mL at Week 52.

Adequacy of Carcinogenicity Study

The carcinogenicity study was adequately designed and conducted.

Appropriateness of Test Models

The test model was appropriate to assess the carcinogenic potential of LY2189265.

Reference ID: 3527260

Evaluation of Tumor Findings

The administration of LY2189265 twice weekly by subcutaneous injection to Sprague-Dawley rats for approximately two years resulted in an increased incidence of thyroid C-cell tumors. Statistically significant increases in the incidence of thyroid C-cell adenomas or adenomas plus carcinomas were observed at ≥0.5 mg/kg in males and at 5.0 mg/kg in females. Numerical increases in C-cell adenomas were observed in females at 0.5 and 1.5 mg/kg and these increases were considered to be treatment related. A numerical increase in C-cell carcinomas was observed for both sexes at 5.0 mg/kg; these increases were also felt to be treatment related, even in the absence of statistical significance by pair-wise analysis. Trend analysis for C-cell carcinomas was statistically significant for females but was slightly above statistical significance for males. The NOEL for neoplasia was considered to be the low dose of 0.05 mg/kg by this reviewer and the sponsor.

Methods

Species/Strain: Rat/Sprague-Dawley

Study Design: (sponsor-generated table)

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg LY2189265/kg)	Dose Concentration (mg LY2189265/mL)
Toxicity Animals				
1 (Control)	60	60	0	0
2 (Low)	60	60	0.05	0.1
3 (Mid)	60	60	0.5	1
4 (Mid-High)	60	60	1.5	3
5 (High)	60	60	5	10
Toxicokinetic Animals				
6 (Control)	6	6	0	0
7 (Low)	12	12	0.05	0.1
8 (Mid)	12	12	0.5	1
9 (Mid-High)	12	12	1.5	3
10 (High)	12	12	5	10

Frequency of dosing: Twice weekly

Dose volume: 0.5 mL/kg

Route of administration: Subcutaneous

Formulation/Vehicle: (b) (4) Na citrate (b) (4) mannitol, and (b) (4) polysorbate 80 in sterile water for injection.

Basis of dose selection: 6-month toxicology study

Age: 7 weeks

Animal housing: Individually in metal cages. Males switched to solid-bottom cages at Day 496

Paradigm for dietary restriction: NA

Dual control employed: No

Interim sacrifice: No

Deviation from study protocol: Multiple, but none considered to affect study integrity or overall study interpretations

Observations and Results

Mortality: There were no treatment-related mortalities. The study was terminated during Week 93 based on low survival in the control groups. Survival of drug-treated groups was longer than controls, with the differences achieving statistical significance for several groups as summarized in the sponsor table below:

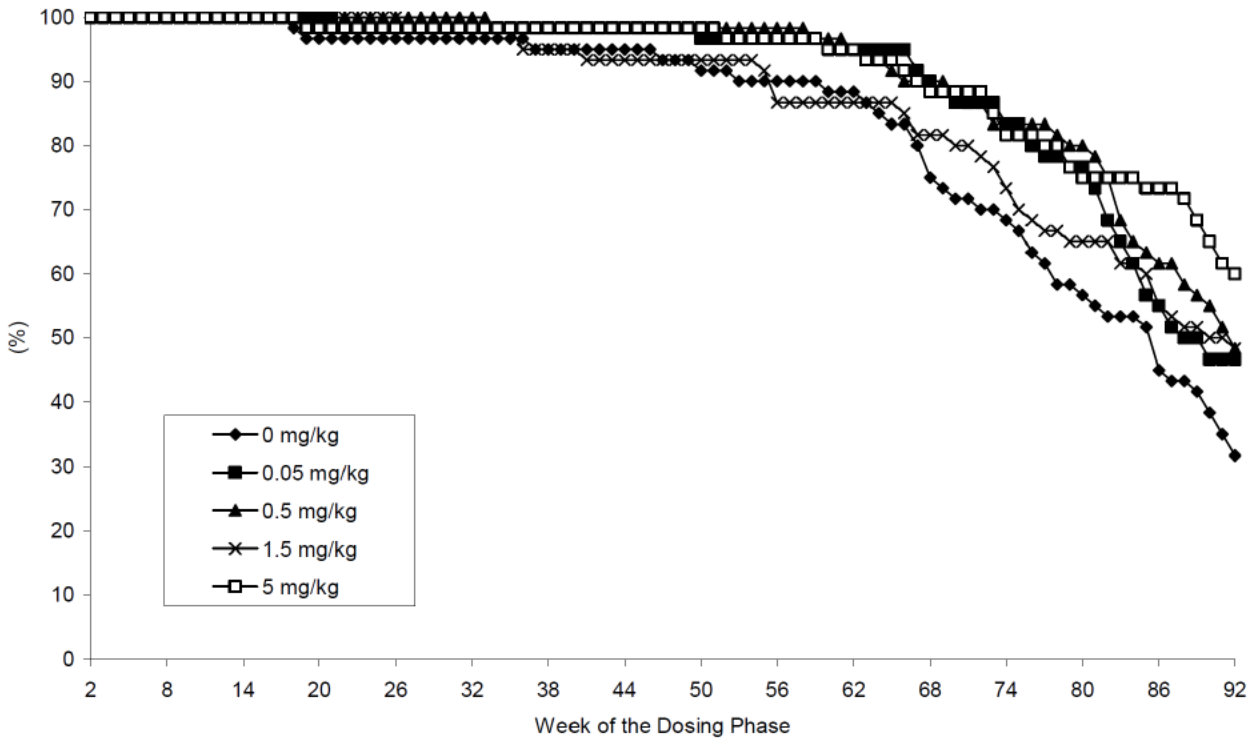
Week 93 Percentage Survival - Toxicity Animals

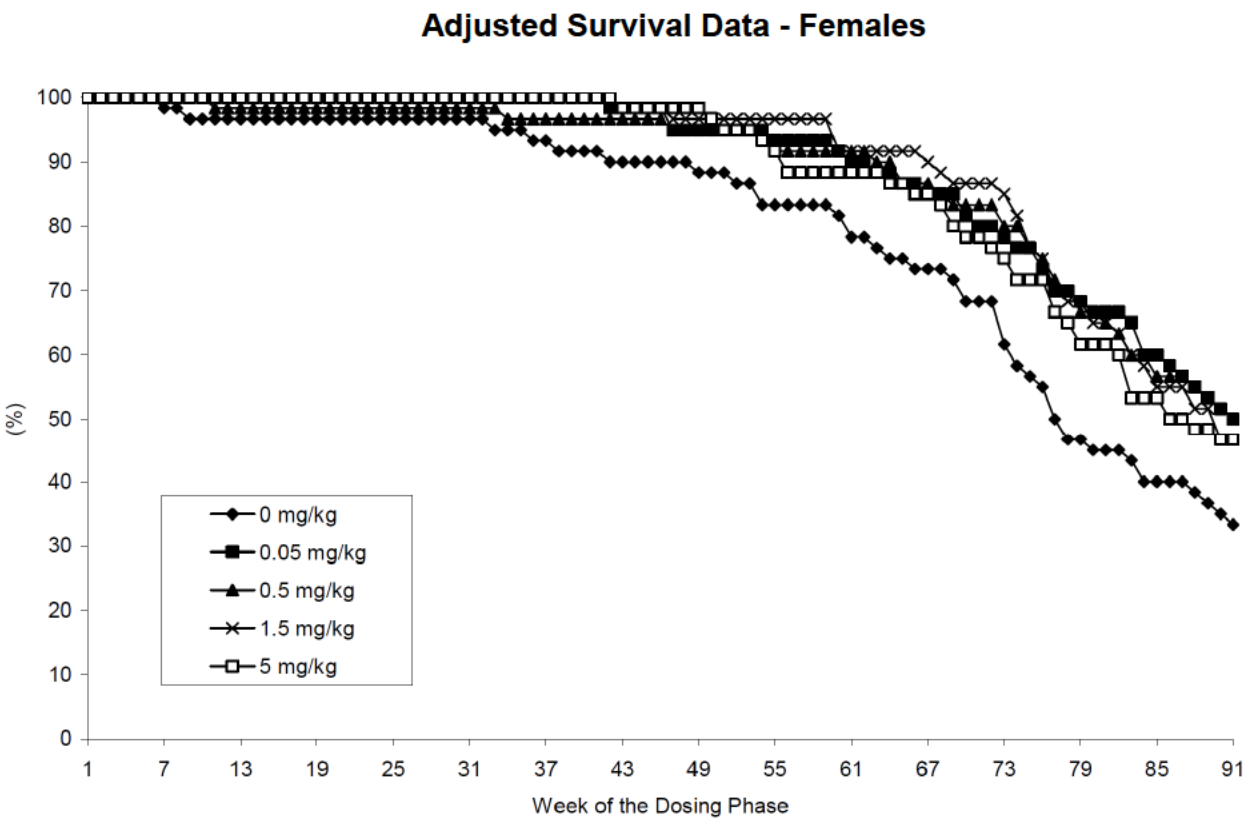
Sex	Males					Females				
LY2189265 mg/kg	0	0.05	0.5	1.5	5	0	0.05	0.5	1.5	5
Total No. of Animals	60	60	60	60	60	60	60	60	60	60
No. of Surviving Animals	19	27	29	29	35	20	30	30	28	27
Percentage	32	45	48*	48	58**	33	50*	50*	47*	45

* = Statistically significant at 5% level.
** = Statistically significant at 1% level.

Although not the only reason, a common cause for early termination of control animals was inflammation and sores/scabs on the foot/footpads, likely associated with their heavier weights. At the higher dose levels administered, animals weighed less leading to fewer issues with the feet that could necessitate early termination. Additional observations in animals terminated early revealed pituitary masses, microscopically identified as adenomas, occurring at a comparable incidence amongst the various groups and were not considered drug related. Survival curves are shown in the sponsor-generate figures below.

Adjusted Survival Data - Males





Historical control survival data from the study site revealed that early study termination due to poor control group survival ranged from 92 to 102 weeks, and that the percentage of control animals surviving at the time of early termination was in the range of 31% to 34% and 25% to 38% in males and females, respectively. Thus, the time of termination due to poor control group survival for the current study (Week 93) and the percentage surviving (32% and 33% for males and females, respectively) were within the historical ranges, albeit at the lower end. Historical control data for the laboratory are shown below:

Table 2: (b) (4) Historical Control Data: Survival at Study Termination for Carcinogenicity Studies in the Sprague Dawley Rat

	Males	Females
Total Number of Studies Completed	15	15
Number of Studies Terminated Early Due to Low Control Group Survival	5	6
Mean Number of Weeks Completed for Studies Terminated Early Due to Low Control Group Survival	96	97
Median Number of Weeks Completed for Studies Terminated Early Due to Low Control Group Survival	97	97
Number of Weeks Completed for Studies Terminated Early Due to Low Control Group Survival (Range)	92-98	93-102
Control Group Survival (%) for Studies Terminated Early Due to Low Control Group Survival (Range)	31-34	25-38
Control Group Survival (No. of Animals) for Studies Terminated Early Due to Low Control Group Survival (Range)	17-20	15-23

Note: (b) (4) 5-year Historical Control Search Conducted January 2014.

Clinical Signs:
Clinical signs of thin appearance and decreased feces secondary to decreased body weight and feed consumption occurred most often in the drug-treated groups, but not always in a dose-related manner. Swelling, red skin, scaly skin and/or sores/scabs were seen on fore- or hind-paws of animals in all groups, although not necessarily following a dose-response pattern. Injection site observations (e.g., sores/scabs, skin discoloration) were present at comparable incidences in all groups, including controls. Hunched posture was also evident, generally occurring at a higher incidence in the drug-treated groups. There were no differences in the incidence of masses between the groups.

Body Weights:
Drug-related decreases in body weight gain leading to lower body weights were evident at all doses, with the effects reaching statistical significance at ≥0.5 mg/kg in males and at all doses in females. The effects on body weight parameters were anticipated pharmacologic effects. Body weight change data are summarized in the reviewer created tables below; body weight data are shown in the sponsor-generated figures following the tables.

Males - Mean Body Weight/Body Weight Gain (% difference from controls)

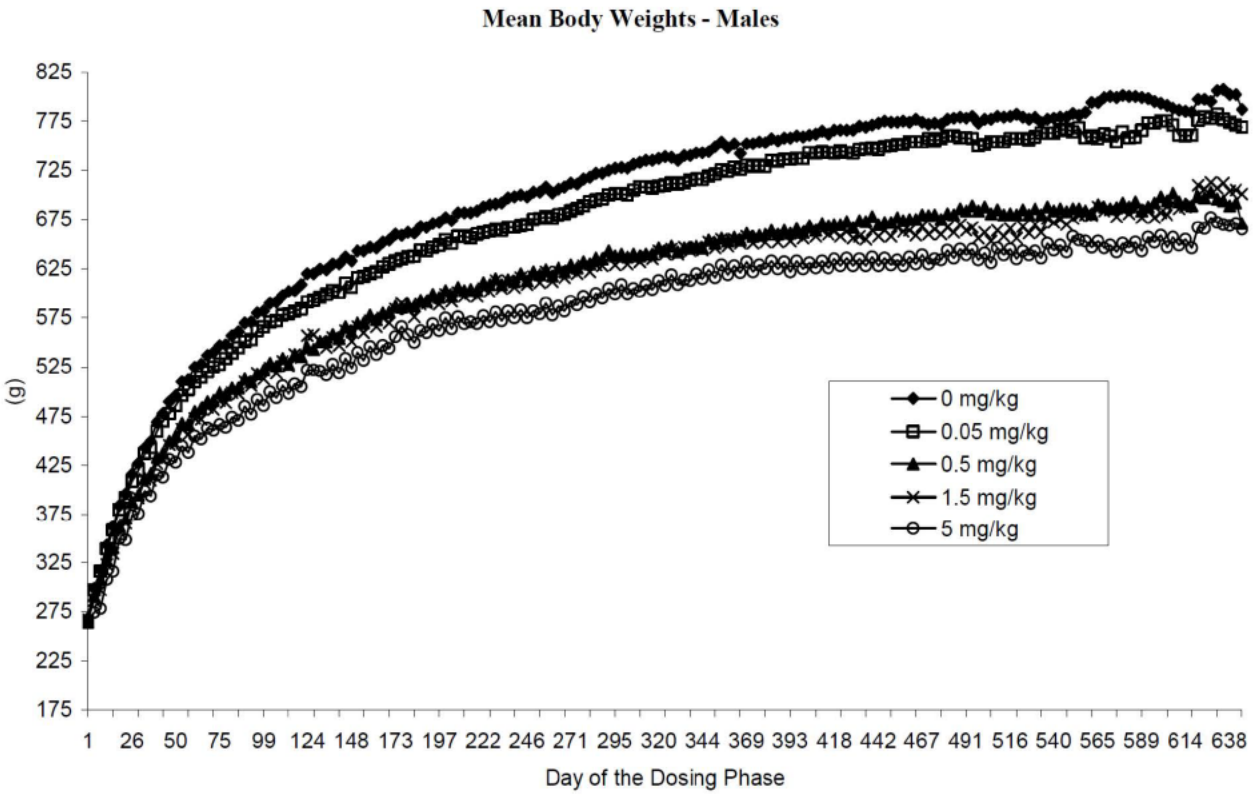
Day or Interval	Dose Level (mg/kg)				
	0	0.05	0.5	1.5	5
1	268	265	264	267	266
96	580	562	518* (↓11%)	519* (↓11%)	492* (↓15%)
180	662	637	589* (↓11%)	584* (↓12%)	558* (↓16%)
362	752	728	657* (↓13%)	655* (↓13%)	628* (↓16%)
645	787	769	671* (↓15%)	701* (↓11%)	665* (↓16%)
1 - 96	312	297	254* (↓19%)	252* (↓19%)	226* (↓28%)
96 - 180	82	75	71* (↓12%)	65* (↓20%)	65* (↓20%)
180 - 362	90	91	70* (↓24%)	71* (↓23%)	70* (↓24%)
1 - 645	524	510	412* (↓21%)	434* (↓17%)	402* (↓23%)

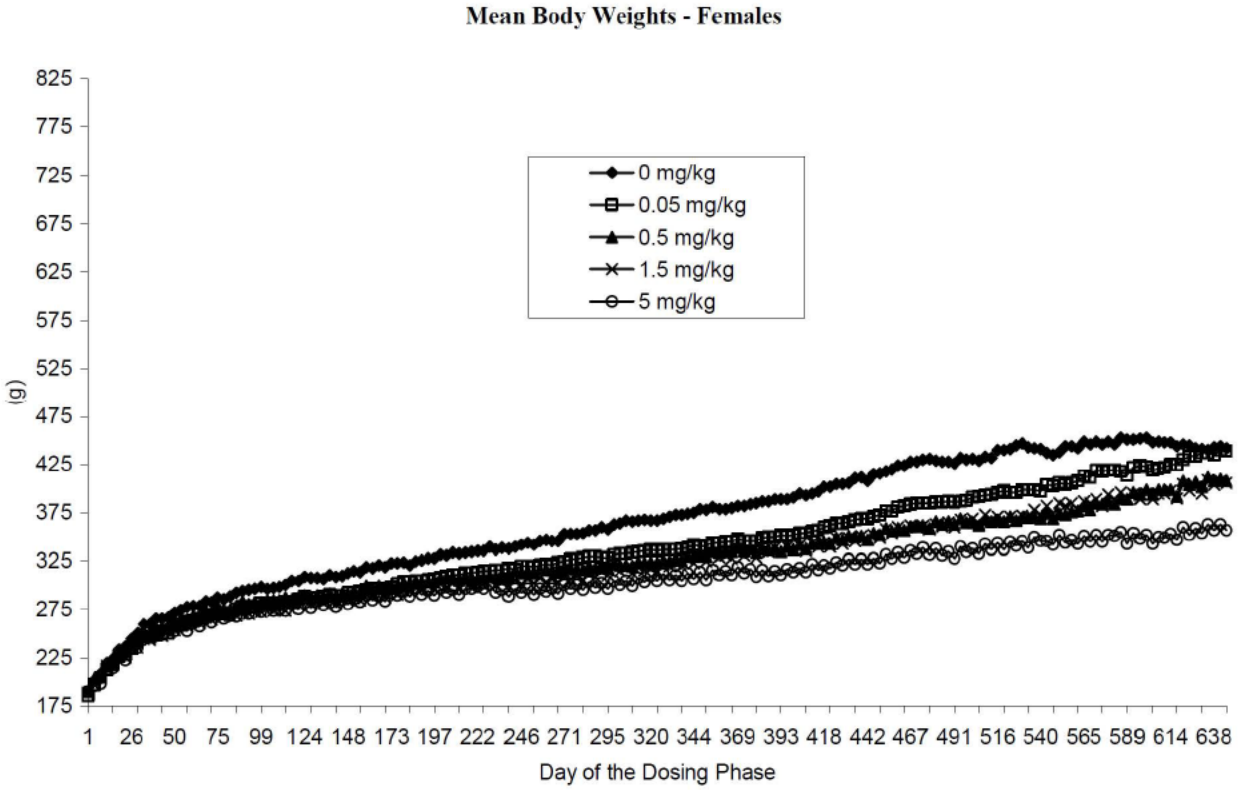
* p≤0.05%

Females - Mean Body Weight/Body Weight Gain (% difference from control)

Day or Interval	Dose Level (mg/kg)				
	0	0.05	0.5	1.5	5
1	190	186	190	189	190
96	297	279*	283*	275*	277*
180	324	304*	303*	296*	294*
362	379	345*	336*	331*	315*
645	441	439	408	406	357*
1 - 96	107	92* (↓14%)	93* (↓13%)	85* (↓21%)	87* (↓19%)
96 - 180	27	25	20* (↓26%)	21* (↓22%)	17* (↓37%)
180 - 362	55	41* (↓25%)	33* (↓40%)	35* (↓40%)	21* (↓63%)
1 - 645	251	253	218	217	167* (↓33%)

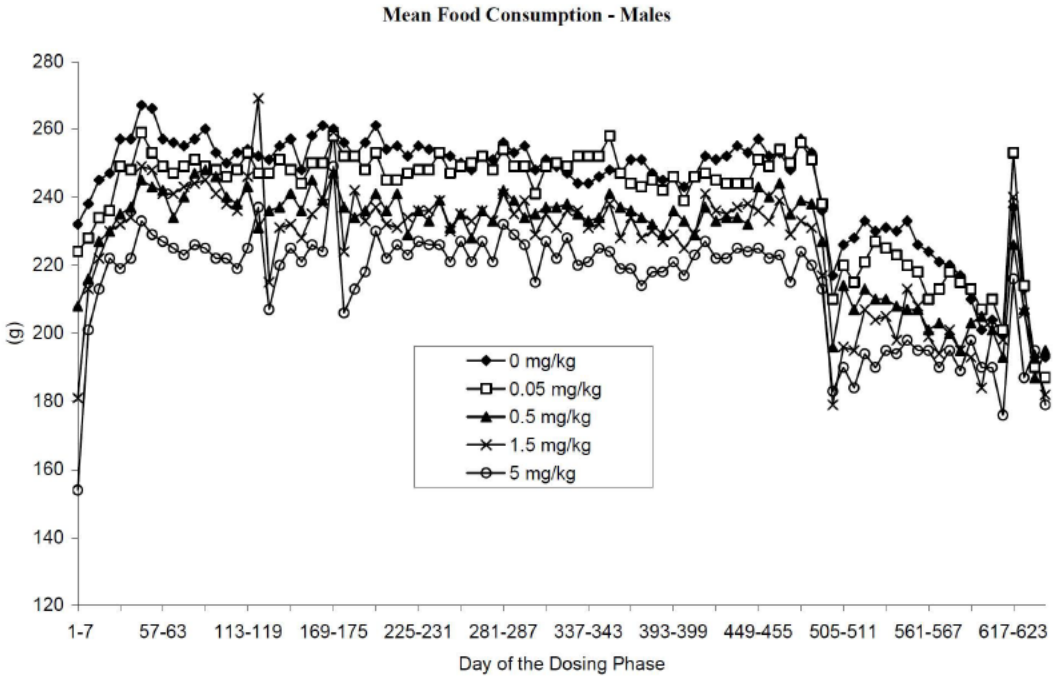
* p≤0.05%

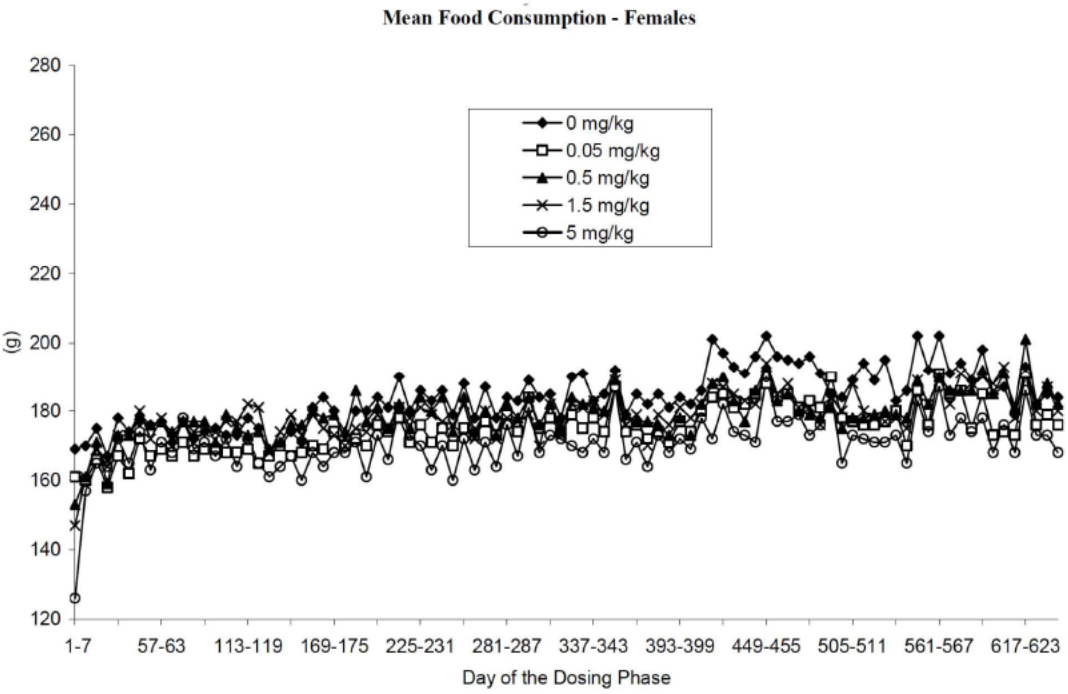

Mean Body Weights - Males
825
775
725
675
625
575
525
475
425
375
325
275
225
175
(g)
1 26 50 75 99 124 148 173 197 222 246 271 295 320 344 369 393 418 442 467 491 516 540 565 589 614 638
Day of the Dosing Phase
0 mg/kg
0.05 mg/kg
0.5 mg/kg
1.5 mg/kg
5 mg/kg


Mean Body Weights - Females
825
775
725
675
625
575
525
475
425
375
325
275
225
175
(g)
1 26 50 75 99 124 148 173 197 222 246 271 295 320 344 369 393 418 442 467 491 516 540 565 589 614 638
Day of the Dosing Phase
0 mg/kg
0.05 mg/kg
0.5 mg/kg
1.5 mg/kg
5 mg/kg

Feed Consumption:

Feed consumption was decreased in all drug-treated groups. The reductions were statistically significant for some intervals in males administered 0.05 mg/kg and for most intervals at ≥0.5 mg/kg. In females, feed consumption was also lower for all groups during many of the intervals, but statistical significance was consistently reached only at 5 mg/kg. Data are shown in the sponsor-generated figures below.





Gross Pathology:
For animals examined following an unscheduled euthanasia or found dead there was no indication of a treatment-related effect on any tissue. Tissues with the most observations were the brain (depressed area), pituitary (masses), female mammary (masses. cysts), and adrenal cortex (discolored). For all these observations, the incidence was similar between the various drug-treated groups and controls. An additional observation was an increase in the number of males with foot/foot pad findings, which were similarly higher in the control, 0.05, and 0.5 mg/kg groups compared with that seen in the 1.5 and 5 mg/kg groups; this effect likely reflected the generally higher weights of the animals in Groups 1-3 and the use of metal caging.

There was no drug-related increase in the occurrence of macroscopic observations in animals euthanized as scheduled. As with the unscheduled animals, the tissues with the most observations in this subset of animals included the brain of females (depressed area), pituitary (discolored, masses), adrenal cortex of females (discolored, large), female mammary (masses, cysts), and uterus (cysts). The incidence of foot/foot pad observations was comparable between groups.

When the incidence of observations was combined for the scheduled and unscheduled groups, no increases were noted for any observation.

Histopathology
Battery Considered Adequate? Yes
Peer Review Performed? Yes

Non-neoplastic observations
Hyperplasia of thyroid C-cells was observed in all groups (including controls). This observation is discussed in more detail with the neoplastic findings later in this review.

In other tissues, the occurrence of non-neoplastic lesions was generally similar in incidence and severity between the various drug-treated groups and controls in animals subjected to unscheduled sacrifice. In animals evaluated following the scheduled termination, there was a slight increase in the incidence of lymphocyte/macrophage infiltrates at the injection site of HD males, also leading to an increase when the unscheduled and scheduled groups were combined. This was likely related to the injection process based on no differences in severity, lack of a dose-response, and absence of a similar increase in HD females. The data were also somewhat confounded by the fewer injections administered to control males based on their lower survival.

Corresponding to the macroscopically observed foot pad lesions, there was an increased incidence of erosions/ulcers, inflammation, and hyperplasia/new bone formation (periosteum) in the feet of control and the lower dose group animals. Inflammation of the footpad was one of the primary causes for the unscheduled sacrifice of animals.

Reference ID: 3527260

In all drug-treated male, but not female, groups surviving to study termination, there was an increase in the incidence of observations in the pituitary (hyperplasia), prostate (inflammation), and pancreas (islet cell hyperplasia, lymphocyte/macrophage infiltrates). In males, the incidence of pituitary carcinomas was higher in the 1.5 mg/kg groups than in any other group, but the absence of a dose-response suggests this observation is incidental. In both males and females sacrificed as scheduled, the incidence of chronic progressive nephropathy, pelvic inflammation and/or hemorrhage and accompanying transitional epithelial hyperplasia occurred at a higher incidence in all drug treated groups than in controls, but the severity of these findings was generally minimal to slight and did not appear to differ between the groups. A similar pattern was not seen in these organs in the unscheduled death/sacrificed animals nor when the unscheduled and scheduled groups were combined, suggesting that these effects may be a reflection of the longer survival of the drug-treated groups.

Neoplastic observations

Tumors considered drug-related were limited to the thyroid gland. A statistically significant increase in the incidence of C-cell adenomas plus carcinomas was seen in males at ≥0.5 mg/kg and in females at 5 mg/kg. A non-statistically significant, numerical increase in C-cell adenomas was observed in females at 0.5 and 1.5 mg/kg, which was considered treatment related. Animals administered 5 mg/kg also had a non-statistically significant (pair-wise analysis), numerical increase in the incidence of thyroid C-cell carcinomas; the effect was statistically significant by trend analysis for females.

> *Reviewer comment: The incidences of C-cell adenomas alone at 0.5 and 1.5 mg/kg did not reach the cut-off of p≤0.01 for a common tumor for either sex. Regardless, given the 2 to 3-fold increase in incidence over controls and the expected occurrence of these tumors with this class of compound, these tumors are considered drug-related.*

Additionally, both focal and diffuse C-cell hyperplasia was evident in the thyroid. Focal C-cell hyperplasia was predominant in controls and low dose groups while diffuse C-cell hyperplasia was predominant at the higher dose levels at unscheduled and scheduled sacrifices. When the unscheduled and scheduled sacrifice groups were combined, the incidence and severity of diffuse C-cell hyperplasia was increased in males at ≥1.5 mg/kg and in females at 5 mg/kg; females receiving ≥0.5 mg/kg also had an increased incidence and severity of focal C-cell hyperplasia. There was a lower incidence of focal C-cell hyperplasia seen in males at dosages ≥0.5 mg/kg, occurring in conjunction with an increase in the incidence of thyroid C-cell adenomas, suggesting a progression from focal hyperplasia to adenomas in these groups. An overall summary of the thyroid observations are shown in the sponsor-generated Table 5 below. Note that the statistical results for tumor incidence were slightly different than for the FDA statistical reviewer's analysis. However, the overall conclusions regarding treatment-related tumorigenesis are consistent between the sponsor and FDA reviewers.

Reference ID: 3527260

Table 5. Incidence of Thyroid C-Cell Hyperplasia, Adenoma, and Carcinoma - Unscheduled Deaths and Dosing Phase Necropsy

Sex	Males					Females				
LY2189265 mg/kg	0	0.05	0.5	1.5	5	0	0.05	0.5	1.5	5
No. Examined	60	59	60	60	60	60	59	60	60	60
Diffuse C-cell Hyperplasia (I)	9+(A)**	9NA	16+(A)	19+(A)*	28+(A)**	7+(A)**	6NA	12+(A)	11+(A)	25+(A)**
Focal C-cell Hyperplasia (I)	39-(A)**	28-(A)*	25-(A)**	25-(A)**	15-(A)**	7+(A)*	11+(A)	20+(A)**	20+(A)**	20+(A)**
C-Cell Adenoma (I)	10+(A)#	14+(A)	25+(A)*	24+(A)**	31+(A)^	7+(A)#	6NA	19+(A)**	19+(A)*	32+(A)^
C-Cell Carcinoma (I/F)	1+(E)@	0NA	2NA	2NA	4+(E)	1+(E)@	0NA	1NA	1NA	4+(E)
C-Cell Adenoma and Carcinoma (I/F)	11+(A)#	14+(A)	27+(A)**	24+(A)**	33+(A)^	8+(A)#	6-(A)	20+(A)*	20+(A)*	35+(A)^

I = Incidental tumor. F = Fatal tumor.
* = Significant at 5% level using the IARC method; ** = Significant at 1% level, using the IARC method.
+/- = Effect in the increased/decreased direction.
(A) = Asymptotic test using the IARC method. (E) = Exact test using the IARC method. NA = Not analyzed.
= Significant trend at 0.005 level; ^ = Significant high-dose group comparison at 0.01 for a common tumor.
@ = Not a significant trend at 0.005 level.

The historical control data from the study site are shown in the table below. Note that the incidence of C-cell adenoma in males (17%) and diffuse C-cell hyperplasia in both males (15%) and females (12%) occurring in controls from the current study are above the range seen historically.

Table 3: (b) (4) Historical Control Data: Thyroid C-cell Hyperplasia and Neoplasia and Thyroid Follicular Neoplasia in Sprague Dawley Rat Carcinogenicity Studies

Number of Studies =15	Males Number Examined = 1144		Females Number Examined = 1140	
Observation	Mean Incidence[a]	Range[b]	Mean Incidence[a]	Range[b]
Hyperplasia – C-Cell, focal	17.3%	8.3% to 78.5%	20.2%	0% to 80.0%
Hyperplasia – C-Cell, diffuse	0.2%	0.0% to 3.3%	0.4%	0.0% to 8.3%
Adenoma, C-Cell	12.6%	8.3% to 14.6%	12.9%	0.0% to 23.3%
Carcinoma, C-Cell	1.3%	0.0% to 2.6%	1.0%	0.0% to 2.3%
Adenoma, Follicular Cell	2.4%	0.0% to 10%	0.5%	0.0% to 3.3%
Carcinoma, Follicular Cell	0.7%	0.0% to 3.3 %	0.1%	0.0% to 1.5%

Note: (b) (4) 5-year Historical Control Search Conducted January 2014.
[a] Mean Incidence: Total number of animals recorded with observation/Total number of animals examined.
[b] Range: Range of control incidence across studies. For example, the incidence of Thyroid C-cell focal hyperplasia in males ranged from 8.3% to 78.5%in a different study.

Details of the statistical significance of the thyroid tumors performed by the FDA reviewer (Dr. Matthew Jackson) are shown below, including the combination of adenomas and carcinomas (composite endpoints):

Animal carcinogenicity study
Male rats

Organ or tissue name	*Tumor name*	*Quantity*	*Control Size = 60*	*Low dose Size = 60*	*Mid dose Size = 60*	*Mid-high dose Size = 60*	*High dose Size = 60*
Thyroid	Adenoma, C-cell	P-value of test of trend or comparison	.0018	.4020	.0141	.0139	.0017
		Number of animals reported with tumor	10	14	25	24	31
	Adenoma, Follicular Cell	P-value of test of trend or comparison	.9222	.2309	.7986	.2201	1
		Number of animals reported with tumor	1	4	1	4	0
	Carcinoma, C-cell	P-value of test of trend or comparison	.0399	1	.5691	.5246	.2503
		Number of animals reported with tumor	1	0	2	2	4
	Carcinoma, Follicular Cell	P-value of test of trend or comparison	.9943	.8978	1	1	1
		Number of animals reported with tumor	2	1	0	0	0
Composite endpoints							
	Thyroid C-cell tumors	P-value of test of trend or comparison	.0010	.4724	.0100	.0207	.0011
		Number of animals reported with tumor	11	14	27	24	33
	Thyroid follicular cell tumors	P-value of test of trend or comparison	.9644	.2685	.9071	.3831	1
		Number of animals reported with tumor	2	5	1	4	0

Animal carcinogenicity study
Female rats

Organ or tissue name	*Tumor name*	*Quantity*	*Control Size = 60*	*Low dose Size = 60*	*Mid dose Size = 60*	*Mid-high dose Size = 60*	*High dose Size = 60*
Thyroid	Adenoma, C-cell	P-value of test of trend or comparison	<0.0001	.8093	.0399	.0399	<0.0001
		Number of animals reported with tumor	7	6	19	19	32
	Adenoma, Follicular Cell	P-value of test of trend or comparison	.3128		.2983		.5424
		Number of animals reported with tumor	0	0	2	0	1
	Carcinoma, C-cell	P-value of test of trend or comparison	.0177	1	.8017	.8017	.2458
		Number of animals reported with tumor	1	0	1	1	4
	Carcinoma, Follicular Cell	P-value of test of trend or comparison	.8585	1	.8017	1	1
		Number of animals reported with tumor	1	0	1	0	0
Composite endpoints							
	Thyroid C-cell tumors	P-value of test of trend or comparison	<0.0001	.8798	.0499	.0499	<0.0001
		Number of animals reported with tumor	8	6	20	20	35
	Thyroid follicular cell tumors	P-value of test of trend or comparison	.5801	1	.3985	1	.7949
		Number of animals reported with tumor	1	0	3	0	1

Similarly, details of the statistical analysis of the hyperplasia findings in the thyroid are summarized in the sponsor-generated tables below (sponsor tables modified by reviewer to only show thyroid observations):

Results of Statistical Analyses of Categorical Incidence Data - Males

		Unadjusted Lifetime Incidence Rate				
	Group	1	2	3	4	5
	Dose: mg/kg	0	0.05	0.5	1.5	5
Tissue and Lesion	p-value	Trend	2 vs. 1	3 vs. 1	4 vs. 1	5 vs. 1
Thyroid: Diffuse Hyperplasia, C-cell (I)		9/60	9/59	16/60	19/60	28/60
1 vs. 2, 3, 4, and 5 p-value (one-sided)		0.0000+(A)**	NA	0.0943+(A)	0.0210+(A)*	0.0005+(A)**
Thyroid: Focal Hyperplasia, C-cell (I)		39/60	28/59	25/60	25/60	15/60
1 vs. 2, 3, 4, and 5 p-value (two-sided)		0.0001-(A)**	0.0388-(A)*	0.0098-(A)**	0.0072-(A)**	0.0000-(A)**

* = Significant at 5% level.using the IARC method; ** = Significant at 1% level.using the IARC method.
+/- = Effect in the increased/decreased direction. (A) = Asymptotic test using the IARC method (E) = Exact test using the IARC method.

Results of Statistical Analyses of Categorical Incidence Data – Females

		Unadjusted Lifetime Incidence Rate				
	Group	1	2	3	4	5
	Dose: mg/kg	0	0.05	0.5	1.5	5
Tissue and Lesion	p-value	Trend	2 vs. 1	3 vs. 1	4 vs. 1	5 vs. 1
Thyroid: Diffuse Hyperplasia, C-cell (I)		7/60	6/59	12/60	11/60	25/60
1 vs. 2, 3, 4, and 5 p-value (one-sided)		0.0000+(A)**	NA	0.3136+(A)	0.2393+(A)	0.0004+(A)**
Thyroid: Focal Hyperplasia, C-cell (I)		7/60	11/59	20/60	20/60	20/60
1 vs. 2, 3, 4, and 5 p-value (one-sided)		0.0212+(A)*	0.2847+(A)	0.0028+(A)**	0.0056+(A)**	0.0041+(A)**

* = Significant at 5% level.using the IARC method; ** = Significant at 1% level.using the IARC method.
+/- = Effect in the increased/decreased direction. (A) = Asymptotic test using the IARC method (E) = Exact test using the IARC method.
NA = Not Analyzed.

The severity determinations of the thyroid observations are shown in the sponsor-generated table below:

Controls from group(s): 1	Animal sex:	-- Males --					-- Females --				
	Dosage group:	Ctls	2	3	4	5	Ctls	2	3	4	5
Tissues With Diagnoses	No. in group:	60	60	60	60	60	60	60	60	60	60
Thyroid .....Number examined:		60	59	60	60	60	60	59	60	60	60
B-Adenoma, C-cell											
	->	50	45	35	36	29	53	53	41	41	28
	2>	10	14	25	24	31	7	6	19	19	32
.....Total Incidence of Finding Observed:		10	14	25	24	31	7	6	19	19	32
Thyroid .....Number examined:		60	59	60	60	60	60	59	60	60	60
B-Adenoma, Follicular Cell											
	->	59	55	59	56	60	60	59	58	60	59
	2>	1	4	1	4	0	0	0	2	0	1
.....Total Incidence of Finding Observed:		1	4	1	4	0	0	0	2	0	1
C-Hematopoietic Neoplasm, see Body, Whole for type											
	->	60	59	60	60	60	60	59	59	60	60
	^>	0	0	0	0	0	0	0	1	0	0
.....Total Incidence of Finding Observed:		0	0	0	0	0	0	0	1	0	0
M-Carcinoma, C-cell											
	->	59	59	58	58	56	59	59	59	59	56
	1>	0	0	0	0	1	0	0	0	0	0
	2>	1	0	2	2	3	1	0	1	1	4
.....Total Incidence of Finding Observed:		1	0	2	2	4	1	0	1	1	4
M-Carcinoma, Follicular Cell											
	->	58	58	60	60	60	59	59	59	60	60
	2>	2	1	0	0	0	1	0	1	0	0
.....Total Incidence of Finding Observed:		2	1	0	0	0	1	0	1	0	0

There were no treatment-related tumor findings in other tissues. Although hyperplasia or neoplasms were also observed in multiple other organs/tissues, the incidence and severity of these effects, as well as any other microscopic effects, was similar between LY2189265-treated and control groups and showed no dose-response in the unscheduled or scheduled sacrifice groups or when these two populations were combined. The data were also evaluated for various combinations of tumor types,

which revealed no increases in incidence for tumor types other than in the thyroid as mentioned previously.

General Comment

The incidence of tissues identified as autolyzed or as not being examined was comparable between groups, and the inability to evaluate the autolyzed/missing tissues is not considered to have an impact on overall study interpretations (see sponsor-generated table below). The only possible exception is for the evaluation of the mammary gland of male rats as this tissue was not evaluated in 19% of the animals across all groups. However, the groups with the highest proportion not examined were the control and low dose groups, and there was no indication of a drug related effect for any mammary observation. For these reasons, this reviewer considers the examination of the male mammary to be adequate to conclude there are no drug-related effects on this tissue.

Organs reported as unexamined
IND 70930
Animal carcinogenicity study
Male Rats

Organ or tissue name	*Control(count)*	*Control(%)*	*Low dose(count)*	*Low dose(%)*	*Mid dose(count)*	*Mid dose(%)*	*Mid-high dose(count)*	*Mid-high dose(%)*	*High dose(count)*	*High dose(%)*	*Total(count)*	*Total(%)*
Adrenal, Medulla	1	1.7%	1	1.7%	.	.	.	.	.	.	2	0.7%
Bone, Femur	1	1.7%	1	1.7%	.	.	.	.	1	1.7%	3	1.0%
Bone, Sternum	1	1.7%	.	.	.	.	.	.	.	.	1	0.3%
Colon	.	.	.	.	.	.	.	.	1	1.7%	1	0.3%
Foot/Foot Pad	2	3.3%	.	.	2	3.3%	.	.	1	1.7%	5	1.7%
Jejunum	.	.	.	.	1	1.7%	1	1.7%	.	.	2	0.7%
Joint, Other	.	.	.	.	1	1.7%	.	.	.	.	1	0.3%
LN, Mesenteric	.	.	1	1.7%	.	.	.	.	.	.	1	0.3%
Mammary, Male	14	23%	20	33%	6	10%	9	15%	9	15%	58	19%
Marrow, Femur	1	1.7%	1	1.7%	.	.	.	.	1	1.7%	3	1.0%
Marrow, Sternum	1	1.7%	.	.	.	.	.	.	.	.	1	0.3%
Muscle, Bi Fem	.	.	1	1.7%	.	.	.	.	1	1.7%	2	0.7%
Nerve, Sciatic	.	.	1	1.7%	.	.	.	.	1	1.7%	2	0.7%
Prostate	.	.	1	1.7%	.	.	.	.	.	.	1	0.3%
Seminal Vesicle	.	.	1	1.7%	.	.	.	.	.	.	1	0.3%
Spinal Cord	.	.	.	.	.	.	1	1.7%	.	.	1	0.3%
Thymus	.	.	1	1.7%	.	.	1	1.7%	1	1.7%	3	1.0%
Urinary Bladder	.	.	1	1.7%	.	.	.	.	.	.	1	0.3%

Toxicokinetics: Exposure increased with dose in an approximately dose-proportional manner. Exposures reached steady-state by approximately Week 13 and stayed constant through study termination (sponsor-generated Figure 1 below). Accumulation was seen as exposures were higher following repeated dosing as compared to that seen following the initial dose-administration (sponsor-generated Table 1 below). There were no differences in exposure between the sexes.

Table 1. Summary of TK data

Parameter	Administered Dose (mg LY2189265/kg)							
	0.05		0.5		1.5		5	
Sex	M	F	M	F	M	F	M	F
LY2189265								
Day 1								
C_{max} (µg/mL)	0.0751	0.0875	0.765	0.836	2.25	2.75	6.05	6.42
Mean $AUC_{0\text{-}96hr}$ (µg·hr/mL)	2.04	2.31	23.3	27.8	76.6	90.3	208	236
SEM $AUC_{0\text{-}96hr}$ (µg·hr/mL)	0.278	0.141	1.27	2.16	2.63	4.23	7.88	18.9
Week 52								
C_{max} (µg/mL)	0.0914	0.120	1.11	1.16	2.88	3.25	10.0	8.46
$AUC_{0\text{-}96hr}$ (µg·hr/mL)	4.05	4.07	62.4	56.7	162	155	366	563
SEM $AUC_{0\text{-}96hr}$ (µg·hr/mL)	0.385	0.414	5.94	7.16	9.91	14.7	55.6	60.4

Note: Values represent 3 animals/sex/group.

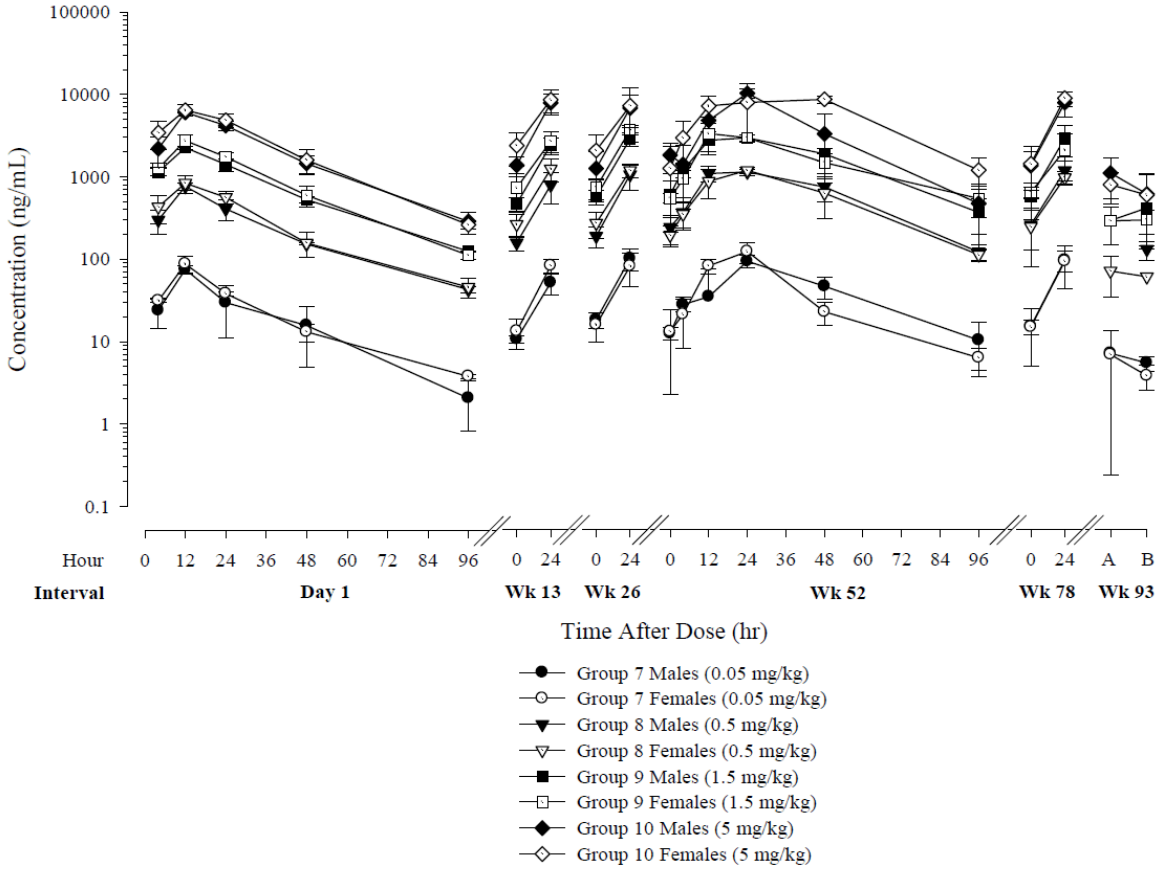


Figure 1. Mean (±SD) concentrations (ng/mL) of LY2189265 in male and female rats following twice weekly subcutaneous administration of 0.05, 0.5, 1.5, or 5 mg/kg LY2189265 for 93 weeks

Note: For Week 93, sacrifice time A was 27 June 2011 for males and 29 June 2011 for females. Sacrifice time B was 28 June 2011 for males and 30 June 2011 for females. Sacrifice times A and B occurred approximately 4 days after the final dose for each sex.

Dosing Formulation Analysis:
Dose formulations were stable under the conditions of use. Dosing formulations ranged from (b) (4) of claim, with the exception of the L dose formulation at week 78 which was at (b) (4) of claim. Based on the consistency of observations with those made in preceding weeks, the higher than desired test-article formulation is not considered to have affected study interpretations.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: A male fertility study in Sprague Dawley rats of LY2189265 administered via subcutaneous injection every third day

Study number:	(b) (4) 353113, Amendment 1
Study report location:	Module 4.2.3.5.1
Conducting laboratory:	(b) (4)
Date of study initiation:	21 August 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, purity, and % unmodified peptide:	LY2189265, Lot # (b) (4) C1301-78, 65.9% (b) (4) %

Key Study Findings

- There was no mortality during the study.
- Clinical signs included decreased defecation at 4.9 and 16.3 mg/kg and red material around the nose at all dose levels.
- Decreased body weight gain and food consumption were noted at all doses.
- Decreased weights were noted for the testes, epididymides, seminal vesicles, and prostate, which were likely secondary to decreased food consumption and body weight gain.
- There were no direct test article effects on gross pathology or histopathology.
- There were no effects on male mating performance or fertility; on sperm concentration, motility, or morphology; or on early embryonic viability.
- Although there were decreased body weight gains at all dose levels, this is an expected pharmacologically-mediated effect and was not considered to be adverse. Accordingly, the NOAEL for this study is 16.3 mg/kg.

Methods

Species/Strain: Rat/Sprague-Dawley

Study design: Male rats were dosed every third day by bolus subcutaneous injection (injection site rotated clockwise between 4 sites in the scapular region) for 4 weeks prior to cohabitation and during a 2-week cohabitation period. (sponsor-generated table)

Group Number	Compound	Dosage Level (mg/kg)[a]	Dosage Volume (mL/kg)	Number of Males	Number of Females[b]
1	Vehicle[c]	0	1.0	20	20
2	LY2189265	1.63	1.0	20	20
3	LY2189265	4.89	1.0	20	20
4	LY2189265	16.3	1.0	20	20

a Doses were expressed as total LY2189265 protein content.

b Females were not dosed.

c Vehicle control is [(b) (4)] citrate [(b) (4)] (pH [(b) (4)] Polysorbate 80 with [(b) (4)] sodium chloride prepared in sterile water for injection, USP (Diluent B)

Satellite groups: TK sampling was not conducted

Immunogenicity: Not assessed

Parameters and endpoints: Survival and clinical signs; body weight; food consumption; mating, fertility, copulation, and conception indices; sperm evaluation; organ weight, macroscopic, and microscopic assessments of the male reproductive organs; and early embryonic development endpoints (Gestation Day 15 laparohysterectomy)

Protocol deviations: No GLP or protocol deviations occurred that impacted the validity or interpretability of the study data. After the study report was finalized, it was determined that the extinction coefficient of the test article was different than previously calculated resulting in lower administered dose levels than planned. The actual dose levels were 0, 1.63, 4.89, and 16.3 mg/kg rather than 2, 6, and 20 mg/kg as planned.

Observations and Results

Mortality: (assessed twice daily)

No mortality occurred during the study.

Clinical signs: (assessed twice daily)

Dosage (mg/kg):	0[a]	1.63	4.89	16.3
Clinical signs[b]				
Red material around the nose	1/1	10/7	16/12	58/18
Decreased defecation	0/0	0/0	22/20	21/20

a Vehicle.

b Total occurrence/number of animals.

(sponsor-generated table)

Body weight: (assessed predose and twice weekly thereafter)
A decrease in body weight gain was noted at all dose levels, with body weight loss occurring between Day 0 and 3. Respective mean body weights in the 1.6, 4.9, and 16.3 mg/kg groups were 8.8%, 8.1%, and 15.2% lower than the control group mean at the start of the mating period on Day 27, and 7.4%, 6.8%, and 15.9% lower than the control group mean at the conclusion of the dose administration period on Day 42. A summary is presented in the sponsor-generated table below.

Dose (mg/kg):	0[a]	1.63	4.89	16.3
Body weight change (g)				
Study Days 0-3	22	-1**	-18**	-37**
Study Days 3-6	15	12	16	8**
Study Days 12-15	19	15	21	24*
Study Days 0-27	136	99**	101**	70**
Study Days 0-42	175	141**	143**	100**

[a] Vehicle.
**p<0.01.
* p<0.05

Food consumption: (Predose and twice weekly thereafter)

Dose (mg/kg):	0[a]	1.63	4.89	16.3
Food consumption (g/animal/day)				
Study Days 0-3	27	17**	11**	6**
Study Days 3-6	27	22**	20**	16**
Study Days 6-9	26	23**	23**	20**
Study Days 9-12	26	24**	24**	21**
Study Days 12-15	26	23**	24**	23**
Study Days 15-18	26	23	24	23**
Study Days 18-21	28	26	27	25**
Study Days 21-24	27	26	26	24**
Study Days 24-27	28	26**	25**	23**
Study Days 0-27	27	23**	23**	20**

[a] Vehicle.
**p<0.01.
(sponsor-generated table)

Necropsy
Gross Pathology: A small prostate was noted for 2 HD males and small seminal vesicles were noted for 1 HD male. These findings were considered secondary to the compound-related decreases in body weight gain observed in these males. The profile of lower body weight and food consumption noted in this study was similar to feed restriction in adult males where absolute seminal vesicle and prostate weights were significantly reduced (Chapin et al., 1993).

Organ Weight: The absolute organ weights were decreased for several organs; however, when evaluated in relation to body weight, many of the relative organ weights were greater than control, suggesting that the decreased organ weights were secondary to the overall lower final mean body weights.

Histopathology:
There were no compound-related microscopic findings at any dose level.

Mating Performance and Fertility:
There were no test article effects on male mating performance or fertility. Data for the key parameters are presented in the sponsor-generated table below.

Dose (mg/kg):	0[a]	1.63	4.89	16.3	(b) (4) HC mean (range)
Male mating index, % (incidence)	100.0 (20/20)	100.0 (20/20)	100.0 (20/20)	85.0 (17/20)	96.8 (86.7-100.0)
Male fertility index, % (incidence)	95.0 (19/20)	100.0 (20/20)	95.0 (19/20)	85.0 (17/20)	89.8 (72.0-100.0)
Male copulation index, % (incidence)	95.0 (19/20)	100.0 (20/20)	95.0 (19/20)	100.0 (17/17)	92.7 (75.0-100.0)
Female conception index, % (incidence)	95.0 (19/20)	100.0 (20/20)	95.0 (19/20)	100.0 (17/17)	92.9 (75.0-100.0)
Mean pre-coital intervals (days)	1.8	2.4	2.1	2.0	3.0 (1.8-4.7)

[a] Vehicle.
HC Historical Control.

Sperm motility, concentration, and morphology:
There were no test article-effects on spermatogenesis (mean testis and epididymal sperm numbers), sperm motility, or sperm morphology.

Gestation Day 15 laparohysterectomy (treated males mated with untreated females):
There were no test article-related effects on the number of viable embryos, dead embryos, early or late resorptions, implantation sites, corpora lutea, pre- or post-implantation losses, or gravid females.

Toxicokinetics: TK analyses were not conducted; the same high-dose level (16.3 mg/kg) was administered twice weekly to Sprague-Dawley rats in a 3-month toxicology study, which yielded mean C_{max} and $AUC_{0\text{-last}}$ values of 22.3 µg/mL and 1042 µg·h/mL, respectively, for males on Day 92.

Dosing Solution Analysis:
The compound stock solution was stable throughout the dosing period. The LY2189265 concentration of each sampled dosing solution did not deviate substantially from its theoretical value. Mean assayed concentrations of LY2189265 were 100% of their theoretical values.

9.2 Embryonic Fetal Development

Study title: A female fertility and early embryonic development study in Sprague Dawley rats of LY2189265 administered via subcutaneous injection every third day

Study number:	(b) (4) 353112, Amendment 1
Study report location:	Module 4.2.3.5.1
Conducting laboratory:	(b) (4)
Date of study initiation:	7 August 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, purity, and % unmodified peptide:	LY2189265, Lot # (b) (4) C1301-78, 65.9% (b) (4) %

Key study findings:

- There were no treatment-related mortalities.
- A dose-related increase in red material around the nose, decreased defecation, and loss of hair in the ventral abdominal and/or thoracic regions.
- Maternal body weight loss during the pre-mating period and a decrease in body weight gain during the gestation treatment period were noted at all dose levels, which correlated with reduced food intake. A dose-related compensatory increase in food intake and corresponding body weight gain was noted during the gestation non-treatment period for all dose levels.
- An increase in the number of females with prolonged diestrus was noted at 4.9 and 16.3 mg/kg.
- A dose-related decrease in the mean number of corpora lutea, implantation sites, and viable embryos was seen at all dose levels; however these decreases were only statistically significant for the 4.9 and 16.3 mg/kg groups.
- There were no compound-related effects on maternal mating, fertility, or conception, or embryonic survival.
- On the basis of prolonged diestrus and statistically significant decreased number of corpora lutea, implantation sites, and viable embryos noted at ≥4.9 mg/kg, the NOAEL for female reproductive performance was 1.6 mg/kg. On the basis of maternal weight loss at all dose levels, the NOAEL for maternal toxicity was <1.6 mg/kg.

Methods

Species/Strain: Rat/Sprague Dawley

Study Design: Female rats were dosed every third day by bolus subcutaneous injection for 2 weeks prior to cohabitation and during the cohabitation period until mating was confirmed; rats with evidence of mating were dosed during gestation on either GD 0, 3, 6; GD 1 and 4; or GD 2 and 5, depending on which Study Day evidence of mating was observed. Females with no evidence of mating were dosed every 3 calendar days with the last dose administered on Study Day 33. Females with evidence of mating were euthanized on GD 15. Females without evidence of mating

Reference ID: 3527260

were euthanized once all of the females that had mated had been euthanized. (sponsor-generated table)

Group Number	Test Article	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Females
1	Vehicle[a]	0	1.0	20
2	LY2189265	1.63	1.0	20
3	LY2189265	4.89	1.0	20
4	LY2189265	16.3	1.0	20

[a] = Vehicle control is [(b) (4)] citrate [(b) (4)] (pH [(b) (4)] Polysorbate 80 with [(b) (4)] sodium chloride prepared in sterile water for injection, USP (Diluent B).

Satellite groups: TK sampling not conducted

Immunogenicity: Not assessed

Age: 11 weeks

Weight: 205 to 267 g

Parameters and endpoints: Survival, clinical signs, and external gross examination (i.e., injection site), body weight, food consumption. Macroscopic examinations (GD 15): A complete macroscopic examination was conducted on all females. The cervix, ovaries and oviducts, pituitary gland, uterus with vagina, and all gross lesions were collected and placed in fixative. Laparohysterectomy: the number of viable embryos, dead embryos, early or late resorptions, implantation sites, corpora lutea, pre- or post-implantation losses, and gravid females.

Protocol deviations: No GLP or protocol deviations occurred that impacted the validity or interpretability of the study data. After the study report was finalized, it was determined that the extinction coefficient of the test article was different than previously calculated resulting in lower administered dose levels than planned. The actual dose levels were 0, 1.63, 4.89, and 16.3 mg/kg rather than 2, 6, and 20 mg/kg.

Observations and Results

Mortality: One female in the 4.9 mg/kg group was euthanized in extremis before the second dose administration (Day 3). At necropsy, macroscopic findings consisted of calculi in the urinary bladder, distended and thickened urinary bladder and urethra, distended ureters, and an enlarged iliac lymph node. This animal was suspected of having a pre-existing renal condition, and because there were no mortalities at 16.3 mg/kg, this death was not considered to be test article related.

One female in the 4.9 mg/kg group was accidently allowed to deliver because mating was not detected and it was believed that this female was not pregnant; therefore cesarean sectioning was not conducted on this animal.

Clinical signs:

Dose (mg/kg):	0[a]	1.63	4.89	16.3
Clinical signs[b]				
Hair loss- ventral abdominal area	0/0	3/2	50/5	86/7
Hair loss- ventral thoracic area	2/1	0/0	14/3	78/6
Red material around the nose	10/6	20/11	31/12	54/16
Decreased defecation	0/0	0/0	1/1	30/12

[a] Vehicle control.
[b] Incidence/animal.
(sponsor-generated table)

Body weight (Pre-mating): A mean body weight loss was noted in the 16.3 mg/kg group after administration of the second dose and in all dose groups at the end of the pre-mating period. Mean body weights for the 1.6, 4.9, and 16.3 mg/kg groups were 4.5%, 8.6%, and 12.2% lower, respectively, than the control group at the end of the pre-mating period. The respective mean body weight gains during the pre-mating period for the 1.6, 4.9, and 16.3 mg/kg groups were 129%, 286%, and 443% lower than the control group. A summary of the body weight data during the pre-mating period is shown in the sponsor-generated table below.

Dose (mg/kg):	0[a]	1.63	4.89	16.3
Mean body weight gain (g)				
Study Days 0-3	-3	-9*	-16**	-24**
Study Days 3-6	5	6	1	-2*
Study Days 6-9	1	4	3	7**
Study Days 9-12	5	1	5	4
Study Days 12-14	0	-4	-8**	-8**
Study Days 0-14	7	-2*	-13**	-24**
Mean body weight (g)				
Study Day 0	238	236	238	239
Study Day 3	234	227	222*	214**
Study Day 6	240	233	224**	213**
Study Day 9	240	236	227*	220**
Study Day 12	246	238	232*	223**
Study Day 14	245	234*	224**	215**

[a] Vehicle control.
*p<0.05.
*p<0.01.

Body weight (gestation) – The respective mean body weights for the 1.6, 4.9, and 16.3 mg/kg groups were 7.7%, 12.1%, and 13.2% lower than the control group during the gestation treatment period (GD 0 to 6). The respective mean body weight gains for the 1.6, 4.9, and 16.3 mg/kg groups were 10%, 31%, and 24% lower than the control group. During the post-treatment period (GD 6 to 15), significant compensatory increases in body weight gain were noted in the 4.9 and 16.3 mg/kg groups. As a result of these increases, mean body weight was only 6.7% and 3.7% lower than the control group for the 4.9 and 16.3mg/kg groups, respectively. A summary of the body weight data during the gestation period is shown in the sponsor-generated table below.

Dose (mg/kg):	0[a]	1.63	4.89	16.3
Mean body weight gain (g)				
Gestation Days 0-6	29	26	20**	22*
Gestation Days 6-15	46	49	58**	69**
Mean body weights (g)				
Gestation Day 0	251	241	227**	224**
Gestation Day 1	257	239**	230**	223**
Gestation Day 2	264	250	236**	234**
Gestation Day 3	269	257	240**	236**
Gestation Day 4	273	252**	240**	239**
Gestation Day 5	276	260*	243**	244**
Gestation Day 6	280	267	247**	245**
Gestation Day 9	292	282	270**	268**
Gestation Day 12	309	300	287**	294
Gestation Day 15	326	316	304*	314

[a] Vehicle control.
*p<0.05.
*p<0.01.
Note: the last dose was administered on Gestation Day 4, 5, or 6.

Food consumption: Mean food consumption was decreased at all dose levels during the pre-mating period and during the gestation treatment period. A compensatory increase in mean food consumption was noted for all dose levels once dosing ended.

Dose (mg/kg):	0[a]	1.63	4.89	16.3
Food consumption (g/animal/day)				
Study Days 0-3	20	12**	10**	6**
Study Days 3-6	21	16**	12**	10**
Study Days 6-9	21	18**	16**	15**
Study Days 9-12	24	20**	18**	17**
Study Days 12-14	22	18**	16**	15**
Food consumption (g/kg/day)				
Study Days 0-3	86	53**	45**	27**
Study Days 3-6	90	71**	56**	48**
Study Days 6-9	89	77**	70**	71**
Study Days 9-12	99	85**	80**	78**
Study Days 12-14	90	74**	69**	67**

[a] Vehicle control.
*p<0.01.

Dose (mg/kg):	0[a]	1.63	4.89	16.3
Food consumption (g/animal/day)				
Gestation Days 0-6	29	25**	22**	22**
Gestation Days 6-15	29	28	29	30
Food consumption (g/kg/day)				
Gestation Days 0-6	108	98**	93**	93**
Gestation Days 6-15	97	99	103	107**

[a] Vehicle control.
*p<0.01.
Note: the last dose was administered on Gestation Day 4, 5, or 6.
(sponsor-generated table)

Necropsy:
No compound-related macroscopic findings were observed.

Mating performance and fertility:
Throughout the pre-mating treatment period, 3, 0, 5, and 7 females in the control, 1.6, 4.9, and 16.3 mg/kg groups, respectively, had abnormal estrous cycles of greater than 5 days in length or had no complete cycles. There were no compound-related effects on maternal mating, fertility, or conception indices. A summary of the mating and fertility data is presented in the Sponsor's table below.

Dose (mg/kg):	0[a]	1.63	4.89	16.3	(b) (4) HC Mean (range)
Time to mating (days)	2.8	2.8	2.2	2.7	3.0 (1.8-4.7)
Mating index (%)	100	100	100	100	97.8 (86.7-100)
Fertility index (%)	85	95	100	100	91.3 (72.0-100)
Conception index (%)	85	95	100	100	92.9 (75.0-100)
Mean estrous cycle length (days)	4.0	4.1	4.6	4.8	4.5 (4.0-5.8)
Number of females with prolonged diestrus[b]	3	0	5[c]	7	NA

[a] Vehicle control.
[b] Cycles that were >5 days in length or no complete cycles during the premating period.
[c] Excludes female that was euthanized in extremis.
None significantly different from the control group.
NA = Not applicable

Maternal reproductive and embryonic parameters:
A compound-related decrease in the mean number of corpora lutea, resulting in lower mean numbers of implantation sites and viable embryos, occurred in the 1.6, 4.9, and 16.3 mg/kg groups. These decreases were statistically significant and outside of the lab's historical control range for the 4.9 and 16.3 mg/kg groups. There were no compound-related effects on embryonic survival (pre- or post-implantation loss) at any dose level.

Dose (mg/kg):	0[a]	1.63	4.89	16.3	(b) (4) HC Mean (range)
Pregnant females	17	19	18	20	NA
Corpora lutea	16.5	14.8	13.5**	13.5**	16.7 (15.3-18.8)
Implantation sites	15.1	14.0	13.1*	12.4**	15.3 (13.9-16.8)
Preimplantation loss (% per litter)	8.2	4.6	3.8	7.3	8.2 (2.7-13.8)
Postimplantation loss (% per litter)	4.2	3.8	4.8	6.5	5.1 (2.9-10.0)
Mean no. of viable embryos	14.5	13.5	12.3*	11.6**	14.9 (13.5-16.3)

All data expressed as mean values for the group, except for the number of pregnant females.
[a] Vehicle control.
HC = Historical control data.
NA = Not applicable.
*p<0.05.
*p<0.01.

Toxicokinetics: TK assessments were not performed in this study. The same dose levels were administered to rats twice weekly in a 3-month toxicology study. In that study, mean for $AUC_{0\text{-}96h}$ values on Day 92 for female groups receiving 1.63, 4.89, and 16.3 mg/kg were 134, 255, and 1319 µg•h/mL, respectively.

Dosing Solution Analysis: The LY2189265 concentration of each sampled dosing suspension did not deviate substantially from its theoretical value. Mean assayed concentrations of LY2189265 ranged from 95% to 100% of theoretical values.

Study title: An embryo-fetal development and toxicokinetic study in Sprague-Dawley Rats of LY2189265 administered via subcutaneous injection every third day

Study number:	(b) (4) 353116, Amendment 2
Study report location:	Module 4.2.3.5.2
Conducting laboratory:	(b) (4)
Date of study initiation:	25 August 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, % purity, and % unmodified peptide:	LY2189265, Lot # (b) (4) C1301-78, 65.9% (b) (4) %

Key Study Findings

- One death occurred in the HD group on GD 8 that was possibly test article related.
- Clinical signs, which were mainly observed in the 1.6 and 4.9 mg/kg group animals, consisted of hunched posture, decreased defecation, hair loss on ventral abdominal and thoracic regions, and red material around the eyes, nose, mouth, and forelimbs.
- A significant reduction in maternal body weight gain was noted for all test article groups, with body weight loss occurring at 1.6 and 4.9 mg/kg from GD6 to 9. The effects on body weight correlated with decreased food consumption.
- An increase in percent post-implantation loss and a decrease in percent viable fetuses were noted for the 4.9 mg/kg group.
- Fetal body weight was significantly decreased at 1.6 and 4.9 mg/kg.
- An increase in mean litter proportions of unossified sternebrae, reduced skull ossification, unossified hyoid, and reduced vertebral arch ossification were observed at 4.9 mg/kg.
- On the basis of decreased maternal weight gain, the maternal NOAEL was considered to be <0.49 mg/kg. On the basis of decreased fetal body weights at ≥1.6 mg/kg and increased post-implantation loss and decreased skeletal ossification at 4.9 mg/kg, the NOAEL for fetal development was 0.49 mg/kg.

Methods

Species/strain: Rat/Sprague-Dawley

Study design: Pregnant female rats were dosed every third day by bolus subcutaneous injection (injection site rotated clockwise between 4 sites in the scapular region) starting on GD 6 (e.g., 6, 9, 12, and 15) with the last dose occurring on GD 15. Necropsy was performed on GD 20. (sponsor-generated table)

Embryo-Fetal Development Phase ([(b) (4)]353116)

Group Number	Test Article	Dosage Level[a] (mg/kg)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle[b]	0	1.0	25
2	LY2189265	0.49	1.0	25
3	LY2189265	1.63	1.0	25
4	LY2189265	4.89	1.0	25

Toxicokinetic Phase ([(b) (4)]-353116T)

Group Number	Test Article	Dosage Level[a] (mg/kg)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle[b]	0	1.0	4
2	LY2189265	0.49	1.0	8
3	LY2189265	1.63	1.0	8
4	LY2189265	4.89	1.0	8

[a] = Dosage levels are expressed as total LY2189265 protein content.

[b] = Vehicle control is [(b) (4)] citrate [(b) (4)] pH [(b) (4)] Polysorbate 80 with [(b) (4)] sodium chloride prepared in sterile water for injection, USP (Diluent B).

Age at GD 0: Approximately 84 days

Weight at GD0: 235 to 301 g

TK sampling: Control – 12 hours post-dose on GD 15; LY2189265-treated groups – pre-dose and 4, 12, 24, 48, and 96 hours post-dose on GD15 (3 rats/time point with no more than 3 time points per rat)

Immunogenicity: Not assessed

Parameters and endpoints: Survival and clinical observations – twice daily
Body weight and food consumption – GD 0, 6, 9, 12, 15, 18, and 20
Macroscopic examination: GD 20
Laparohysterectomy (GD 20) – gravid uterine weight, corpora lutea, number and location of fetuses, early and late resorptions, implantation sites
Fetal morphological examination –weight, sex, external, visceral, and skeletal exams

Protocol deviations: No GLP or protocol deviations occurred that impacted the validity or interpretability of the study data. After the study report was finalized, it was determined that the extinction coefficient of the test article was different than previously calculated resulting in lower administered dose levels than planned. The actual dose levels were 0, 0.49, 1.63, and 4.89 mg/kg rather than 0.6, 2, and 6 mg/kg.

Observations and Results

Mortality (dams): One 4.9 mg/kg group female was found dead on GD 8, approximately 48 hours following the first dose administration. Approximately 5 to 6 hours before death, this animal was noted as having hunched posture, hypoactivity, hypothermia (cool to touch), decreased defecation, and red material around the eyes, nose, mouth, and forelimbs. No macroscopic findings were noted and histopathology was not conducted. The cause of death was undetermined. This death may have been test article related.

Clinical signs (dams):

Dose (mg/kg):	0[a]	0.49	1.63	4.89
Clinical signs[b]				
Hunched posture	0/0	0/0	3/3	14/12
Hair loss ventral abdominal area	0/0	19/2	124/12	169/18
Hair loss ventral thoracic area	0/0	7/1	72/10	77/11
Red material around nose	3/3	11/4	13/10	46/19
Red material around the eye(s)	0/0	1/1	0/0	19/4
Red material around the mouth	0/0	0/0	3/3	8/8
Red material on forelimb(s)	2/1	0/0	8/4	31/12
Decreased defecation	0/0	3/3	18/11	50/23

[a] Vehicle control.
[b] Total occurrence/No. of animals.
(sponsor-generated table)

Body weight (dams): Decreased body weight gain was noted in all compound-treated groups and body weight loss was observed for the 1.6 and 4.9 mg/kg groups from GD 6 to 9. A compensatory increase in body weight gain was noted between GD 18 and 20 for the 1.6 and 4.9 mg/kg groups. Between GD 6 and 18, the 0.49, 1.6, and 4.9 mg/kg group dams gained 16%, 33%, and 67% less weight than the control animals, respectively. The respective mean weights for those groups were 5%, 9%, and 16% less than controls on GD 18 and 5%, 7%, and 13% less than controls on GD 20. A decrease in mean gravid uterine weight was seen at 4.9 mg/kg. Maternal body weight data are summarized in the sponsor-generated table below.

Reference ID: 3527260

Dose (mg/kg):	0[a]	0.49	1.63	4.89
Mean body weight gain (g/day)				
Gestation Days 6-9	15	5**	-10**	-27**
Gestation Days 9-12	15	10	10	9
Gestation Days 12-15	20	19	18	13**
Gestation Days 15-18	42	44	44	36*
Gestation Days 6-18	93	78**	62**	31**
Gestation Days 18-20	38	35	40	47**
Gestation Days 6-20	130	113**	102**	77**
Mean body weight (g)				
Gestation Day 6	299	294	295	296
Gestation Day 9	313	299*	285**	270**
Gestation Day 12	329	310**	295**	278**
Gestation Day 15	348	328**	313**	291**
Gestation Day 18	391	372*	357**	328**
Gestation Day 20	429	407*	397**	374**
Mean gravid uterine weight (g)	89.7	84.4	83.0	68.8**
Mean net body weight gain (g)	70.1	54.2**	45.2**	37.8**
Mean net body weight (g)	339.3	320.5**	314.0**	305.3**

[a] Vehicle control.
*p<0.05.
**p<0.01.

Food consumption (dams): A decrease in mean food consumption was noted for all dose levels compared with control animals. A compensatory increase in food consumption was observed between GD 18 and 20. Effects on food consumption are summarized in the sponsor-generated table below.

Dose (mg/kg):	0[a]	0.49	1.63	4.89
Mean food consumption (g/animal/day)				
Gestation Days 6-9	27	19**	14**	9**
Gestation Days 9-12	28	22**	19**	16**
Gestation Days 12-15	29	24**	23**	18**
Gestation Days 15-18	31	29*	28**	24**
Gestation Days 6-18	28	24**	21**	17**
Gestation Days 18-20	32	33	36**	37**
Gestation Days 6-20	29	25**	23**	19**
Mean food consumption (g/kg/day)				
Gestation Days 6-9	87	65**	49**	32**
Gestation Days 9-12	86	72**	67**	57**
Gestation Days 12-15	84	76**	74**	64**
Gestation Days 15-18	83	82	84	76**
Gestation Days 6-18	85	74**	69**	57**
Gestation Days 18-20	79	84*	96**	105**
Gestation Days 6-20	82	74**	71**	63**

[a] Vehicle control.
*p<0.05.
**p<0.01.

Maternal reproductive parameters: A slight, non-significant increase in percent post-implantation loss and a decrease in percent viable fetuses were noted in the 4.9 mg/kg group compared with controls. These changes were considered compound related because the values were outside of the lab's historical control range. Fetal body weight was significantly decreased in a dose-dependent manner at 1.6 and 4.9 mg/kg. A summary of the maternal reproductive parameter data is presented below in the sponsor-generated table.

Dose (mg/kg):	0[a]	0.49	1.63	4.89	HC Mean (Range)
Pregnant females	25	24	25	23	NA
Corpora lutea	16.4	16.1	16.9	17.3	17.5 (15.8-19.4)
Implantation sites	16.1	15.6	16.3	16.6	15.9 (14.5-17.5)
Viable fetuses	15.4	14.6	15.4	15.0	15.2 (13.9-16.6)
Early resorptions (% Per Litter)	3.9	7.7	5.8	8.9	4.4 (1.5-8.6)
Late resorptions (% Per Litter)	0.0	0.0	0.0	1.0	0.1 (0.0-0.8)
Viable fetuses (% Per Litter)	96.2	92.3	94.2	90.1	95.5 (91.4-98.0)
Postimplantation loss (%)	3.9	7.7	5.8	9.9	4.5 (2.0-8.6)
Combined fetal weight (g)	3.8	3.7	3.5**	2.7**	3.6 (3.4-3.8)

All data expressed as mean values for the group, except for the number of pregnant females.
HC = (b) (4) historical control data.
NA = Not applicable.
[a] Vehicle control.
**p<0.01.

Fetal parameters: At 4.9 mg/kg, some fetal skeletal variations were considered to be compound related. The mean litter proportions of unossified sternebrae numbers 1, 2, 3, and/or 4, reduced ossification of the skull, unossified hyoid, and reduced ossification of the vertebral arches were higher (not significant) than the control group and the maximum mean values in the lab's historical control data. It was felt that these findings were secondary to the significant intrauterine growth retardation observed at 4.9 mg/kg. The mean litter proportion of reduced ossification of the vertebral arches was also higher in the low dose group (0.49 mg/kg). This finding was not considered adverse at this dose level because it was not observed in the 1.6 mg/kg group and because the observations were generally limited to single fetuses with low body weights in otherwise normal litters. A summary of the effects on fetal development is presented in the sponsor-generated table below.

Dose (mg/kg):	0[a]	0.49	1.63	4.89	HC mean (Range)
Skeletal developmental variations (% Per Litter)					
Sternebrae nos. 1, 2, 3, and/or 4 unossified	0.0	0.0	0.0	4.9	0.2 (0.0-1.3)
Reduced ossification of the skull	0.0	0.3	0.5	5.5	0.1 (0.0-1.0)
Hyoid unossified	0.0	0.7	2.2	3.5	1.5 (0.0-4.2)
Reduced ossification of the vertebral arches	0.0	5.4	0.0	3.5	0.1 (0.0-1.1)

- No compound-related findings.
HC = (b) (4) historical control data.
NA = Not applicable.
[a] Vehicle control.

Toxicokinetics: TK data following the fourth dose administration (GD 15) showed that exposure (C_{max} and AUC) increased in a dose-proportional manner. A summary of the TK data is presented in the sponsor-generated table below.

Dose (mg/kg)[a]:	0.49	1.63	4.89
Gestation Day 15			
C_{max} (µg/mL)	0.96	2.79	10.31
T_{max} (h)	12.0	12.0	12.0
$AUC_{0\text{-}96\ hr}$ (µg·h/mL)	35.00	110.06	348.14

Abbreviations: $AUC_{0\text{-}96\ h}$ = plasma concentration time curve between 0 and 96 hours, C_{max} = maximum plasma concentration, and T_{max} = time of maximum plasma concentration.
[a] = Doses expressed as total LY2189265 protein content.

Dosing formulation analysis: The LY2189265 concentration of each sampled dosing suspension did not deviate substantially from its theoretical value. Mean assayed concentrations of LY2189265 ranged from 98.3% to 100% of theoretical values.

Study title: Embryo-fetal development and toxicokinetic study in New Zealand White rabbits of LY2189265 administered via subcutaneous injection every third day

Study number:	(b) (4) 353119, Amendment 1
Study report location:	Module 4.2.3.5.2
Conducting laboratory:	(b) (4)
Date of study initiation:	02 November 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	LY2189265, Lot # (b) (4) C1301-78, 65.9% (b) (4), reduced)

Key Study Findings

- No mortality or adverse clinical signs were noted.
- Initial significant weight loss followed by decreased body weight gain occurred at ≥0.12 mg/kg; decreased body weight gain was also noted for the 0.04 mg/kg group throughout the treatment period. These effects on weight correlated with a significant decrease in food consumption at all dose levels.
- A compensatory increase in body weight and food consumption occurred once dosing ended, which lasted until the end of the study.
- There were no effects on maternal reproductive parameters.
- Increased fetal malformations (lung lobular agenesis, vertebral anomaly with or without assoc. rib anomaly, and costal cartilage anomaly) occurred at 0.41 mg/kg.
- Increased fetal skeletal variations occurred at 0.41 mg/kg (increased incidence of 27 presacral vertebrae, 13th full rib, bent hyoid arches, malaligned sternebrae, and reduced ossification of the ribs. Also, a decreased incidence of having a 13th rudimentary rib was noted for kits from the 0.12 and 0.41 mg/kg litters.
- Anti-LY2189265 antibodies may have been produced in some animals during the treatment period because plasma concentrations on GD 19 were quite variable. If present, it is uncertain whether the presence of the antibodies significantly impacted the activity the test compound in vivo; exposure values on GD 19 seemed to be only marginally affected, if at all.
- On the basis of decreased body weight gain at 0.04 mg/kg, the NOAEL for maternal toxicity is <0.04 mg/kg, although the Sponsor felt that this was not an adverse effect and determined the NOAEL to be 0.12 mg/kg. On the basis of the fetal malformations and increase in skeletal variations observed at 0.41 mg/kg, the NOAEL for fetal development is 0.12 mg/kg. Overall, the adverse effects on fetal development were observed only in the presence of maternal toxicity.

Methods

Species/strain:	Rabbit/New Zealand White
Study design:	Pregnant female rabbits were dosed every third day by bolus subcutaneous injection (injection site rotated clockwise between 4 sites in the scapular region) starting on GD 7 with the last dose occurring on GD 19 (i.e., GD 7, 10, 13, 16, & 19). Necropsy was performed on GD 29.

Reference ID: 3527260

(sponsor-generated table)

Embryo-Fetal Development Phase ([(b) (4)]-353119)

Group Number	Test Article	Dosage Level[a] (mg/kg)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle	0	0.25	20
2	LY2189265	0.04	0.25	20
3	LY2189265	0.12	0.25	20
4	LY2189265	0.41	0.25	20

Toxicokinetic Phase ([(b) (4)]353119T)

Group Number	Test Article	Dosage Level[a] (mg/kg)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle	0	0.25	4
2	LY2189265	0.04	0.25	4
3	LY2189265	0.12	0.25	4
4	LY2189265	0.41	0.25	4

[a] = Dosage levels are expressed as total LY2189265 protein content.

Age at GD 0:	Approximately 5 months
Weight at GD 0:	2.99 to 4.04 kg
TK sampling:	Pre-dose and 4, 8, 12, 24, 48, and 72 hours post-dose on GD7 and 19 (4 rabbits/time point)
Immunogenicity:	Not assessed
Parameters and endpoints:	Survival - twice daily Clinical observations, injection site exams, and food intake – daily Body weights – GD 0, 5, 6, 7, 10, 13, 16, 19, 22, 25, 29 Gross pathology (maternal) and gravid uterine weights – GD 29 Laparohysterectomy (litter number and fetal morphological evaluations) – GD 29
Protocol deviations:	No GLP or protocol deviations occurred that impacted the validity or interpretability of the study data. After the study report was finalized, it was determined that the extinction coefficient of the test article was different than previously calculated resulting in lower administered dose levels than planned. The actual dose levels were 0, 0.04, 0.12, and 0.41 mg/kg rather than 0.05, 0.15, and 0.5 mg/kg.

Results

Mortality (dams): There were no mortalities during the study.

Clinical signs (dams): The noteworthy clinical signs observed during the study are presented in the sponsor-generated table below.

Dose (mg/kg):	0[a]	0.04	0.12	0.41
Clinical signs[b]				
Decreased Defecation	37/11	27/9	66/15	158/20
Feces Small	2/1	12/7	17/11	24/12

[a] Vehicle.
[b] Total Occurrences/No. of Animals.

Body weight (dams): Mean body weight loss was observed between GD 7 and 13 for the 0.41 mg/kg group and between GD 7 and 10 for the 0.12 mg/kg group. A decrease in body weight gain was observed for the 0.04 and 0.12 mg/kg groups from GD 7 to 19 and GD 10 to 19, respectively. Total mean body weight gain during the treatment period (GD 7 to 19) for the 0.04, 0.12, and 0.41 mg/kg groups was 48%, 63%, and 104% less than control animals, respectively. An increase in body weight gain relative to controls was noted for all treatment groups once the administration of the test article had ended (GD 19 to 29). Interestingly, an increase in body weight gain was noted for the high dose group from GD 13 to 19, even though food consumption was lower than control animals. A summary of the body weight and body weight gain data is presented in the sponsor-generated table below.

Dose (mg/kg):	0[a]	0.04	0.12	0.41
Mean body weight gain (g/day)				
Gestation Days 7-10	65	33	-63**	-205**
Gestation Days 10-13	45	18	41	-1
Gestation Days 13-16	76	58	63	96
Gestation Days 16-19	84	64	59	98
Gestation Days 19-22	45	71	60	92
Gestation Days 7-22	314	244	160**	80**
Gestation Days 22-29	6	104*	163**	166**

[a] Vehicle.
*p<0.05.
**p<0.01.

Food consumption (dams): A dose-related decrease in mean food consumption was observed at all dose levels from GD 7 to 22. A compensatory increase in food consumption was observed between GD 22 and 29.

Dose (mg/kg):	0[a]	0.04	0.12	0.41
Mean food consumption (g/animal/day)				
Gestation Days 7-10	175	134**	73**	27**
Gestation Days 10-13	167	121**	91**	35**
Gestation Days 13-16	161	117**	99**	62**
Gestation Days 16-19	177	150	116**	108**
Gestation Days 19-22	162	157	135	147
Gestation Days 7-22	169	136*	103**	75**
Gestation Days 22-29	86	128**	134**	146**

[a] Vehicle.
*p<0.05.
**p<0.01.

Macroscopic examinations: There were no compound-related internal or external macroscopic findings at any dose level.

Maternal reproductive parameters: There were no compound-related effects on intrauterine growth and survival parameters, including implantation sites, viable fetuses, resorptions, pre- or post-implantation losses, or combined fetal weights.

Dose (mg/kg):	0[a]	0.04	0.12	0.41
Pregnant females	19[b]	19	19	20
Corpora lutea	9.9	9.2	9.3	10.3
Implantation sites	9.7	8.4	8.9	9.6
Viable fetuses	9.3	7.8	8.6	9.3
Early Resorptions (% per litter)	2.2	4.3	1.0	1.4
Late Resorptions (% per litter)	1.5	1.1	1.6	1.5
Pre-implantation loss (%)	2.5	10.2	5.7	6.6
Postimplantation loss (%)	3.7	5.4	2.6	3.4
Viable fetuses (% per litter)	96.3	94.6	97.4	96.6
Dead fetuses (% per litter)	0.0	0.0	0.0	0.6
Combined fetal weight	41.3	45.5	44.5	40.8

All data expressed as mean values for the group.
NA = Not applicable
[a] Vehicle.
[b] Does not include female no. 47814 that delivered on Gestation Day 28.

Fetal parameters: A summary of the malformations and variations observed in the offspring is presented in the sponsor-generated table below. The number of fetuses (litters) for which malformations were observed was 2(2), 0(0), 2(2), and 14(7) in the control, 0.04, 0.12, and 0.41 mg/kg groups, respectively. A statistically significant increase in the total percent of variations per litter was seen at 0.41 mg/kg (95.4%) compared with that observed for the control group (71.8%). In addition to the effects noted below, an increase in the prevalence of bent hyoid arches (10.6% per litter for the 0.41 mg/kg group compared with 5.3% for the control group) and a slight increase in malaligned sternebrae (2.0% compared with 0.5%, respectively).

Filamentous tail and short tail were noted for one fetus each in the 0.41 mg/kg group; skeletally, both of these findings consisted of absent sacral and/or caudal vertebrae and fused and/or misshapen caudal, lumbar, and /or sacral vertebrae. In addition to short tail, one fetus from the high-dose group also had several visceral malformations and developmental variations, consisting of absent kidney and ureter, misshapen liver, a small gallbladder, cystic chordae tendineae, pale spleen, retrocaval ureter, and ovarian adhesions. Vertebral anomalies with associated rib anomalies were also observed for this kit.

Dose (mg/kg):	0[a]	0.04	0.12	0.41	HC Mean[b] (Range)
Malformations (% per litter)					
Lungs - Lobular agensis	0.6	0.0	0.5	2.6	0.4 (0.0-2.6)
Vertebral anomaly with or without associated rib anomaly	0.0	0.0	0.0	2.3	0.4 (0.0-2.0)
Costal cartilage anomaly	0.0	0.0	0.6	1.0	0.1 (0.0-0.7)
Variations (% per litter)					
27 presacral vertebrae	7.2	19.9	23.1	52.5++	18.1 (7.5-33.1)
13th full rib	35.8	52.8	53.5	82.0++	39.1 (26.9-58.6)
13th rudimentary rib	18.9	15.5	7.2+	6.6+	18.5 (8.1-29.7)
Reduced ossification of the rib(s)	0.0	0.0	0.0	1.2	0.0 (0.0-0.6)

All data expressed as mean values for the group.
[a] Vehicle.
[b] (b) (4) historical control data of NZW rabbits (b) (4) facility.
+ $p<0.05$.
++ $p<0.01$.

Toxicokinetics: A dose-related increase in systemic exposure was observed on both GD 7 and GD 19. Exposure after either a single dose (GD 7) or repeated doses (GD 19) increased in a reasonably dose-proportional manner. There was no apparent accumulation after repeated dosing; however, a definitive conclusion concerning potential accumulation was confounded by high variability in plasma concentrations following the GD 19 administration. This observed variability, particularly evident in one animal per group, may have resulted from possible presence of anti-LY2189265 antibodies. If present, the Sponsor feels that the impact of an antibody response with regard to LY2189265 exposure was minimal because it was shown that adequate exposure over the duration of the major organogenesis period was observed and evidence of a drug effect (decreased food consumption) was observed throughout the treatment period. However, the drug-related effects at the high dose level may have been diminished after GD 13 because these animals tended to gain more weight than the control animals even though they were exposed to the test article during this time period. A summary of the TK data is provided in the sponsor-generated table below.

Note that $AUC_{0\text{-}24hr}$ values in the TK table should be listed as $AUC_{0\text{-}last}$, for which the last sampling time point was 72 hours after dosing. This is an important distinction for calculating clinical exposure margins, as the AUC values below should be divided by 72 rather than 24 to standardize to the human $AUC_{0\text{-}168h}$ values.

Dose (mg/kg):	0.04	0.12	0.41
Day 7			
C_{max} (µg/mL)	0.15	0.45	1.31
T_{max} (hr)	12.0	20.0	16.0
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	6.62	22.30	65.38
Day 19			
C_{max} (µg/mL)	0.12	0.51	1.63
T_{max} (hr)	13.3	16.0	20.0
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	4.81	21.81	64.59
Day 19 [a]			
C_{max} (µg/mL)	0.17	0.56	1.74
T_{max} (hr)	18.0	18.0	24.0
$AUC_{0\text{-}24\ hr}$ (µg•hr/mL)	7.05	26.52	77.94

Abbreviations: $AUC_{0\text{-}24\ hr}$ = plasma concentration time curve between 0 and 24 hours, C_{max} = maximum plasma concentration, and T_{max} = time of maximum plasma concentration.

[a] = Means exclude animal data that indicated likely antibody formation.

Dose formulation analysis: The LY2189265 concentration of each sampled dosing solution did not deviate substantially from its theoretical value. Mean assayed concentrations of LY2189265 were 100% of their theoretical values.

Reference ID: 3527260

9.3 Prenatal and Postnatal Development

Study title: A prenatal and postnatal development and toxicokinetic study, including maternal function, of LY2189265 administered via subcutaneous injection every third day

Study number:	(b) (4) 353253
Study report location:	Module 4.2.3.5.3
Conducting laboratory:	(b) (4)
Date of study initiation:	23 May 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, concentration:	LY2189265, Lot #17804-1, 40.2 mg/mL

Key Study Findings

F_0 Dams

- There were no treatment related mortalities or adverse clinical signs.
- A dose-related decrease in mean body weight gain was noted for all treated groups. HD females lost weight between GD 6 and 9 followed by body weight gains similar to or slightly greater than the MD group, but less than the control group. During lactation, body weight gains were similar to controls. The effects on body weight correlated with decreased food consumption during the gestation and lactation periods.
- Treatment did not affect mean values for gestation duration, litter size, live litter size, number of implantation sites, or pup sex ratio.

F_1 Pups

- Survival rates during the lactation period for pups from HD dams were lower than historical control values, but were similar to the concurrent control values.
- An increased incidence of pups showing small stature, paleness, or cool to touch was noted for pups from HD dams.
- Mean body weights for male and female pups from HD dams were statistically significantly lower than controls at all time points from LD 1 to 21. Weights remained lower throughout the remainder of the study, with the differences being statistically significant up to PND 63 for males and up to PND 84 for females. Mean body weights remained lower for HD F_1 pregnant females during gestation, although body weight gains during gestation were similar to controls. There were no noteworthy effects on food consumption during the post-natal period.
- HD males had a slightly longer mean time to balano-preputial separation (47.0 days vs. 45.6 days for control), which was likely secondary to the smaller size of the HD male pups.
- A decrease in forelimb and hindlimb grip was noted for male and female HD pups on PND 20, and mean total and ambulatory motor activity was increased for male

and female HD pups during a 60-minute observation period on PND 21. HD female pups also showed a slight decrease in maximum startle response on PND 22. The effects on grip strength, motor activity, and startle response were not statistically significantly different from control values and were not observed during test occurring from PND 60 to 62. These findings may be indicative of a slight developmental delay, possibly due to the smaller size of the pups.

- A statistically significant increase in mean Biel maze straight channel swim trial escape time was noted for HD female pups on PND 70 (6.8 sec vs. 5.0 sec for control). An increase in mean Biel maze memory escape time was also noted for HD female pups during trial number 12 (62.6 sec vs. 31.9 sec for control), which was associated with a greater number of memory errors during this trial. No effects in the Biel maze tests were noted for male pups.
- There were no effects on F1 generation mating, fertility, or reproductive organs (microscopic findings or organ weights).

F_2 Pups

- There were no effects on the mean number of pups born, live litter size, sex ratio, body weight, or postnatal survival (to PND 1). There were no external findings suggestive of a test article effect.

Conclusions

- On the basis of effects on body weight and food consumption for F_0 females, the NOAEL for maternal toxicity was considered to be 0.2 mg/kg.
- On the basis of effects on body weight and body weight gain of the F_1 pups, the NOAEL for neonatal toxicity was considered to be 0.49 mg/kg.
- Because effects on memory were observed for F_1 females derived from HD dams, the NOAEL for neurological developmental was considered to be 0.49 mg/kg for females and 1.63 mg/kg for males.
- The NOAEL for F_1 reproductive parameters was 1.63 mg/kg.

Methods

Species/Strain: Rat / Sprague-Dawley

Study design: (sponsor-generated table)

Group Number	Treatment	Dose Level (mg/kg)	Dose Volume (mL/kg)	Number of Females PPN Phase	Number of Females TK Phase
1	Vehicle Control	0	1	25	4
2	LY2189265	0.2	1	25	8
3	LY2189265	0.49	1	25	8
4	LY2189265	1.63	1	25	8

PPN Phase = Pre- and postnatal development phase.
TK Phase = Toxicokinetic phase.

Route of administration:	Subcutaneous
Frequency of dosing:	Every 3rd day from GD 6 through LD 19
Formulation/Vehicle:	(b) (4) citrate (b) (4) (pH (b) (4) mannitol, and (b) (4) polysorbate 80 in sterile water for injection
Study design:	Assumed pregnant F_0 females were dosed every third day from GD 6 through LD 19. All F_0 females were allowed to deliver and rear their offspring until LD 21. Litters were culled to 4 pups/sex/litter, when possible, on PND 4. Negative geotaxis was assessed starting on PND 6. One male and 1 female per litter per dose group were randomly selected for the F_1 generation evaluations of detailed clinical observations (PND 11, 20, and 60), locomotor activity (PND 21 and 61), acoustic startle (PND 22 and 62), learning and memory (PND 70), sexual maturation (starting at PND 35 [males] and PND 25 [females], and reproductive performance (minimum of PND 85, avoiding sibling matings). All females were allowed to deliver and rear their young to PND 1, at which time F_2 pups were euthanized and examined externally (no internal exam).
Protocol deviations:	There were no protocol or GLP deviations that impacted the quality or integrity of the study data.

Observations and Results

F_0 Dams

Survival: (assessed twice daily)
No treatment-related mortality occurred. One gravid LD female was found dead on GD 22.

Clinical signs: (assessed twice daily)
No treatment-related adverse clinical signs were observed.

Body weight: (measured on GD 0, 6, 9, 12, 15, 18, 20 and LD 1, 4, 7, 10, 13, 16, 19, and 21)
A dose-related decrease in mean body weight gain was noted for all treated groups. HD females lost weight between GD 6 and 9, followed by body weight gains similar to or slightly greater than the MD group, but less than the control group. An initial decrease in body weight gain occurred from LD 1 to 4, increased body weight gain occurred from LD 4 to 7, and similar body weight gains occurred from LD 7 to 21, resulting in overall mean body weight gains during lactation that were similar to controls. Because of the lower body weights at parturition, final body weights of treated groups remained lower than controls at the end of lactation.

Reference ID: 3527260

Bodyweight Gain during Gestation

Dose (mg/kg)	0	0.2	0.49	1.63
BW Gain from GD 6 to GD 20 (g)	124	114	91**	78**
Diff from control (g)		-10	-33	-46
Diff from control (%)		↓8%	↓27%	↓37%

Food consumption: (assessed on GD 0, 6, 9, 12, 15, 18, 20 and LD 1, 4, 7, 10, and 13)
A dose-related decrease in food consumption was noted for all treated groups compared with control, with the difference being statistically significant for most time points. This trend continued during lactation, with differences being statistically significant for the HD group.

Gestation:
There were no treatment-related effects on the duration of gestation. The mean length of gestation for all groups ranged from 21.8 to 22.0 days.

Necropsy and uterine content: (All females including dead and moribund animals; females with viable pups were euthanized on PND 21; females with entire litter loss were euthanized with 24 hours of litter loss; females that did not deliver were euthanized on post-mating Day 25)

There were no treatment-related macroscopic findings observed. There were no apparent treatment-related effects on mean litter size, mean live litter size, implantation sites, or sex ratio.

Toxicokinetics: (Samples collected pre-dose and at 4, 12, 24, 48, and 96 hours after dosing on GD 6 and LD 4)

Dose (mg/kg):	0.2	0.49	1.63
Gestation Day 6			
AUC_{last} (µg•hr/mL)	10.4	28.5	95.0
C_{max} (µg/mL)	0.389	1.13	3.65
T_{max} (hr)	12	12	12
Lactation Day 4			
AUC_{last} (µg•hr/mL)	15.3	35.7	131
C_{max} (µg/mL)	0.497	1.04	3.95
T_{max} (hr)	12	12	12

Abbreviations:
AUC_{last} = Area under the plasma curve from time of dosing to 48 hours post-dosing on Gestation Day 6 and 96 hours post-dosing on Lactation Day 4
C_{max} = maximum plasma concentration, and T_{max} = time of maximum plasma concentration.
(Sponsor-generated table)

Dosing Solution Analysis:
LY2189265 was found to be stable throughout the dosing period. The concentration of each sampled dosing suspension did not deviate substantially from its theoretical value, with mean concentration values ranging from 103% to 105% of theoretical values.

F_1 Generation

Survival: (Twice daily)
Postnatal survival was slightly lower for the HD group compared with control. Fifty and 55 pups from the HD group were found dead or missing (presumed cannibalized), respectively, compared with 37 and 32 pups in the control group. However, the values for both control and HD groups were below the laboratory's historical control values (sponsor-generated table below). All total litter loss in the control and HD groups occurred after culling during PNDs 5 to 16.

Dose (mg/kg)	0[a]	0.2	0.49	1.63	(b) (4) HC mean (range)
Mean no. of pups born	15.2	14.4	14.5	15.5	14.8 (12.6-16.6)
Percentage of males at birth (per litter)	45.6	52.5	48.9	50.5	49.0 (42.9-55.3)
Live litter size (PND 0)	15.2	14.0	14.5	15.4	14.7 (12.6-16.4)
Postnatal survival (% per litter)					
PND 0 relative to no. born	99.5	92.3	100.0	99.4	98.7 (96.1-100.0)
PND 0-1	97.6	99.0	98.9	97.5	99.1 (96.5-100.0)
PND 1-4	91.5	92.9	93.5	85.5	98.7 (95.7-100.0)
PND 4-7	84.5	95.2	95.1	79.5	98.8 (95.8-100.0)
PND 7-14	96.9	97.7	100.0	93.0	99.3 (96.2-100.0)
PND 14-21	95.5	100.0	100.0	95.5	99.4 (95.7-100.0)
Birth to PND 4	89.3	84.8	92.7	83.3	96.6 (91.9-99.3)
PND 4-21	78.0	93.1	95.1	73.0	97.1 (86.7-100.0)

[a] = Vehicle control.
HC = (b) (4) reproductive historical control data.

Clinical signs: (Twice daily with detailed exams on PND 1, 4, 7, 10, 14, 17, and 21)
A slight increase in the incidences of small stature, pale body, and cool body were noted for the HD group (sponsor-generated table).

CATEGORY FINDING	GROUP:	1	2	3	4
SMALL STATURE		9/ 6	4/ 3	5/ 3	17/ 15
	LITTERS:	6	2	3	12
BODY PALE		2/ 2	0/ 0	1/ 1	8/ 8
	LITTERS:	2	0	1	6
BODY COOL		1/ 1	0/ 0	0/ 0	5/ 5
	LITTERS:	1	0	0	3

Body weight: (Weekly until necropsy for males and until signs of mating for females, after which on GD 0, 6, 9, 12, 16, and 20 and LD 1)

Pre-Weaning Body Weights (sponsor-generated table)

Dose (mg/kg):	0[a]		0.2		0.49		1.63		(b) (4) HC Mean (Range)	
	M	F	M	F	M	F	M	F	M	F
Mean body weight (g; %)										
PND 1	6.9	6.6	6.9 (0.0)	6.5 (-1.5)	6.7 (-2.9)	6.3 (-4.5)	6.3 (-8.7)*	5.8 (-12.1)**	7.0 (6.6-7.5)	6.6 (6.1-7.0)
PND 4	9.1	8.8	9.0 (-1.1)	8.5 (-3.4)	9.0 (-1.1)	8.4 (-4.5)	7.3 (-19.8)**	6.8 (-22.7)**	9.7 (8.7-10.8)	9.2 (8.1-10.4)
PND 7	14.9	14.4	14.1 (-5.4)	13.3 (-7.6)	14.3 (-4.0)	13.3 (-7.6)	11.9 (-20.1)**	10.6 (-26.4)**	15.0 (12.9-17.4)	14.3 (12.1-16.5)
PND 10	21.5	20.8	20.6 (-4.2)	19.5 (-6.3)	20.8 (-3.3)	19.3 (-7.2)	18.0 (-16.3)**	16.0 (-23.1)**	22.0 (18.3-24.1)	21.0 (17.4-23.8)
PND 14	31.6	30.8	30.7 (-2.8)	29.3 (-4.9)	30.1 (-4.7)	28.1 (-8.8)	26.8 (-15.2)**	24.1 (-21.8)**	31.0 (24.6-34.5)	29.7 (24.2-32.8)
PND 17	39.7	38.5	37.7 (-5.0)	36.0 (-6.5)	37.4 (-5.8)	35.0 (-9.1)*	33.8 (-14.9)**	31.7 (-17.7)**	38.5 (30.7-42.0)	37.0 (30.0-40.3)
PND 21	50.8	49.1	49.3 (-3.0)	46.6 (-5.1)	48.1 (-5.3)	45.5 (-7.3)	43.9 (-13.6)**	41.3 (-15.9)**	49.0 (37.8-55.7)	46.9 (36.9-53.0)

[a] Vehicle control.

HC = (b) (4) historical control data.

% = Percent difference from the vehicle control group.

*p<0.05 **p<0.01

Post-Weaning Body Weights

Dose (mg/kg):	0[a]	0.2	0.49	1.63
F_1 Males mean body weight (g, %)				
PND 28	89	89 (0.0)	86 (-3.4)	75 (-15.7)**
PND 35	152	151 (-0.7)	146 (-3.9)	131 (-13.8)**
PND 42	216	217 (0.5)	210 (-2.8)	189 (-12.5)**
PND 49	280	279 (-0.4)	273 (-2.5)	249 (-11.1)**
PND 56	341	338 (-0.9)	334 (-2.1)	310 (-9.1)*
PND 63	388	385 (-0.8)	382 (-1.5)	356 (-8.2)*
PND 70	428	426 (-0.5)	426 (-0.5)	398 (-7.0)
PND 77	449	447 (-0.4)	449 (0.0)	423 (-5.8)
PND 84	481	480 (-0.2)	483 (0.4)	456 (-5.2)
PND 91	500	499 (-0.2)	495 (-1.0)	476 (-4.8)
PND 98	530	527 (-0.6)	518 (-2.3)	504 (-4.9)
PND 105	550	551 (0.2)	537 (-2.4)	529 (-3.8)
PND 112	573	574 (0.2)	554 (-3.3)	551 (-3.8)
PND 119	586	593 (1.2)	574 (-2.0)	565 (-3.6)
PND 126	601	608 (1.2)	589 (-2.0)	579 (-3.7)

Dose (mg/kg):	0[a]	0.2	0.49	1.63
F_1 Females mean body weight (g, %)				
PND 28	82	80 (-2.4)	77 (-6.1)	67 (-18.3)**
PND 35	128	127 (-0.8)	123 (-3.9)	108 (-15.6)**
PND 42	166	165 (-0.6)	161 (-3.0)	144 (-13.3)**
PND 49	196	196 (0.0)	191 (-2.6)	174 (-11.2)**
PND 56	227	223 (-1.8)	218 (-4.0)	203 (-10.6)**
PND 63	244	241 (-1.2)	234 (-4.1)	218 (-10.7)**
PND 70	261	258 (-1.1)	254 (-2.7)	235 (-10.0)**
PND 77	271	263 (-3.0)	261 (-3.7)	245 (-9.6)**
PND 84	288	279 (-3.1)	276 (-4.2)	260 (-9.7)**

[a] Vehicle control.
% = Percent difference from the vehicle control group.
**p<0.01

Bodyweights and Bodyweight Gains for Pregnant F_1 Females

Dose (mg/kg):	0[a]	0.2	0.49	1.63
Mean body weight (g, %)				
Gestation Day 0	293	284 (-3.1)	285 (-2.7)	264 (-9.9)**
Gestation Day 6	324	316 (-2.5)	315 (-2.8)	292 (-9.9)**
Gestation Day 9	336	327 (-2.7)	325 (-3.3)	302 (-10.1)**
Gestation Day 12	351	343 (-2.3)	342 (-2.6)	318 (-9.4)**
Gestation Day 16	378	370 (-2.1)	366 (-3.2)	342 (-9.5)**
Gestation Day 20	436	430 (-1.4)	426 (-2.3)	391 (-10.3)**
Mean body weight gain (g)				
Gestation Days 0-6	31	32	30	28
Gestation Days 6-9	12	11	10	10
Gestation Days 9-12	16	16	17	16
Gestation Days 12-16	27	27	24	24
Gestation Days 16-20	58	60	60	49
Gestation Days 0-20	143	146	141	127

[a] Vehicle control.
% = Percent difference from the vehicle control group.

Feed consumption: (GD 0, 6, 9, 12, 16, and 20; pregnant F1 females only)
No noteworthy differences in mean food consumption were noted for pregnant F_1 females.

Sex determination: (PND 0, 4, 10, and 21)
There were no effects on sex ratio of the F_1 generation at any time point.

Physical development:
There were no treatment-related effects on the time until negative geotaxis was achieved. HD males had a slightly longer mean time to balano-preputial separation (47.0 days vs. 45.6 days for control), which was likely related to the treatment-related effect on decreased mean pup weight for the HD group. There were no apparent effects on the time to vaginal patency even though mean body weight was also lower for HD female pups.

Neurological and neuromuscular assessments:
There was a slight reduction in mean forelimb and hindlimb grip strength for HD males and females on PND 20. This difference did not reach statistical significance. A similar trend was not observed on PND 60.

MALES

GROUP:	1	2	3	4
NUMBER OF ANIMALS TESTED	22	19	23	19
FORELIMB GRIPSTRENGTH (AVG)				
MEAN	113.7	123.6	124.2	107.2
S.D.	28.10	37.29	32.11	30.26
N	22	19	23	19
HINDLIMB GRIPSTRENGTH (AVG)				
MEAN	74.7	69.2	67.9	60.4
S.D.	18.57	14.78	13.26	19.37
N	22	19	23	19

FEMALES

FORELIMB GRIPSTRENGTH (AVG)				
MEAN	119.9	115.5	108.3	101.3
S.D.	32.60	31.04	32.07	35.44
N	22	20	23	21
HINDLIMB GRIPSTRENGTH (AVG)				
MEAN	64.9	70.3	56.5	57.3
S.D.	12.03	16.46	12.51	14.23
N	22	20	23	21

Mean motor activity counts (total and ambulatory) on PND 21 were higher for HD males for the 60 minute test period, with the most noteworthy differences occurring between 11 and 60 minutes. In females, mean motor activity counts were higher from 21 to 60 minutes. However, these effects were not statistically significantly different from control values. This trend was not as noteworthy on PND 61.

Mean Motor Activity

Dose (mg/kg):	0[a]		0.2		0.49		1.63	
	M	F	M	F	M	F	M	F
PND 21 Total counts (%)								
0-10	1650	1554	1515 (-8.2)	1555 (0.1)	1657 (0.4)	1605 (3.3)	1684 (2.1)	1531 (-1.5)
11-20	606	694	662 (9.2)	468 (-32.6)	659 (8.7)	610 (-12.1)	866 (42.9)	623 (-10.2)
21-30	366	350	322 (-12.0)	205 (-41.4)	359 (-1.9)	305 (-12.9)	511 (39.6)	392 (12.0)
31-40	191	110	157	146	197	172	274	276
41-50	136	103	72	58	182	115	259	214
51-60	133	85	61	38	169	73	347	148
Overall	3081	2896	2789 (-9.5)	2469 (-14.7)	3223 (4.6)	2880 (-0.6)	3940 (27.9)	3183 (9.9)
PND 21 Ambulatory counts (%)								
0-10	695	665	646 (-7.1)	677 (1.8)	707 (1.7)	700 (5.3)	794 (14.2)	709 (6.6)
11-20	188	227	215 (14.4)	130 (-42.7)	222 (18.1)	198 (-12.8)	388 (106.4)	232 (2.2)
21-30	119	88	99 (-16.8)	58 (-34.1)	121 (1.7)	94 (6.8)	217 (82.4)	144 (63.6)
31-40	60	21	40	36	60	50	121	100
41-50	41	24	9	11	65	26	122	73
51-60	36	21	8	3	62	7	167	51
Overall	1138	1046	1016 (-10.7)	914 (-12.6)	1238 (8.8)	1075 (2.8)	1808 (58.9)	1309 (25.1)

[a] Vehicle control.

% = Percent difference from the vehicle control group.

On PND 22, female pups from treated dams had a slight decrease in mean maximum startle response. Faster mean TMax and average values were also noted for these females compared to the control group. These findings were considered to be secondary to the developmental delay observed in these pups (15.9% lower weight than controls). This was not observed on PND 62.

A statistically significant increase in mean Biel maze straight channel swim trial escape time was noted for HD female pups on PND 70 (6.8 seconds vs. 5.0 seconds for control). An increase in mean Biel maze memory escape time was noted for HD female pups for trial number 12 only (62.6 seconds vs. 31.9 seconds for control), which was associated with a greater number of memory errors during this trial (see sponsor-generated table below). No effect was noted for male pups for Biel maze straight channel, learning escape times or learning errors, or memory escape times or memory errors.

Biel Maze Memory

Dose (mg/kg):	0[a]		0.2		0.49		1.63	
	M	F	M	F	M	F	M	F
PND 70 Path A Memory Trial Escape Time (seconds; %)								
11	76.8	86.6	76.7	71.8	65.4	71.3	69.7	73.9
			(-0.13)	(-17.09)	(-14.84)	(-17.67)	(-9.24)	(-14.67)
12	32.3	31.9	43.7	39.9	33.0	45.7	26.7	62.6*
			(35.29)	(25.08)	(2.17)	(43.26)	(-17.34)	(96.24)
Overall	54.6	59.3	60.2	55.8	49.2	58.5	48.2	68.3
PND 70 Path A Memory Trial Number of Error (%)								
11	8	8	7	7	6	8	6	7
			(-12.5)	(-12.5)	(-25.0)	(0.0)	(-25.0)	(-12.5)
12	3	3	4	3	3	5	3	6**
			(33.3)	(0.0)	(0.0)	(66.7)	(0.0)	(100.0)
Overall	5	5	6	5	5	6	5	7

[a] Vehicle control.
% = Percent difference from the vehicle control group.
*p=0.008 **p=0.003

Reproduction:

There were no treatment-related effects on F_1 male or female mating or fertility indices, male copulation index, female conception index, mean pre-coital interval, mean estrous cycle length, total normal estrous cycles, or total estrous cycles.

Although there were some statistical significant differences in the mean gestational length for F_1 pregnant females, the differences from the concurrent control and historical control values were not considered to be biologically meaningful (sponsor-generated table)

Dose (mg/kg):	0[a]	0.2	0.49	1.63	(b) (4) HC Mean (Range)
Mean gestation length (days)	21.6	21.8	21.9*	22.1**	21.8 (21.3-22.0)

[a] Vehicle control.
*p<0.05 **p<0.01
HC = (b) (4) historical control data.

A slight decrease in mean number of implantation sites, number of corpora lutea, and number of pups born was observed for the HD F_1 females (sponsor-generated table below). Although these values were slightly below the historical control range, the differences from the concurrent control group were not statistically significantly different.

Dose (mg/kg):	0[a]	0.2	0.49	1.63	(b) (4) HC Mean (Range)
Mean no. implantation sites	14.6	15.1	14.4	13.0	15.4 (13.9-17.1)
Mean no. corpora lutea	16.3	15.6	16.5	14.6	16.9 (15.3-18.4)
Mean no. pups born	13.5	14.1	13.6	12.5	14.8 (12.6-16.6)

[a] Vehicle control.
HC = (b) (4) historical control data.

There were no apparent effects on F_1 male sperm motility or motility or mean left epididymal concentration at any dose level.

Necropsy: (fresh dissection technique for pups found dead on PND 0 to 7; detailed gross necropsy for any pup dying after PND 7; pups not selected for physical development evaluations were euthanized on PND 21)

There were no treatment-related macroscopic findings noted for F_1 pups.

Organ weights: (All F_1 parental animals (for females, only those that delivered: brain, epididymides, ovaries, prostate, seminal vesicles, testes, and uterus with cervix and oviducts)

HD F_1 females had decreased mean absolute ovary (↓12%) and uterus (↓20%) weights, although this effect was considered to be secondary to the 9% difference in body weight for this group compared with control at the time of euthanization. No meaningful differences in organ weights were noted for F_1 males.

Histopathology: (All F_1 parental animals from vehicle and HD groups and all F_1 males found dead or euthanized moribund: ovaries and oviducts, prostate, seminal vesicles, testes with epididymides and vas deferens, uterus with vagina and cervix)

Peer Review: Yes

No treatment-related changes were observed in histologic sections of reproductive organs from F_1 males and females.

F_2 Generation

Survival: (Daily)

The mean number of F_2 pups born, mean live litter size, sex ratio, and postnatal survival was unaffected by F_0 female exposure to the test article. For pups that were found dead, there were no external or internal findings suggestive of a test article effect.

General Physical Condition:

The general physical condition of all F_2 pups was unaffected by F_0 maternal exposure to the test article.

Body Weight: (PND 1)

Mean body weights for F_2 pups were unaffected by F_0 maternal exposure to the test article.

Reference ID: 3527260

10 Special Toxicology Studies

Study title: An investigational time course study of thyroid C-cell mass in rats treated with LY2189265 (compound 2189265) for up to 12 months

Study number:	8236888
Study report location:	Module 4.2.3.7.3
Conducting laboratory:	(b) (4)
Date of study initiation:	01 April 2011
GLP compliance:	Yes (except for thyroid morphometrics, and calcitonin and thyroid morphometric data interpretation)
QA statement:	Yes
Drug, lot #, and % purity:	LY2189265, Lot #17804-1, 40.2 mg/mL

Key Study Findings

- No treatment-related mortalities or adverse clinical signs occurred.
- Treated animals gained less weight than controls throughout the study, with final mean body weights being 20% less than controls. The effect on body weight generally correlated with decreased food consumption.
- An age-related increase in plasma calcitonin was observed in both control and treated animals. No difference in CaCl-stimulated calcitonin was observed between control and treated groups.
- There were no noteworthy differences in the incidence or severity of C-cell hyperplasia after treatment for up to 9 months. After 1 year of treatment, an increase in incidence and severity (slight) of focal/multifocal C-cell hypertrophy/hyperplasia was noted for the treated group. No difference in incidence or severity of diffuse C-cell hyperplasia was observed between the two groups. One C-cell adenoma was observed in each of the control and treated groups. One animal from the LY2189265-treated group had a C-cell carcinoma.
- A higher severity (slight) for Ki67/calcitonin double staining was observed for treated animals after 1 year of treatment. However, at earlier time points, more control animals had severity scores of slight or moderate for double staining.
- Tissue morphometery was conducted on thyroids to determine if C-cell volume or number increased over time in treated animals and if that correlated with increased plasma calcitonin levels. No statistically significant difference was noted between the two groups for percent calcitonin positive cells, volume of calcitonin positive tissue, thyroid tissue volume, or thyroid organ volume. Although there was some correlation between the number/size of calcitonin positive cells and plasma calcitonin values, this was not always the case. Histopathology findings of focal hyperplasia or carcinoma also did not correlate well with plasma calcitonin values.
- Plasma LY2189265 levels initially increased from Day 94 to Day 185, but a large decrease in plasma values were noted by Day 276. It is uncertain whether the decreases occurred due to the presence of anti-drug antibodies because the samples were not analyzed. Based on the body weight effects, it does appear that pharmacological activity was maintained throughout the treatment period.

Reference ID: 3527260

Methods

Species/Strain: Rat/Sprague-Dawley (males only)

Study design: (sponsor-generated table)

Group	No. of Males	Dose Level (mg LY2189265/kg)[a]	Dose Concentration (mg LY2189265/mL)[a]
1 (Control)[b]	80	0	0
2 (Treated)[c]	80	5	10
3 (Untreated)[d]	25	NA	NA

NA = Not applicable.

a The dose volume was 0.5 mL/kg.

b Group 1 (control) animals received the control article only.

c Group 2 (treated) animals received the test article prepared in Diluent A and Diluent B.

d Five of the 25 animals were used as replacements, if necessary, and dosed with calcium chloride according to the Dosing and Sacrifice Schedule.

Dose justification: 5 mg/kg induced an increased incidence of C-cell adenomas and carcinomas in a 2-year bioassay

Frequency of dosing: Twice weekly

Dosing schedule: (sponsor-generated table)

Animals[a]	Day of Last Test/Control Article Dose	Day of $CaCl_2$ Formulation Dose[b]	Day of Calcitonin Sample Collection[c]	Day of Scheduled Sacrifice	Sacrifice Number
Group 3	NA	1	1 (predose and 4 minutes postdose)	3	1
First 20/group from Groups 1 and 2	88	92	92 (predose and 4 minutes postdose)	94	2
Second 20/group from Groups 1 and 2	179	183	183 (predose and 4 minutes postdose)	185	3
Third 20/groupfrom Groups 1 and 2	270	274	274 (predose and 4 minutes postdose)	276	4
Last 20/group from Groups 1 and 2	361	365/372[d]	365/372[d] (predose and 4 minutes postdose)	374	Final

Note: All days were in the dosing phase.

a The first, second, third, and last 20 males/group, survival permitting, were dosed and sacrificed according to the schedule.

b Animals received an intravenous dose of 45 mg calcium chloride/kg (5 mL/kg of a 9-mg/mL solution of calcium chloride).

c Sample collection times were approximate.

d Due to deaths following $CaCl_2$ dosing on Day 365, dosing with $CaCl_2$ was discontinued. A limited number of animals received $CaCl_2$ and had samples collected approximately 4 minutes postdose on Day 365. Dosing with $CaCl_2$ was attempted on Day 372, but a death occurred and dosing was discontinued. A limited number of animals received $CaCl_2$ and had samples collected approximately 4 minutes postdose on Day 372.

Route of administration: Subcutaneous

Formulation/Vehicle: (b) (4) citrate (b) (4) (pH (b) (4) (w/v) mannitol, and (b) (4) (w/v) polysorbate 80 prepared in sterile water for injection, USP

Age: 6 to 7 weeks

Weight: 138 to 273 g

Unique study design: $CaCl_2$ injections to induce calcitonin secretion
Thyroid cell morphometric analysis

Protocol deviations: There were no GLP or protocol deviations that impacted the integrity of the study or interpretation of the study results.

Observations and Results

Mortality: (assessed twice daily)

No drug-related mortalities occurred. Six control and two LY2189265-treated animals were found dead or sacrificed in moribund condition.

Clinical Signs: (assessed twice daily with detailed exams occurring once weekly)

There were no treatment-related adverse clinical signs.

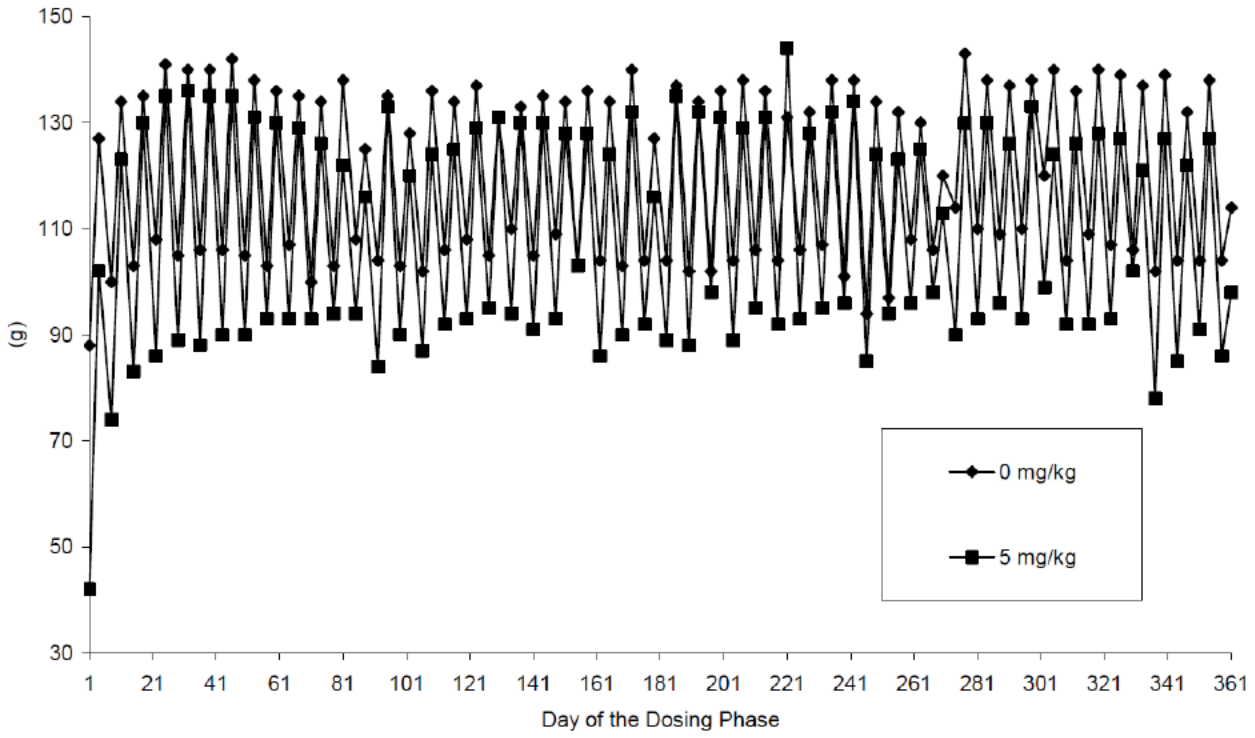
Body Weights: (assessed twice weekly)

Dose Level (mg/kg)	Day of Dosing Phase			
	92	183	274	365
	Mean Body Weight (Percent Difference from Control)			
0	571	650	696	722
5	490*(-14.2)	549*(-15.5)	587*(15.7)	579*(-19.8)

* = Statistically significant at $p \leq 0.05$.

Feed Consumption: (assessed twice weekly)

Figure 2
Mean Food Consumption



Note: food consumption was measured twice weekly, with the first measurement including 4 days' worth of food and the second weekly measurement including 3 days' worth of food consumption, which is why the values for each group are not consistent from time point to time point in the figure.

Clinical Chemistry: (assessed on the day of schedule sacrifice; standard endpoints)
There were no treatment-related adverse effects on clinical chemistry parameters when compared with mean control values. Trends noted for treated animals included:

-Slightly lower mean glucose values at all time points (up to 10%).
-Lower mean triglyceride values starting at Day 185 (up to 41%).
-Slightly lower mean cholesterol values at all time points (up to 13%)
-Higher mean aspartate dehydrogenase values at Day 185 and 276 (up to 53%).
-Higher mean alanine dehydrogenase values starting at Day 285 (up to 200%).

Calcitonin Analysis/$CaCl_2$ Injection: (a single bolus IV injection of 45 mg/kg $CaCl_2$ was administered on the days stated in the dosing schedule table above; animals were fasted overnight. Samples for calcitonin measurements were collected before and ~4 minutes after $CaCl_2$ injection)
As shown in the sponsor-generated figure below, mean plasma calcitonin levels were similar between control and treated groups, either prior to or after $CaCl_2$ injection. On Day 365 and 374, several deaths occurred in the control group after $CaCl_2$ injection. Because of this, the LY2189265-treated animals did not undergo the calcitonin induction treatment with $CaCl_2$, and therefore, data are not available for these animals.

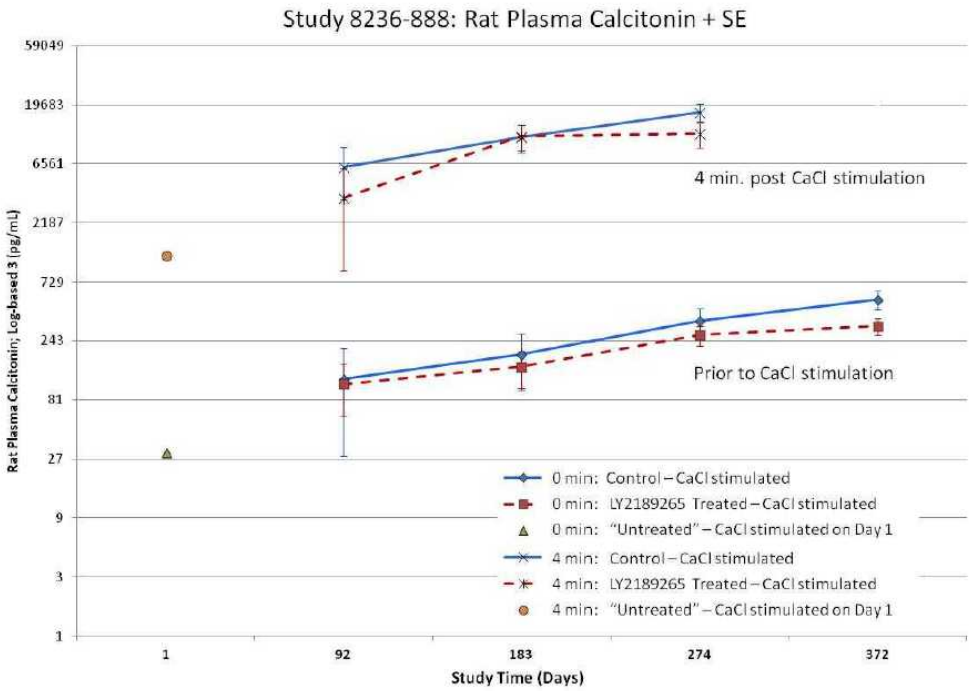


Figure 1: Basal and calcium chloride (CaCl) stimulated plasma calcitonin concentration in rats administered vehicle or LY2189265.

The Day 1 values represent the values in a group of untreated rats (Group 3) used to provide baseline values at study initiation. At the 12-month interval, Day 365 or 372, adequate post-stimulated calcitonin data were not available due to complications arising from the calcium chloride dosing procedure. The pre-stimulated calcitonin levels were similar for Days 365 and 372; therefore, Day 365 data are not included in the graph.

Gross Pathology: (conducted on all animals that died or were sacrificed, either at the scheduled time point or at an unscheduled interval)

There were no treatment-related macroscopic findings observed.

Organ Weights: (conducted for all animals at scheduled sacrifice and included: brain, kidneys, and thyroid (two lobes) with parathyroid; paired organs were weight together)

There were no biologically relevant differences in organ weights between the treated and untreated groups at any of the sacrifice time points.

Histopathology:

Adequate Battery: Yes, a limited number of tissues was collected because of the specific investigative nature of this study. Tissues included: kidneys and thyroid with parathyroid. L4 vertebra and tibia with bone marrow were collected and fixed but not analyzed.

Thyroid glands were exhaustively sectioned through at 5 µm. A series of 6 serial sections were collected at 100 µm intervals through the entire pair of thyroid glands. Three slides with the 6 thyroid sections were stained with H&E; and an additional 3 slides with 6 thyroid sections were co-labeled with calcitonin and Ki67. Quantitative image analysis included determinations of group mean total C-cell mass, and individual C-cell mass. Image analysis software was used for thyroid morphometric analysis to determine the thyroid calcitonin-stained area.

Peer Review: Yes, by the sponsor at (b) (4)

Reference ID: 3527260

Histological Findings:

Incidence and Severity of Selected Microscopic Findings in the Thyroid

	LY2189265	
Sex	Males	
Dose Level (mg/kg)	0	5
Scheduled Sacrifice 3 (6 months) - Thyroid		
Number Examined	18	19
Hypertrophy/Hyperplasia, C-cell, Focal/Multifocal		
Not Present	18	18
Minimal	0	1
Scheduled Sacrifice 4 (9 months) - Thyroid		
Number Examined	20	20
Hypertrophy/Hyperplasia, C-cell, Focal/Multifocal		
Not Present	19	18
Minimal	1	2
Hyperplasia, C-cell, Diffuse		
Not Present	16	18
Minimal	4	2
Terminal Sacrifice (12 months) - Thyroid		
Number Examined	16	19
Hypertrophy/Hyperplasia, C-cell, Focal/Multifocal		
Not Present	14	8
Minimal	2	6
Slight	0	5
Hyperplasia, C-cell, Diffuse		
Not Present	8	8
Minimal	8	11
Adenoma, C-cell		
Not Present	15	18
Present	1	1
Carcinoma, C-cell		
Not Present	16	18
Present	0	1

Reference ID: 3527260

Relative Frequency of Ki67-Calcitonin Co-labeled Cells - Thyroid

	LY2189265	
Sex	Males	
Dose Level (mg/kg)	0	5
Scheduled Sacrifice 1 (3 days) Thyroid Ki67 Calcitonin		
Number Examined	20[a]	0
Ki-67-Calcitonin Co-labeled Cells		
Not Present	0	NA
Minimal	14	NA
Slight	5	NA
Moderate	1	NA
Scheduled Sacrifice 2 (3 months) - Thyroid Ki67-Calcitonin		
Number Examined	20	20
Ki-67-Calcitonin Co-labeled Cells		
Not Present	0	0
Minimal	13	19
Slight	5	1
Moderate	2	0
Scheduled Sacrifice 3 (6 months) - Thyroid Ki67-Calcitonin		
Number Examined	18	19
Ki-67-Calcitonin Co-labeled Cells		
Not Present	0	0
Minimal	9	19
Slight	9	0
Scheduled Sacrifice 4 (9 months) - Thyroid Ki67-Calcitonin		
Number Examined	20	19
Ki-67-Calcitonin Co-labeled Cells		
Not Present	0	0
Minimal	10	18
Slight	8	1
Moderate	2	0
Terminal Sacrifice (12 months) - Thyroid Ki67-Calcitonin		
Number Examined	16	19
Ki-67-Calcitonin Co-labeled Cells		
Not Present	0	0
Minimal	15	9
Slight	1	10

NA = Not applicable.
Severity scores: minimal = 1 to 8 co-labeled cells/10 C-cell-rich 400x fields, slight = 9 to 16 co-labeled cells/10 C-cell-rich 400x fields, and moderate = 17 or more co-labeled cells/10 C-cell-rich 400Xxfields.
a Animals in this group did not receive control or test article.

Morphometry

A summary of the morphometery, histopathology, and plasma calcitonin data for individual animals after 1 year is presented in the table below. Highlighted data cells represent the 4 or 5 highest values or most severe histopathology findings for each parameter. A clear correlation between volume of calcitonin positive cells, histopathology findings, and plasma calcitonin values was not observed. Although calcitonin values increased with age (sponsor-generated Figure 1), there was no meaningful difference between the percentage/volume of calcitonin positive cells or plasma calcitonin values between control and treated groups after 1 year of treatment.

Summary of Thyroid C-cell and Calcitonin Data for Individual Animals at Day 365

Animal Number	% Tissue Calcitonin Positive	Volume of Calcitonin Positive Tissue (mm^3)	Thyroid Tissue Volume (mm^3)	Thyroid Organ Volume (mm^3)	Diffuse Hyperplasia Severity	Focal Hyperplasia Severity	Tumor	Plasma Calcitonin on Day 365 (before $CaCl_2$)
Group 1 (Vehicle Control)								
B03747	16.27	1.29	7.65	14.43	-	-	-	190
B03748	24.21	3.08	12.71	19.98	Minimal	-	-	248
B03749	18.46	1.53	8.27	12.36	-	Minimal	-	234
B03750	24.50	2.63	10.73	21.37	-	-	-	1200
B03751[a]	12.93	0.87	6.69	10.42	-	-	-	NE
B03752	17.38	1.39	8.00	13.71	Minimal	-	-	170
B03753	21.16	1.57	7.41	13.00	-	-	-	606
B03754	22.13	1.78	8.03	12.38	Minimal	-	-	442
B03755[a]	26.78	2.99	11.18	15.51	-	-	-	280
B03756	27.01	2.99	11.07	19.39	Minimal	-	-	714
B03757	15.41	1.81	11.78	17.90	Minimal	-	-	185
B03758	14.82	0.63	4.28	7.34	-	-	-	57.8
B03759[a]	18.56	1.81	9.76	14.39	-	-	-	140
B03760[a]	38.34	3.76	9.81	14.69	Minimal	-	-	559
B03761	27.31	3.79	13.86	20.91	Minimal	Minimal	-	1920
B03762	31.91	4.68	14.66	27.46	-	-	-	673
B03763	15.79	1.11	7.05	11.54	-	-	-	217
B03764	39.43	6.84	17.34	28.09	Minimal	-	Adenoma	1160
B03765	19.05	1.69	8.85	15.18	-	-	-	154
B03766	28.60	3.47	12.15	18.26	Minimal	-	-	551
Mean	23.00	2.49	10.06	16.42				511

Animal Number	% Tissue Calcitonin Positive	Volume of Calcitonin Positive Tissue (mm^3)	Thyroid Tissue Volume (mm^3)	Thyroid Organ Volume (mm^3)	Diffuse Hyperplasia Severity	Focal Hyperplasia Severity	Tumor	Plasma Calcitonin on Day 365 (before $CaCl_2$)
Group 2 (5 mg/kg LY2189265)								
B03827	17.32	1.57	9.09	18.22	-	Minimal	-	358
B03828	23.77	1.82	7.64	11.64	Minimal	-	-	467
B03829	14.76	1.05	7.12	11.56	-	-	-	220
B03830	21.14	2.08	9.85	18.19		Slight	Adenoma	746
B03831	24.26	2.75	11.34	17.50	Minimal	Minimal	-	602
B03832[b]	NE	NE	NE	NE	-	-	Carcinoma	624
B03833	15.38	1.15	7.47	13.09		Minimal	-	684
B03834	21.81	1.65	7.56	11.51	Minimal	-	-	388
B03836	15.86	1.05	6.62	10.62	-	Slight	-	256
B03837	28.81	2.15	7.45	13.68	Minimal	-	-	689
B03838	37.37	4.75	12.72	22.33	Minimal	-	-	592
B03839	19.78	1.37	6.94	13.05	Minimal	Slight	-	336
B03840	26.98	3.37	12.49	19.36	Minimal	-	-	749
B03841	30.66	2.42	7.91	12.41	Minimal	-	-	482
B03842	21.66	1.65	7.61	12.48	-	-	-	485
B03843[c]	NE	NE	NE	NE	Minimal	Minimal	-	499
B03844	27.90	3.37	12.08	17.78	Minimal	Minimal	-	1430
B03845	34.19	3.70	10.84	15.97	Minimal	Minimal	-	374
B03846	13.04	0.87	6.64	12.01	-	Slight	-	216
Mean	23.22	2.16	8.90	14.79				537

NE = not evaluated; "-" = finding not observed

[a] Animals were sacrificed or found dead between Days 358 and 372.

[b] Animal B03832 contained a calcitonin mass not recognized by the algorithm as thyroid. The algorithm thus gave an incorrect estimate of the parameters.

[c] Animal B03843 contained much calcitonin positive colloid leading to large false positive areas. The algorithm thus gave an incorrect estimate of the parameters.

Note: The highlighted cells represent the 4 or 5 data points with the highest value or most severe histopathology finding for each parameter. This is highlighted to determine whether a correlation could be observed regarding % or volume of calcitonin-positive tissue with histopathology findings and/or plasma calcitonin values.

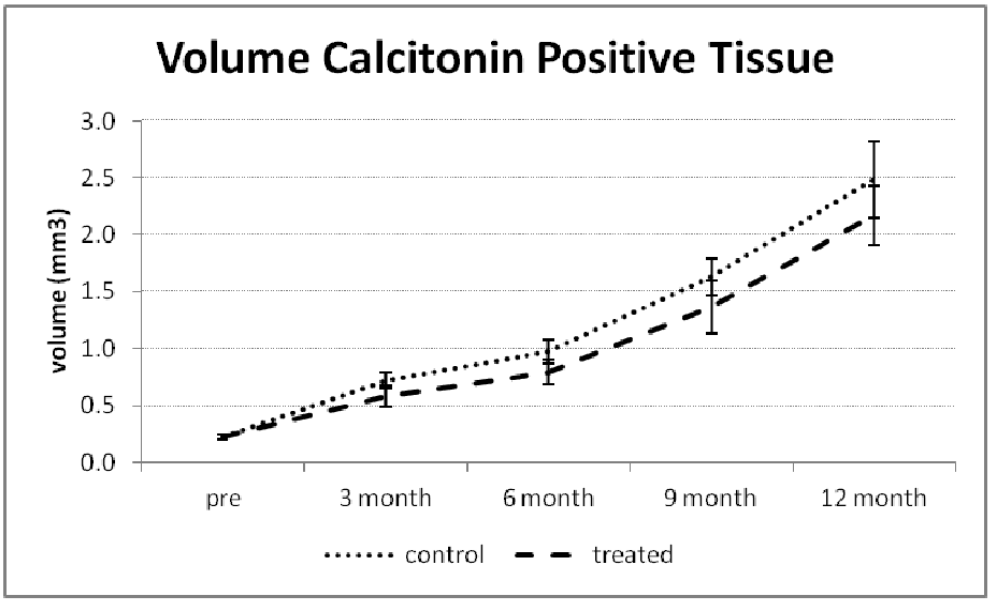


Figure 1: Volume of calcitonin positive tissue in male rats given vehicle or 5 mg/kg of LY2189265 for up to 12 months.

Toxicokinetics: (a single sample was collected from each animal on the scheduled sacrifice day)

Plasma LY2189265 levels measured at interim sacrifices (6 days after the last dose) generally decreased as the study progressed, however there was considerable variation (sponsor-generated Table 2). Plasma levels measured 13 days after the final dose on Day 374 were below the LLOQ of 0.8 ng/mL for all animals except one that have a level of 0.91 ng/mL.

Table 2. Mean LY2189265 Plasma Level for Animals Given 5.0 mg/kg

	Day of Scheduled Sacrifice		
	94	185	276
Mean Plasma Level (ng/mL)	16.8	51.0	0.99
Standard Deviation	15.6	196.5	0.559
No. of Animals with Plasma LY2189265 Level Below LLOQ of 0.8 ng/mL	3	11	15
No. of Animals with Sample Collected	20	19	20

Anti-Drug Antibody Analysis: (a single sample was collected from each animal on the scheduled sacrifice day)
Samples were discarded without analysis.

Dosing Solution Analysis:
Mean assayed concentrations of formulated LY2189265 dosing solutions ranged from 99% to 101% of theoretical values.

Study title: An investigational time-course study of calcitonin responses and thyroid C-cell mass and terminal evaluation of pancreatic structure in male Cynomolgus monkeys treated with LY2189265 twice weekly by subcutaneous injection for 12 months

Study number:	8252199
Study report location:	Module 4.2.3.7.3
Conducting laboratory:	(b) (4)
Date of study initiation:	18 August 2011
GLP compliance:	Yes, (except for thyroid morphometrics, and calcitonin and thyroid morphometric data interpretation)
QA statement:	Yes
Drug, lot #, and conc.:	LY2189265, Lot #17804-1, 40.2 mg/mL

Key Study Findings

- One drug-treated monkey lost approximately 18% of its weight after the first two doses, resulting in a dosing holiday for the next 4 dose administrations. Once the animal's condition improved sufficiently to resume dosing on Days 26 and 29, body weight declined again and this animal was removed from the study.
- The treated group had an increased incidence of few or no feces, low food consumption, and thin appearance compared with the control group.
- Mean body weights for the LY2189265-treated group decreased from baseline by 0.3 kg between Days 1 and 29. By the end of the study, the final mean body weight for the treated group was 20% lower than the control group. Effects on body weight correlated with decreased food consumption.
- Mean serum calcitonin values remained below the level of quantitation for both groups throughout the study. A slight trend for higher calcitonin secretion was noted for the LY2189265-treated group compared with control at 2 and 10 minutes after calcium gluconate treatment. However, these differences were not statistically significant and did not correlate with C-cell morphometry data.
- No treatment-related adverse microscopic findings were noted for the thyroid, parathyroid, pancreas, or kidney (the only organs evaluated). A slight increase in the severity of increased pancreatic goblet cells in interlobular ducts was noted for the treated group. This finding did not correlate with serum chemistry (amylase and lipase) or clinical pathology endpoints.
- There was no increase in the percentage of Ki67-labeled thyroid C-cells or pancreatic ductal cells. Morphometry analysis also did not reveal any treatment-related cell proliferative effects in the thyroid.
- A pathologist working group was assembled to assess for possible treatment-related adverse effects on the pancreas. **The Pancreas Working Group consensus statement was**: "Of the findings recorded for the pancreas in the anatomic pathology report, the consensus of the PWG was that only the increased severity of "goblet cells, increased, interlobular duct" in four treated animals was considered to be treatment related. This effect was considered non-adverse because there was no evidence of an associated inflammatory or proliferative change including pancreatitis, ductal dilatation, ductal blockage, or PanIN."

Reference ID: 3527260

Methods

Species/strain: Monkey/Cynomolgus

Study design: (sponsor-generated table)

Group	No. of Males	Dose Level (mg LY2189265/kg)[a]	Dose Concentration (mg LY2189265/mL)[a]
1 (Control)[b]	20	0	0
2 (Treated)[c]	20	8.15	8.15

a The dose volume was 1 mL/kg.
b Group 1 (control) animals received the control article only.
c Group 2 (treated) animals received the test article prepared in (b) (4) citrate (b) (4) pH (b) (4) (b) (4) mannitol (w/v), and (b) (4) (w/v) polysorbate 80.

Frequency of dosing: Twice weekly

Route of administration: Subcutaneous

Formulation/Vehicle: (b) (4) sodium citrate (b) (4) (pH (b) (4) (b) (4) mannitol (w/v), and (b) (4) polysorbate 80 in sterile water

Age: 2 to 3 years old

Weight: 2.6 to 3.6 kg

Unique study design: Calcium gluconate injections to induce calcitonin secretion
Thyroid cell morphometric analysis

Protocol deviations: There were no GLP or protocol deviations that impacted the integrity of the study or interpretation of the study results.

Observations and Results

Mortality: (assessed twice daily)

One of the LY2189265-treated animals lost approximately 18% of its Day 1 body weight after the first 2 doses. Dosing was suspended for this animal for five of the next seven dose administration days (Days 12, 15, 19, 22, and 33). Body weight rebounded to the Day 1 value during the dosing holiday, so doses were administered on Days 26 and 29; however, the animal lost weight again once dosing resumed. Because of the profound effects on body weight for this animal, it was felt that dosing could not continue and the animal was removed from the study on Day 36.

Clinical Signs: (assessed once daily; detailed observations conducted once weekly)

Summary of Clinical Observations
Dosing Phase

Test Article	(dosage)	1M	2M
LY2189265	mg/kg	0	8.15

Phase: Dosing

Category / Observation	Group/Sex: 1/M	2/M
	Number in Group: 20	20
Excretion		
feces, few	2	20
feces, liquid	6	2
feces, none	1	15
feces, nonformed	19	13
Qualitative food consumption		
low	7	20
none	2	15
Appearance		
thin	0	2

Body Weights: (assessed pre-dose, Days 1, 3, and 5, and twice weekly thereafter)

Mean Body Weight and Body Weight Change Data (kg)

Dose (mg LY2189265/kg)	0	8.15
Mean Body Weight (kg)		
Day 29	3.1	2.6*
Day 365	4.5	3.6*
Mean Body Weight Change (kg)		
Day 1 to Day 29	0.1	-0.3*
Day 1 to Day 365	1.5	0.7*

* = Statistically significant from controls at $p \leq 0.05$.

Feed Consumption: (assessed qualitatively once daily through Day 130 (Set 2 animals) or Day 137 (Set 1 animals)

Incidence of Abnormal Food and Supplement Consumption - Dosing Phase

Total Number of Incidences (Number of Animals with Observation)		
Dose (mg LY2189265/kg)	0	8.15
	Days 1 - 28	
Qualitative Food Consumption		
Low	20 (5)	243 (20)
None	1 (1)	10 (8)
Supplement Consumption		
Some/None	8 (5)	130 (19)
	Days 29 - 137	
Qualitative Food Consumption		
Low		
None	44 (5)	611 (19)
Supplement Consumption	2 (1)	50 (14)
Some/None	19 (11)	198 (20)

Clinical Chemistry: (samples collected at scheduled sacrifice)
There were no apparent treatment-related effects on clinical chemistry parameters. Compared with controls, the LY2189265-treated group had slightly elevated values for mean ALT (70 vs 101 U/L) and AST (64 vs. 115.5 U/L) on Day 367, which was primarily the result of a single monkey with very high values (590 and 507 U/L, respectively). It is uncertain whether the increased liver enzymes correlated with microscopic lesions because the liver was not included in the histopathology evaluation. No effects on serum lipase or amylase were noted.

Calcitonin Analysis/Calcium Gluconate Injection:
Blood samples were collected from fasted animals on Days 35 and 42 of the pre-dose phase (first and second 10 animals/group, respectively) and on Days 88, 179, 270, and 365 of the dosing phase prior to calcium gluconate (5 mg/kg calcium IV bolus over ~3 minutes), immediately after, 2 minutes after, and 10 minutes after sodium gluconate.

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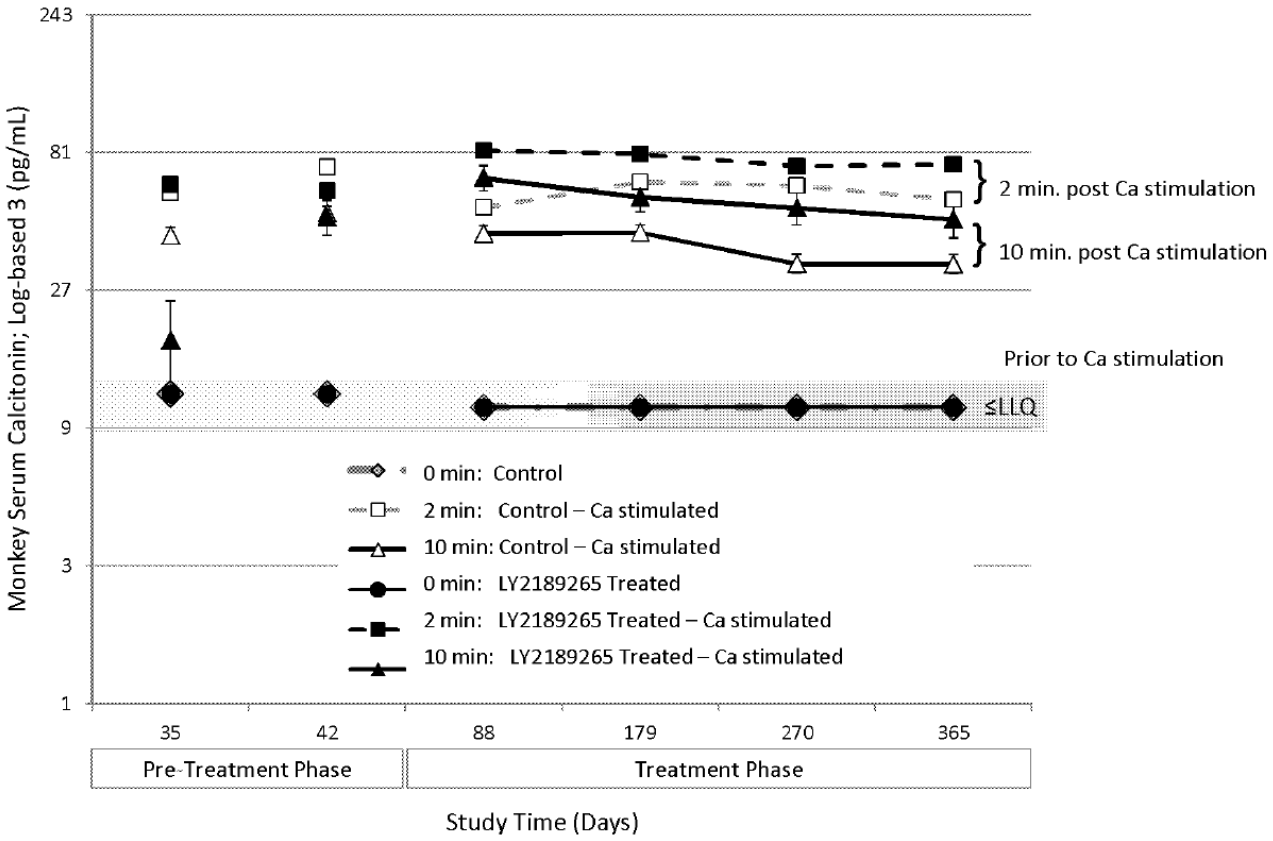
Group	Study Day	Concentration (pg/mL)			
		Pre-stimulated	Post-stimulated (end of infusion)	Post-stimulated (2 min postdose)	Post-stimulated (10 min postdose)
Control	-7	<11.8	38.7	58.8	41.8
LY2189265		<11.8	55.4	62.8	18.2
Control	0	<11.9	57.5	72.2	50.0
LY2189265		<11.9	50.4	59.6	48.4
Control	88	<10.7	52.2	42.5	41.6
LY2189265		<10.7	82.2	66.2	71.6
Control	179	<10.7	64.0	42.7	53.4
LY2189265		<10.7	80.1	56.8	69.5
Control	270	<10.7	62.3	33.4	51.7
LY2189265		<10.7	72.6	52.1	62.0
Control	365	<10.7	55.7	33.3	45.1
LY2189265		<10.7	73.7	47.6	63.1



Gross Pathology: (conducted for all animals on Day 367)
There were no treatment-related macroscopic findings.

Organ Weights: (conducted on brain, kidneys, pancreas, and thyroid with parathyroid for all animals).
There were no treatment-related effects on organ weights.

Histopathology: (a complete list of tissues was collected and archived)

Adequate Battery: Yes, because of the specific investigative nature of this study, only the thyroid (with parathyroid), pancreas, and kidney were evaluated microscopically. Tibia and L4 vertebra were collected and archived. A complete list of tissues was collected and archived for each animal that died or was euthanized at an unscheduled interval.

For the thyroid, three slides, each containing one section of each thyroid lobe from one of the spatial thirds of the thyroid, were stained with H&E; an additional three slides, each containing one section of each thyroid lobe from one of the spatial thirds of the thyroid, were co-labeled for calcitonin and Ki67. Positive and negative control co-labeled slides were included. Slides were evaluated at 400x magnification and up to 200 C-cells were counted in each section on the slide.

For the pancreas, six slides, each containing one section of pancreas (three sections from the head, two sections from the body, and one section from the tail of the pancreas) were stained with H&E; an additional three slides (containing sections from the head) were labeled for Ki67. Positive and negative control Ki67-labeled slides were included. The specifics of how many duct cells and Ki67 stained cells were counted per slide are discussed in the anatomic pathology report. The assessment focused on the pancreatic ducts and cell types other than duct luminal or glandular epithelium were not evaluated. It appears that duct cells were identified by morphology rather than a duct-specific stain.

Peer Review: Yes, by the sponsor

Expert Panel Review: After the primary microscopic evaluation and independent peer review by a (b) (4) contributing scientist and the sponsor's peer review pathologist, stained slides of the pancreas (H&E and Ki67 immunohistochemistry) from each animal were reviewed by an expert working group, consisting of three expert consultant pathologists, the study pathologist, and the sponsor's peer review pathologist.

Reference ID: 3527260

Histological Findings:
No treatment-related adverse effects were noted in the thyroid, parathyroid, kidney, or pancreas. A slight increase in severity of increased goblet cells in interlobular ducts was noted for 4 animals treated with LY2189265. This finding did not correlate with serum chemistry (amylase, lipase) or clinical pathology (inflammation, proliferation change, ductal dilatation, or ductal blockage) endpoints. There was no increase in the percentage of Ki67-labeled thyroid C-cells or pancreatic ductal cells (sponsor-generated tables). Morphometry analysis of the thyroid did not show any treatment-related effects on the percentage or volume of calcitonin-positive cells (sponsor-generated table).

Incidence and Severity of Compound-Related Microscopic Finding in Pancreas

	LY2189265	
Sex	Males	
Dose Level (mg/kg)	0	8.15
Pancreas		
Number Examined	20	19
Goblet cells, increased, interlobular duct		
Not Present	14	12
Minimal	6	3
Slight	0	2
Moderate	0	2

Ki67 Labeling in Pancreatic Duct Luminal and Glandular Epithelium

	LY2189265	
Sex	Males	
Dose Level (mg/kg)	0	8.15
Number Examined	20	19
Mean % Ki67 Labeled Cells - Duct Luminal Epithelium	1.8%	1.5%
Mean % Ki67 Labeled Cells - Duct Glandular Epithelium	1.5%	1.9%

Ki67-Calcitonin Colabeling in Thyroid

	LY2189265	
Sex	Males	
Dose Level (mg/kg)	0	8.15
Number Examined	20	19
Mean % Ki67-Calcitonin Colabeled Cells	0.13%	0.03%

Reference ID: 3527260

Summary of Thyroid Morphometry Data

Animal Number	Group	% of Tissue Calcitonin Positive	Volume of Calcitonin Positive Tissue (mm3)	Thyroid Tissue Volume (mm3)	Thyroid Organ Volume (mm3)
I09467	1	0.128	0.014	10.8	55.0
I09468	1	0.233	0.024	10.3	22.1
I09469	1	0.135	0.013	9.4	35.0
I09470	1	0.337	0.023	6.9	27.6
I09471	1	0.522	0.030	5.8	15.9
I09472	1	0.391	0.036	9.3	49.1
I09473	1	0.154	0.012	8.1	31.2
I09474	1	0.301	0.039	12.9	44.8
I09475	1	0.225	0.026	11.8	31.8
I09476	1	0.204	0.020	10.0	37.1
I09477	1	0.439	0.029	6.7	20.3
I09478	1	0.219	0.019	8.8	35.2
I09479	1	0.081	0.012	14.5	60.7
I09480	1	0.246	0.029	11.6	38.3
I09481	1	0.351	0.015	4.4	11.0
I09482	1	0.600	0.046	7.7	30.9
I09483	1	0.366	0.032	8.8	31.9
I09484	1	0.422	0.039	9.3	35.8
I09485	1	0.549	0.042	7.6	24.1
I09486	1	0.352	0.027	7.6	35.1
	Mean	0.313	0.026	9.1	33.6
	Std	0.1463	0.0104	2.45	12.31
	N	20	20	20	20
I09487	2	0.256	0.021	8.1	38.5
I09489	2	0.170	0.012	7.3	20.3
I09490	2	0.131	0.009	7.1	18.0
I09491	2	0.528	0.034	6.5	25.0
I09492	2	0.264	0.023	8.6	46.9
I09493	2	0.292	0.017	5.8	21.4
I09494	2	0.154	0.010	6.6	33.1
I09495	2	0.370	0.024	6.5	22.3
I09496	2	0.597	0.032	5.4	24.1
I09497	2	0.351	0.025	7.2	20.5
I09498	2	0.233	0.013	5.4	24.5
I09499	2	0.239	0.024	10.2	38.1
I09500	2	0.430	0.025	5.8	19.3
I09501	2	0.166	0.016	9.3	43.2
I09502	2	0.584	0.040	6.9	24.4
I09503	2	0.315	0.021	6.6	18.6
I09504	2	0.158	0.009	5.4	17.1
I09505	2	0.326	0.026	8.0	32.0
I09506	2	0.500	0.047	9.4	36.5
	Mean	0.319	0.023	7.2	27.6
	Std	0.1483	0.0105	1.43	9.24
	N	19	19	19	19

PWG Conclusions:

- The PWG reached consensus on the normal histologic anatomy of the interlobular pancreatic ducts in the monkeys, all findings identified by the study pathologist, grading and interpretation of the finding "goblet cells, increased, interlobular duct" in the pancreas, and the Ki-67 staining frequency in the pancreatic ducts.
- The PWG found no morphologic evidence for an effect of LY2189265 on the acinar or endocrine portions of the pancreas. There was no evidence of pancreatitis or necrosis in the control or treated animals.
- Other than "goblet cells, increased, interlobular duct", the PWG found no morphologic or immunohistochemical evidence for a proliferative effect of LY2189265 on the pancreas. This includes no evidence of morphologic changes consistent with human PanIN lesions grade 1, 2 or 3 (Hruban 2004).
- The PWG agreed that small numbers of goblet cells are an anatomic feature in some interlobular pancreatic ducts of untreated monkeys and utilized the diagnostic criteria below when evaluating the slides. The increase in severity of "goblet cells, increased, interlobular duct" recorded in four treated monkeys was interpreted by the PWG pathologists to be a treatment effect, but not consistent with human PanIN lesions. The increased goblet cell number was not associated with duct dilation, blockage or accumulation of mucin.

The panel established the following diagnostic criteria for the grading of the finding "goblet cell, increased, interlobular duct"

Severity Grade	Diagnostic features
0	No goblet cells were present in the major interlobular pancreatic ducts, or rare single goblet cells were randomly present within the epithelial lining of the ducts.
1	Focal, small (generally 3-5 cells) clusters of goblet cells were randomly present within the epithelial lining of major interlobular pancreatic ducts.
2	Focal, large (generally 6-20 cells) clusters of goblet cells were randomly present within the epithelial lining of major interlobular pancreatic ducts.
3	Goblet cells were prominent in most or all major interlobular ducts in all regions of the pancreas and comprised approximately 30-50% of the epithelial lining of the ducts.

- The PWG identified low numbers of peri-ductal glands in the pancreas of all animals and no treatment effect on these glands was detected.
- The PWG agreed with the interpretation for interlobular pancreatic duct immunohistochemically labeled for Ki-67 in the "primary section" as recorded in the preliminary pathology report provided to the PWG. The PWG concluded that there was inter-animal variability in the staining for Ki-67 and there was no compound-related effect, which agreed with the conclusion of the study pathologist.

- All other findings for the pancreas were not considered treatment related and were consistent with spontaneous or incidental background lesions seen in non-human primates of this age.

Toxicokinetics: (a single sample/animal was collected on Days 88, 179, 270, and 365)

Mean Plasma LY2189265 Level for Male Cynomolgus Monkeys Given 8.15 mg/kg

	Day of Dosing Phase			
	88	179	270	365
Mean Plasma Level (ng/mL)	22833	17416	19436	15150
Standard Deviation	14225	6532	3398	3075
Number of Animals	19	19	19	19

Reference ID: 3527260

Anti-LY2189265 Antibody Analysis:
A single sample/animal was collected on Days 88, 179, 270, and 365; however, analysis was deemed unwarranted based on the exposure data and sustained pharmacodynamic effects.

Dosing Solution Analysis:
Mean assayed concentrations of LY2189265 for the formulations ranged from 99% to 101% of theoretical values.

Study title: An investigative study of pancreatic exocrine biomarkers, histology, and histomorphometry in male Zucker Diabetic Fatty (ZDF) rats given LY2189265 by subcutaneous injection twice weekly for 13 weeks

Study number:	504758
Study report location:	Module 4.2.3.7.3
Conducting laboratory:	(b) (4)
Date of study initiation:	05 June 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and conc.:	LY2189265, Lot #17804-1, 40.2 mg/mL

Key Study Findings

- There were no treatment-related mortalities or adverse clinical signs.
- LY2189265-treated animals lost weight during the first 2 weeks of the study. For the next 4 weeks, body weight gain was similar for all groups. However, after approximately Day 42, the control group gained significantly less weight in spite of eating significantly more food as a result of their worsening hyperglycemia. Toward the end of the study, the low dose group also began eating more food, likely as a result of diabetes progression.
- In addition to the body weight and food consumption data, mean glucose, insulin, and HbA1c values also indicated drug-related efficacy for the mid- and high-dose groups.
- A dose-related increase in mean serum pancreatic-specific amylase was observed for all dose groups, with the increase being statistically significant for the mid- and high-dose groups (↑22% and 33% from controls, respectively). No increases in serum lipase were observed.
- Increased incidence and severity (minimal to moderate) of **increased ductal epithelium** was observed at all dose levels. Only one control animal was noted with this finding, which was of minimal severity. Treated animals also had a dose-related increased incidence of minimal to slight **acinar atrophy**, **with or without mixed cell inflammation**. An increase in the incidence and severity of **neutrophilic acinar inflammation** was observed at 5 mg/kg. An increase in the

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severity, but not incidence, of **neutrophilic periductal inflammation** (minimal to moderate) and islet cell hyperplasia was observed for the treated groups.

- A dose-related trend for increased mean Ki67 staining and decreased mean TUNEL staining in pancreatic duct cells was observed at ≥1.5 mg/kg, indicating an increase in ductal cell proliferation and/or a decrease in ductal cell apoptosis. However, the observed changes in staining were not statistically significant. Additionally, with the exception of a few animals, there generally was not a good correlation for individual animals having both a high percentage of Ki67 positive duct cells and an increased incidence/severity of increased ductal epithelium.
- The microscopic changes observed in this study might suggest that some diabetic patients taking LY2189265 could be more susceptible to morphological changes in the exocrine pancreas. However, the observed changes are not sufficient or severe enough to classify the effects as drug-induced pancreatitis or premalignant lesions.

Methods

Species/Strain: Rat/Zucker Fatty Diabetic (ZDF/Crl-Lep[fa]), males

Study design: (sponsor-generated table)

Group No.	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Dose Solution Concentration (mg/mL)	No. of Animals	
				Main Study	Toxicokinetic Study[a]
				Males	Males
1/ Reference Item	0	0.5	0	27	16
2/ LY2189265	0.5	0.5	1	20	12
3/ LY2189265	1.5	0.5	3	20	12
4/ LY2189265	5.0	0.5	10	20	12

a Toxicokinetic animals were used for toxicokinetic and laboratory investigations only.

Frequency of dosing: Twice weekly

Route of administration: Subcutaneous

Formulation/Vehicle: (b) (4) citrate (b) (4) (pH (b) (4), (b) (4) mannitol (w/v), and (b) (4) polysorbate 80 in sterile water for injection USP

Age: 8 weeks at start of treatment

Weight: 213 to 300 g

Unique study design: Extensive sectioning of the pancreas for microscopic examination. Pancreatic ductal cells stained for Ki67 and TUNEL.

Protocol deviations: There were no GLP or protocol deviations that impacted the validity or interpretation of the study results.

Observations and Results

Mortality: (assessed twice daily)

One animal each from the control and HD groups was found dead on Day 49 and Day 75, respectively. Both animals had gross findings of renal pelvis dilatation and the HD animal also had enlarged kidneys with pale foci and pale areas on the kidney cortex and discoloration with thickened urinary bladder wall. The kidney findings are commonly observed in the ZDF rat. The HD animal also had an area of minimal neutrophilic

inflammation in the acinar tissue of the pancreas. The pancreatic inflammation was considered to be related to treatment with LY2189265.

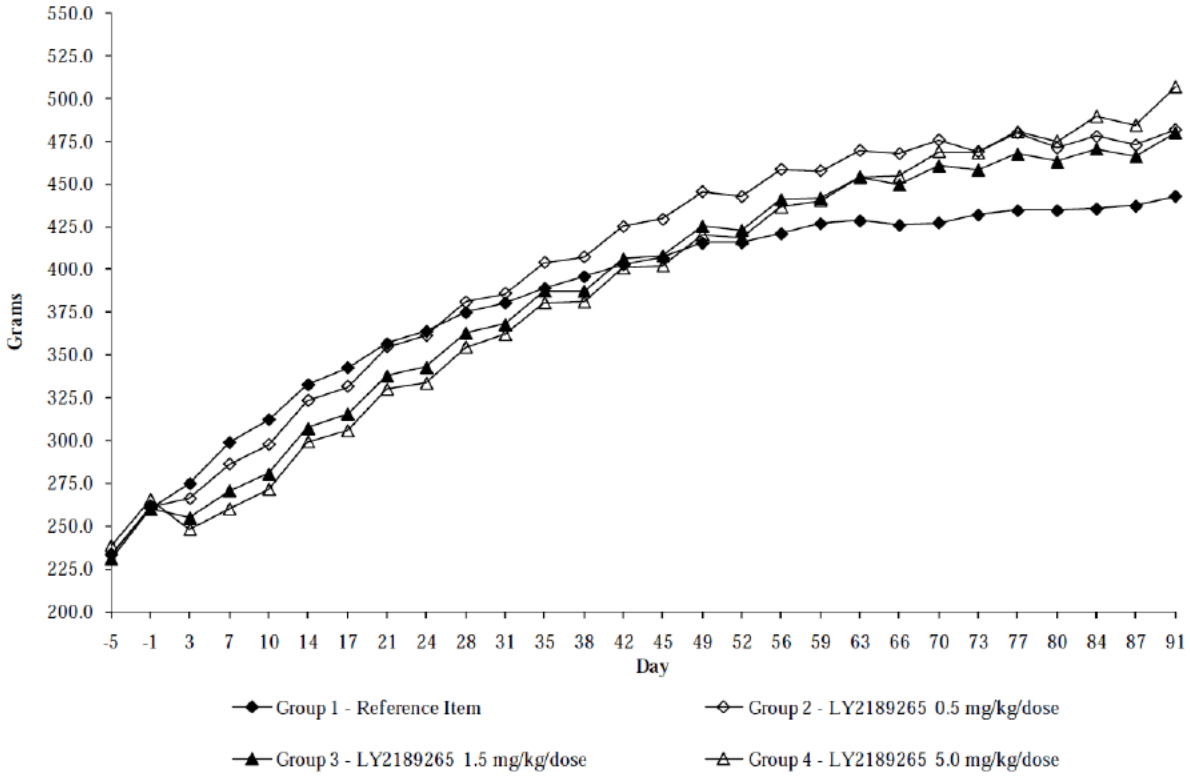
Clinical Signs: (assessed twice daily with detailed exams once weekly)
No treatment-related adverse clinical signs were noted.

Body Weights: (assessed twice weekly)

Dose (mg/kg/d)	0	0.5	1.5	5.0
Weight gain (g): Day -1 to 14	72.3	62.3*	47.4**	33.7**
Diff from control (g)		-10	-24.9	-38.6
% diff from control		↓14%	↓34%	↓53%
Weight gain (g): Day 14 to 91	110.4	158.4	172.9	207.5
Diff from control (g)		48	62.5	97.1
% diff from control		↑43%	↑57%	↑88%
Weight gain (g): Day -1 to 91	182.4	220.7	220.3	241.5††
Diff from control (g)		38.3	37.9	59.1
% diff from control		↑21%	↑21%	↑32%

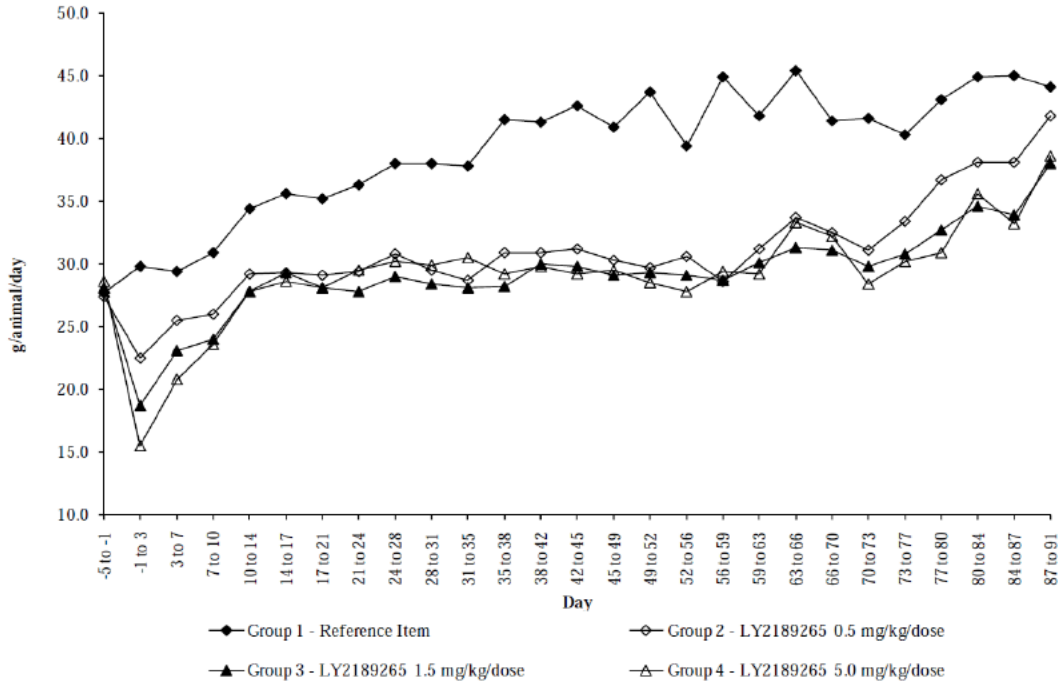
*p≤0.05; **p≤0.001 (Dunnett); ††p≤0.001 (Dunn).

Figure 1: **Body Weights - Summary of Means - Males**



Feed Consumption: (assessed twice weekly)

Figure 2: Summary of Food Consumption - Males



Clinical Chemistry: (samples collected from over-night fasted TK animals pre-treatment and on Days 29, 60, and 88 at 24 hours after dosing; samples were also collected from all main study animals (fasted) at scheduled termination; Endpoints included: total amylase, pancreatic amylase [main study animals only], lipase, glucose, urea, and creatinine)

Table 5: Group Mean Clinical Biochemical Analyses - Main Animals

Day 92
Males

Group 1 - Reference Item
Group 2 - LY2189265 0.5 mg/kg/dose
Group 3 - LY2189265 1.5 mg/kg/dose
Group 4 - LY2189265 5.0 mg/kg/dose

Group	Summary Information	UREAN mg/dL	CREAT mg/dL	GLUC mg/dL	AMYL U/L	PAMYL U/L	LIPASE U/L	GLY HGB %
1	Mean	18.3	0.13	461.2	2601.2	2266.2	10.0	6.51
	SD	4.1	0.05	116.8	491.2	428.5	1.8	1.76
	N	26	26	26	26	26	26	26
2	Mean	16.5	0.15	451.9	2928.0	2541.5	9.5	5.59
	SD	3.8	0.08	91.6	411.6	356.6	0.7	1.34
	N	20	20	20	20	20	20	20
3	Mean	15.4 A	0.19 A	356.9 B	3169.5 C	2757.0 C	9.6	4.63 E
	SD	3.2	0.07	130.5	435.8	374.1	1.1	1.71
	N	20	20	20	20	20	20	20
4	Mean	14.7 B	0.23 C	273.6 C	3447.2 C	3006.7 C	9.6	3.36 F
	SD	3.2	0.08	113.3	508.3	452.3	1.8	0.92
	N	18	18	18	18	18	18	18

Significantly different from control group (group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

Similar trends were observed for the TK animals for urea, glucose, HbA1c, and total amylase. Mean TK group creatinine values were similar across all groups at all time points, including Day 89; therefore the apparent dose-related increase in mean creatinine values for the main study group is likely incidental.

Glucose, HbA1c, and Insulin: (samples collected from over-night fasted TK animals pre-treatment and on Days 29, 60, and 88 at 24 hours after dosing; samples were also collected from all main study animals (fasted) at scheduled termination)

Figure 3: Clinical Biochemical Gulcose Analyses - Summary of Means - Toxicokinetic Males

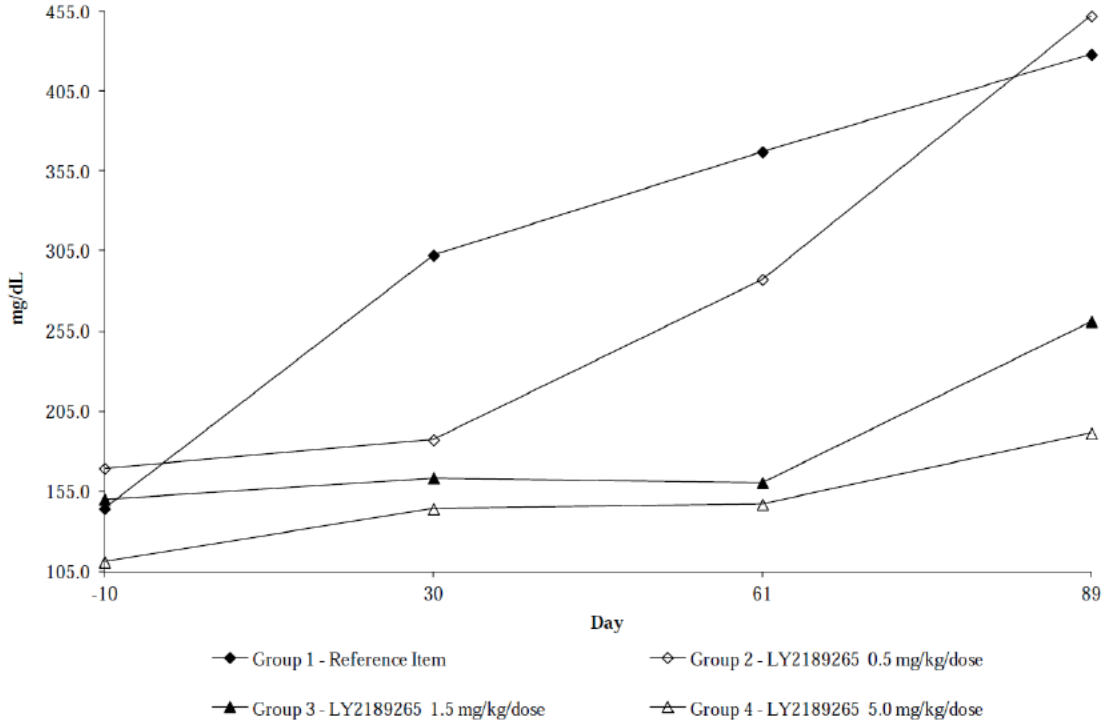


Figure 6: Clinical Biochemical GLY HGB Analyses - Summary of Means - Toxicokinetic Males

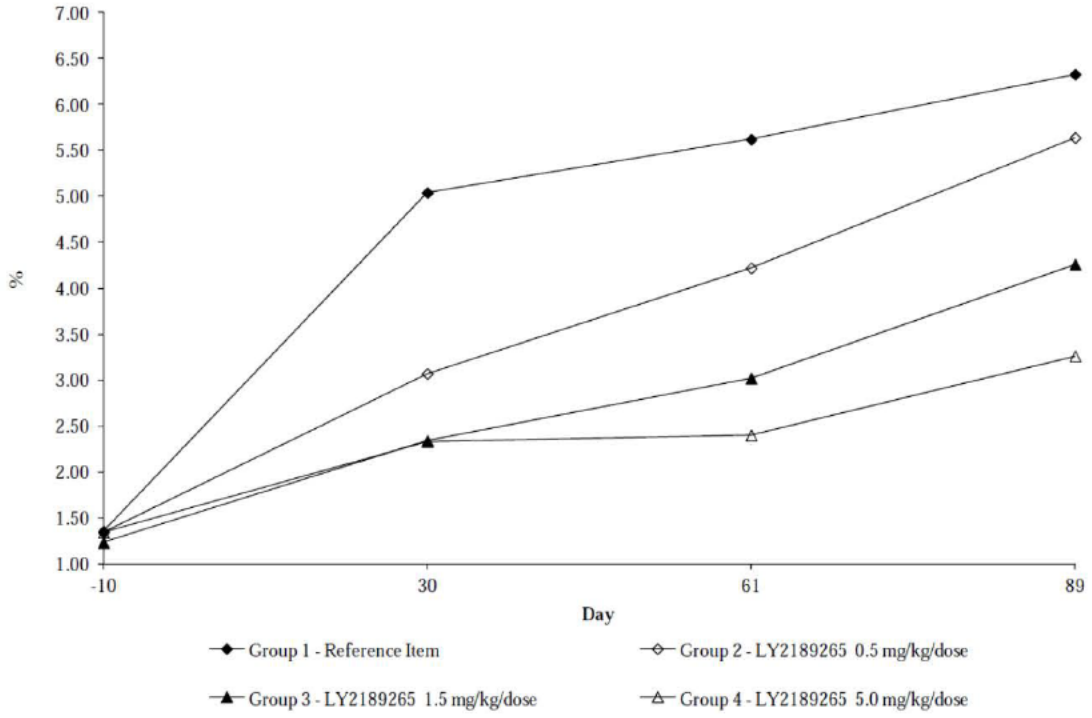
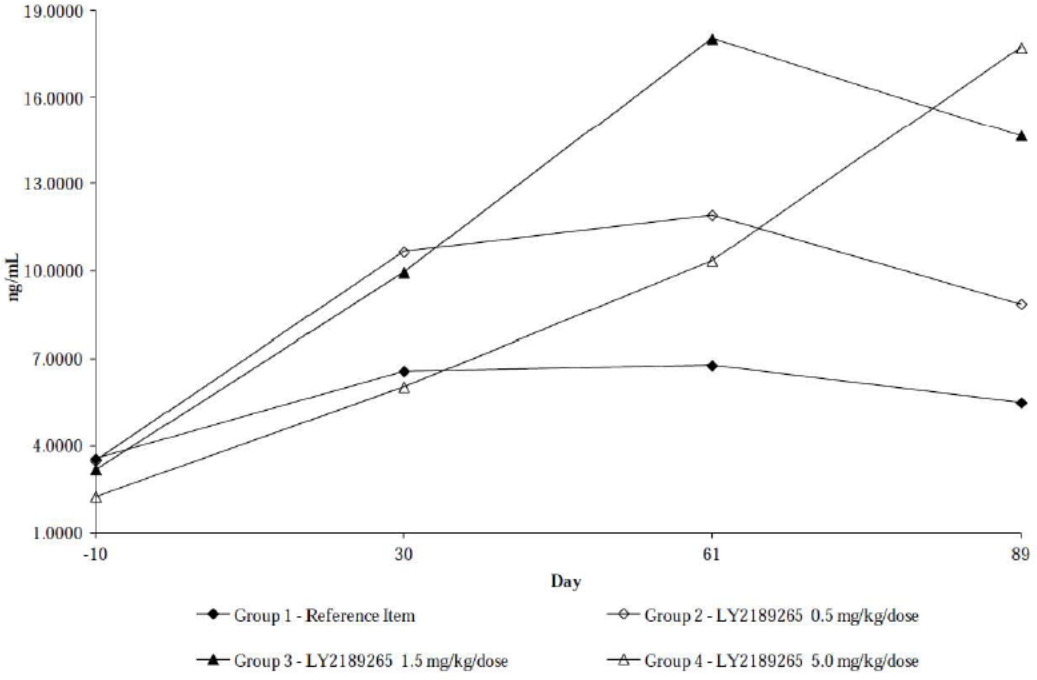


Figure 7: Insulin Analyses - Summary of Means - Toxicokinetic Males



Gross Pathology: (all main study animals)
There were no treatment-related macroscopic findings.

Organ Weights: (all main study animals; brain and pancreas only)
No treatment-related effects on mean pancreas weights were observed.

Histopathology: The pancreas was dissected with the common duct and attached duodenum with papilla. The pancreas was serially trimmed entirely (head, body, tail) into cross sections of approximately 3 mm. For each block containing multiple cross sections of pancreatic tissue, 2 sets of 7 consecutive sections (each set approximately 100 µm apart) were collected. One slide of each set was stained with H&E. The adjacent sections were used for immunohistochemistry. Two sets of consecutive sections as described above for each block were obtained with approximately 100 µm between each pair; one slide from each set was stained for Ki67 (proliferation marker) and cytokeratin-19 (duct epithelial cell marker) and a second selected slide was stained with TUNEL and cytokeratin-19.

Adequate Battery: Yes
Peer Review: Yes, by the sponsor

Text Table 7: Incidence and Severity of Histopathological Changes

Tissue/Finding	Sex	Male			
	(mg/kg/dose)	0	0.5	1.5	5.0
Pancreas	No. examined	27	20	20	20
Increased ductal epithelium					
	Total No. affected	1	7	11	7
	Minimal	1	4	5	3
	Slight	—	3	5	3
	Moderate	—	—	1	1
Inflammation: neutrophilic acinar					
	Total No. affected	1	0	0	5
	Minimal	1	—	—	3
	Slight	—	—	—	2
Atrophy: acinar with mixed cell inflammation					
	Total No. affected	2	1	5	8
	Minimal	2	—	2	6
	Slight	—	1	3	2
Atrophy: acinar					
	Total No. affected	—	1	3	7
	Minimal	—	1	3	6
	Slight	—	—	—	1
Hyperplasia: islet cell					
	Total No. affected	26	20	20	20
	Minimal	12	3	4	—
	Slight	14	17	14	19
	Moderate	—	—	2	1
Inflammation: neutrophilic periductal					
	Total No. affected	9	9	14	7
	Minimal	9	8	9	4
	Slight	—	1	5	2
	Moderate	—	—	—	1

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Histomorphometry: Quantification of proliferation and apoptosis was conducted on pancreata of main study animals from scheduled necropsies. A minimum of 1000 cytokeratin-positive cells were evaluated. Histomorphometry parameters and results are presented in the sponsor-generated tables below.

Measured Histomorphometry Parameters	
Parameter	**Direct Measure**
Ki-67 Cell Count	Total Ki-67 positive duct cells (Cytokeratin-19 positive cells when the cell nucleus was present in the section & Ki-67)
Total Duct Cell Count	Total number of duct cells (1000-2000) (Cytokeratin-19 positive cells when the cell nucleus was present in the section)
Derived Histomorphometry Parameters	
Parameter	**Calculation**
Percent Proliferation	Ki-67 Cell Count/Total Duct Cell Count * 100
Measured Histomorphometry Parameters	
Parameter	**Direct Measure**
TUNEL Cell Count	Total TUNEL positive duct cells (Cytokeratin-19 positive cells when the cell nucleus was present in the section & TUNEL)
Total Duct Cell Count	Total number of duct cells (1000-2000) (Cytokeratin-19 positive cells when the cell nucleus was present in the section)
Derived Histomorphometry Parameters	
Parameter	**Calculation**
Percent Apoptosis	TUNEL Cell Count/Total Duct Cell Count * 100

Group 1 - Reference Item
Group 2 - LY2189265 0.5 mg/kg/dose
Group 3 - LY2189265 1.5 mg/kg/dose
Group 4 - LY2189265 5.0 mg/kg/dose

Group	Summary Information	Percent Proliferation %	Percent Apoptosis %
1	Mean	0.36	0.11
	SD	0.447	0.178
	N	26	26
2	Mean	0.39	0.11
	SD	0.585	0.167
	N	20	20
3	Mean	0.40	0.06
	SD	0.493	0.094
	N	20	20
4	Mean	0.65	0.06
	SD	0.950	0.115
	N	19	19

Significantly different from control group (Group 1) value: A - P <= 0.05, B - P <= 0.01, C - P <= 0.001 (Dunnett)
D - P <= 0.05, E - P <= 0.01, F - P <= 0.001 (Dunn)

Individual Animal Microscopic Findings

Animal Number	Islet Cell Hyperplasia (grade)	Duct Cell Proliferation (% Ki-67+)	Increased Ductal Epithelium (grade)	Inflammation (grade)		Atrophy (grade)		Pancreatic Amylase (U/L)
				Neutrophilic Periductal	Neutrophilic Acinar	Acinar with Mixed Cell Inflammation	Acinar	
Control								
1001	2	0.64	-	1	-	-	-	2290
1002	2	1.19	-	1	-	-	-	2220
1003	1	0.00	-	-	-	-	-	2440
1004	1	0.05	-	-	-	-	-	1970
1005	2	1.13	-	1	-	-	-	2780
1006	1	0.05	-	-	-	-	-	1630
1007	1	0.00	-	1	-	-	-	2320
1008	2	0.00	-	-	-	1	-	3060
1009	1	0.25	-	-	-	-	-	1700
1010	1	0.00	-	-	1	-	-	1820
1011	1	1.17	-	-	-	-	-	1830
1012	2	0.00	-	-	-	-	-	2950
1013	1	0.10	-	-	-	-	-	2640
1014	2	0.20	-	-	-	-	-	2930
1015	1	0.05	-	-	-	-	-	1910
1016	2	0.05	-	1	-	-	-	1990
1017	2	0.10	-	-	-	-	-	2980
1018	2	0.05	-	-	-	1	-	2640
1019	1	0.10	-	-	-	-	-	1770
1020	2	0.11	-	1	-	-	-	2140
1021†	-	NE	-	-	-	-	-	NE
1022	2	0.20	-	1	-	-	-	2270
1023	1	0.91	1	1	-	-	-	1850
1024	2	0.59	-	-	-	-	-	2070
1025	1	0.20	-	-	-	-	-	2020
1026	2	1.26	-	-	-	-	-	2280
1027	2	0.92	-	1	-	-	-	2420

Animal Number	Islet Cell Hyperplasia (grade)	Duct Cell Proliferation (% Ki-67[+])	Increased Ductal Epithelium (grade)	Inflammation (grade)		Atrophy (grade)		Pancreatic Amylase (U/L)
				Neutrophilic Periductal	Neutrophilic Acinar	Acinar with Mixed Cell Inflammation	Acinar	
5 mg/kg LY2189265								
4001	2	1.19	1	1	1	1	-	3320
4002[††]	2	0.73	-	1	-	-	1	3040
4003	2	0.00	-	2	-	-	-	2910
4004	2	0.30	-	-	-	-	-	3460
4005	2	1.01	-	-	-	-	-	3770
4006	2	0.93	2	2	2	-	1	2080
4007	2	0.83	-	-	-	1	-	3710
4008	2	0.33	1	-	-	1	-	3540
4009	2	0.92	1	1	-	2	1	2980
4010	2	0.59	2	-	-	-	-	2550
4011	2	0.30	-	1	-	1	2	2760
4012[†††]	2	NE	-	-	1	-	-	NE
4013	2	0.00	-	-	1	-	-	2910
4014	2	0.19	-	-	-	-	1	2690
4015	2	0.05	-	-	-	-	1	3310*
4016	2	0.26	2	-	-	1	-	2690
4017	2	0.10	-	-	-	-	1	2360
4018	2	0.00	-	-	-	1	-	2800
4019	3	4.24	3	3	2	2	-	2960
4020	2	0.31	-	-	-	-	-	3320

NE = not evaluated; "-" = finding not observed.
*Value excluded from mean.
[†]Animal 1021 was found dead on Day 49; microscopic evaluation revealed acute pancreatic hemorrhage, multifocal, grade 3.
[††]Animal 4002 also had focal acinar cell hyperplasia, grade 2.
[†††]Animal 4012 was found dead on Day 75.
Grade 1 = minimal/very few/very small; Grade 2 = slight/few/small; Grade 3 = moderate/moderate number/moderate size.
Note: The highlighted cells represent the ~7 data points with the highest value or most severe histopathology finding for each parameter. This is highlighted to determine whether a correlation could be observed regarding the percentage of Ki67-positive cells with the incidence of increased ductal epithelium as well as whether a correlation could be observed between incidence/severity of a microscopic lesion with increased serum pancreatic-specific amylase.

Although there was a correlation between Ki67 staining and increased duct epithelium or increased inflammation with increased serum amylase for some animals (i.e., numbers 4001, 4006, 4009, and 4020), a clear correlation could not be made for the majority of the high-dose animals. Overall, the microscopic changes observed in ZDF rats might suggest that some diabetic patients taking LY2189265 could be more susceptible to morphological changes in the exocrine pancreas. However, the changes observed were not sufficient or severe enough to classify the effects as drug-induced pancreatitis or premalignant lesions.

Toxicokinetics: (samples collected from TK animals (3/group/time point) predose and 4, 12, 24, 48, and 96 hours postdose on Days 1 and 88. Additional samples were collected predose and 24 hours postdose on Day 43 and from all main study animals at scheduled sacrifice)

Text Table 4: LY2189265 Exposure Parameters

Administered Dose (mg/kg/dose)			
Parameter	0.5	1.5	5.0
Sex	Male	Male	Male
LY2189265			
Day 1			
T_{max} (Hours)	12.0	12.0	12.0
C_{max} (ng/mL)	765 ± 34.0	2330 ± 136	7110 ± 1160
AUC_{0-96} (ng*Hours/mL)	19,400 ± 1100	56,300 ± 4360	174,000 ± 14,200
Day 88			
T_{max} (Hours)	24.0	24.0	24.7
C_{max} (ng/mL)	591 ± ID	1670 ± 163	7100 ± 336
AUC_{0-96} (ng*Hours/mL)	21,200 ± 2870	65,400 ± 4390	238,000 ± 8360

T_{max} Time of maximum observed concentration.
C_{max} Maximum observed concentration.
AUC_{0-96} Area Under the Curve from 0 to 96 hours.
ID Insufficient Data.
(sponsor-generated table)

Dosing Solution Analysis:
Day 1 and Day 85 dosing formulations were found to be between 100% and 106.7% of the nominal concentrations.

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11 Integrated Summary and Safety Evaluation

Primary Pharmacology

LY2189265 is a long-acting human glucagon-like peptide-1 (GLP-1) receptor agonist. The molecule consists of two identical, disulfide-linked chains, each containing a human GLP-1 analog sequence covalently linked to a modified human immunoglobulin G4 (IgG4) heavy chain fragment (Fc) by a small peptide linker. The GLP-1 analog portion of the molecule has approximately 90% sequence similarity to endogenous human GLP-1 (7-37), (b) (4)

Several in vitro and in vivo studies showed that LY2189265 has high receptor binding potency and induces insulin secretion. LY2189265 was shown to have a similar potency to activate the human GLP-1 receptor in vitro as native GLP-1, with an EC_{50} value of 12.5 pM. Specificity of LY2189265 for the human GLP-1 receptor over human GIP and glucagon receptors was also demonstrated in vitro. LY2189265 potently enhanced insulin secretion from isolated rat and monkey islet cells in a glucose-dependent manner. In vivo, a single SC dose of 0.179 or 1.79 mg/kg LY2189265 decreased mean plasma glucose of Sprague-Dawley rats during an intravenous glucose tolerance test in a dose-related manner. In monkeys, a single administration of 0.1 mg/kg LY2189265 resulted in significant insulinotropic activity during a glucose infusion and was also associated with significant increases in C-peptide levels. These effects were noted for up to 7 days after LY2189265 administration. However, there were no clear effects on glucose, GLP-1, or glucagon levels. Mean serum LY2189265 levels were 325, 47, and 24 ng/mL at 1, 5, and 7 days after administration, respectively. Similar insulinotropic activity was observed after four once weekly doses of 0.1 mg/kg in Cynomolgus monkeys. This effect was also associated with significant increases in C-peptide. Mean serum LY2189265 concentrations 4 days after the final administration was 53 ng/mL. LY2189265 did not show evidence of antibody dependent cell-mediated cytotoxicity (ADCC) in a fully human in vitro test system.

Safety Pharmacology

In an in vitro hERG channel assay, LY2189265 showed a concentration-dependent increase in hERG current inhibition. hERG current was inhibited by up to 33% at the highest concentration of 15.2 µg/mL. The average C_{max} value for type 2 diabetic patients receiving 1.5 mg dulaglutide/week was 0.114 µg/mL.

In a cardiovascular safety pharmacology study, Cynomolgus monkeys received a single SC dose of 1 or 10 mg/kg LY2189265. Treatment resulted in dose-related increases in heart rate, with heart rate being approximately 22% higher than control values at 3 and 5 hours after dosing as well as at later time points. At 10 mg/kg, a statistically significant increase in heart rate (26%) was observed 3 hours after dosing. Statistically

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significant increases of 19% to 44% were also observed at various time points between 11 and 74 hours after treatment (increases of 17 to 25 ms from baseline values). A lower background mean heart rate for the control group led to a bigger increase in heart rate when compared with controls rather than when compared with the baseline value for that particular treatment group. However, the apparent increase in heart rate in the two treated groups was considered to be drug related. The biggest effects on heart rate appeared to occur during the sleeping hours, during which heart rates for control monkeys were lower compared with baseline values whereas heart rates for treated monkeys remained elevated or were similar to baseline values. A dose-responsive increase in left ventricular inotropic state was also noted at both dose levels. As with heart rate, the greatest differences from control tended to occur during the dark cycle, during which values decreased for control animals but remained elevated for treated animals. Noteworthy ECG effects were noted for the 10 mg/kg group (C_{max} ~35 µg/mL; 307X clinical C_{max}) at 24 hours post dose, with a statistically significant 11% increase in mean QTc_B. No physiologically important test article-related effects were noted for systolic, diastolic, pulse, or mean pressures.

In a 1-month monkey study, increases in QTcL interval (Lilly correction method) in relation to control group values occurred in all drug-treated groups. The increases were statistically significant at 3 of 4 time points for the 3 and 10 mg/kg groups and at a single time point for the 1 mg/kg group. Prior to dosing and 2 hours after dosing on Day 4, the effects did not occur in a dose-related manner and the MD group had a similar value as at baseline (Day -16). The largest difference from control or baseline on Day 4 occurred for LD males (+59 msec and +32 msec, respectively). Prior to dosing on Day 28, the MD group had the largest difference from control, although the mean value was similar to the Day -16 baseline value for this group. The most noteworthy change on Day 28 was for HD females, which had a 34 msec increase from control and a 30 msec increase from Day -16. Males had a similar value as baseline and a 19 msec increase from control. Two hours after dosing on Day 28, mean values from all groups were higher than control; however, most values were similar to Day-16 baseline values. MD females had a 34 msec and 19 msec increase and HD females had a 58 msec and 45 msec increase from control and baseline, respectively. Mean C_{max} values on Day 28 for the 1, 3, and 10 mg/kg dose levels were 8.5, 23.8, and 87.4 µg/mL, respectively, which are 75-, 209-, and 767-fold greater than the average clinical C_{max} value in type 2 diabetics after repeated dosing of 1.5 mg dulaglutide.

In a 3-month monkey study, an increase in mean heart rate (up to 45%) was noted for HD groups (16.29 mg/kg) on Day 5 and Day 89 compared with baseline and control values. Some increases in mean QTc values were noted for MD (4.89 mg/kg) and HD groups on Day 5 and/or Day 89 compared with control values. However, the values for these groups were not much higher than at baseline and baseline values were also slightly higher than control values. Therefore, the changes in QTc did not appear to be biologically relevant. The mean C_{max} value for the HD group at Day 92 was 51.6 µg/mL (453X clinical C_{max}). In a 9-month study in which monkeys received twice weekly subcutaneous injections of up to 8.15 mg/kg LY2189265, no toxicologically meaningful effects on neurobehavior, heart rate, or ECG endpoints were noted. Heart rate and

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ECG parameters were measured on Days 5, 29, 92, 180, and 274. Mean C_{max} at the HD level on Day 274 was 73.5 µg/mL (645X clinical C_{max}).

General Toxicology

Standard toxicology studies were conducted in rats and monkeys for up to 6 and 9 months, respectively, with doses being administered by subcutaneous injection twice weekly. A 4-week study was conducted in mice as a dose range-finding study for the 6-month transgenic mouse carcinogenicity study. One-year studies were conducted in rats and monkeys as part of an enhanced analysis of potential drug-related effects on the thyroid and pancreas (monkey only). A 3-month study in a rodent model of diabetes (ZDF rat) was also conducted to evaluate pancreatic safety.

Animals treated with moderate to high dose levels of LY2189265 experienced an initial body weight loss or decreased body weight gain compared with controls that generally correlated with decreased food and water consumption. After the first week or two of treatment, body weight gains became more similar to the control group, although final body weights were generally still lower than controls. During the treatment-free recovery period body weights typically rebounded to values similar to or greater than control values. Initial body weight effects were observed in mice, rats, and monkeys at ≥10, ≥1.6, and ≥0.41 mg/kg twice weekly, respectively. The effect on body weight and food consumption is related to the pharmacodynamic activity of LY2189265, which is also observed with other GLP-1 receptor agonists.

Observed clinical signs were generally associated with the treatment-related effects on food and water consumption and included hunched posture (100 mg/kg in mice); decreased feces, thinness, and rough haircoat (16.3 mg/kg for 3 months and ≥4.9 mg/kg for 6 months in rats); and increased emesis (≥0.41 mg/kg in monkeys). At 8.15 mg/kg, one or two monkeys in each study required supplemental fruit/treat supplements, IV fluids, and/or a dosing holiday due to dehydration and anorexia. In a 1-month study, one female monkey receiving 10 mg/kg exhibited abnormal gait (dragging of hind limb), curling of toes, and decreased skin elasticity and hunched posture.

Clinical pathology assessments showed slight decreases in erythrocyte parameters for mice and rats. In the 1-month mouse study, the 100 mg/kg female group had increases in mean ALT (272%) and AST (270%) values compared with control values, which was primarily due to individual increases for 2 of the 5 animals; there were no correlative histopathology findings in the liver. In monkeys, there were no noteworthy treatment-related effects on clinical pathology parameters for up to 9 months of treatment and there was no indication of complement activation (C3a or Bb) after 28 days of treatment.

Effects on organ weights generally correlated with decreased body weight gains in mice, rats, and monkeys. In the 3-month monkey study, absolute and relative thymus weights were significantly decreased at ≥1.63 mg/kg for males. Decreased thymus weights correlated with increased incidence and/or severity of thymic involution in some males or females given 1.63 or 8.15 mg/kg and some females given 0.41 mg/kg. As

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noted in monkeys treated with other GLP-1 receptor agonists, increased mean testicular weights were noted at ≥1.63 mg/kg (≥85X clinical AUC). Similarly, increased mean weights were noted for epididymis, prostate, and seminal vesicles. In the 9 month monkey study, increased mean absolute and relative prostate (up to 2-fold) and testis weights (up to 2.5-fold) were noted for all male treatment groups (≥0.41 mg/kg; ≥26X clinical AUC). There were no correlative histopathology findings for male reproductive organs. In the 9-month study, decreased mean ovary weights were noted at ≥0.41 mg/kg (up to 39% for absolute weight and 31% for relative weight). At the end of the recovery period, increased testis weights and decreased ovary weights were still noted for the 8.15 mg/kg groups (474X clinical AUC). The effects on reproductive organs correlated with the observation of fewer treated males having immature testes and a greater number of treated females having immature ovaries.

After 1 month of treatment, mice showed decreased vacuolation (glycogen content) in the liver at ≥10 mg/kg (83X clinical AUC); decreased granulation of the granular secretory duct epithelium of the mandibular salivary gland for 100 mg/kg females (675X clinical AUC); decreased zymogen granules in the pancreas for males treated with ≥10 mg/kg; and decreased dilation and increased atrophy were noted in the uterus in females receiving ≥10 mg/kg. These findings appeared to be secondary to the effects on body weight.

In Fischer 344 rats after 1 month of treatment, histopathology revealed an increased incidence of seminiferous tubule atrophy, bilateral epididymal luminal cell debris, oligospermia, and decreased prostate and seminal vesicle secretion in males given 20 mg/kg (215X clinical AUC); an increased incidence of diestrus II in 20 mg/kg females; and thymic atrophy in males given 6 or 20 mg/kg. These changes were consistent with a generalized stress response due to decreased food intake and weight loss; often correlated with decreased organ size and weight; and were not considered adverse.

In Sprague-Dawley rats treated for 3 months, histopathology revealed minimal to slight degeneration of the seminiferous tubules (2/10), slight inflammation of the epididymis (1/10), and minimal hypospermia (1/10) at 16.29 mg/kg (148X clinical AUC). After 6 months of treatment, microscopic findings included a slight increase in focal congestion/hemorrhage of the stomach (2/15 males, 1/15 females) and dilatation of the uterus (6/15) at 16.29 mg/kg (162X clinical AUC). An increase in the number of animals with hepatic bile duct proliferation was noted for the high-dose recovery males (2/10).

In Cynomolgus monkeys treated for 1 month, microscopic findings included hypocellularity and fat atrophy of the bone marrow (≥3 mg/kg for females; 135X clinical AUC), lymphoid depletion in the spleen and mesenteric and submandibular lymph nodes (≥1 mg/kg; 45X clinical AUC), and inflammation at the injection sites (≥1 mg/kg). Other than injection site reactions, the microscopic findings were attributed to secondary effects on body weight and food consumption. There were no adverse microscopic findings attributed to treatment after 3 or 9 months of twice weekly doses up to 8.15 mg/kg (379X to 474X clinical AUC), including the pancreas and thyroid.

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Enhanced Thyroid Analysis

Long-acting GLP-1 receptor agonists induce thyroid C-cell tumors in rats and mice. The relevance of this finding to humans is uncertain. The sponsor attempted to address the question of human relevance by conducting a thorough assessment of rodent and monkey thyroids after different durations of LY2189265 treatment, up to 1 year. The intent of the studies was to correlate drug-induced thyroid C-cell proliferation and serum calcitonin levels with the duration of treatment in rodents and show that similar effects are not observed in monkeys. The sponsor's hypothesis is that humans will respond to GLP-1-induced C-cell proliferation in a manner that is more similar to monkeys than rats. Therefore, the finding of a more robust response in rats compared with monkeys would suggest that rats are more sensitive to the C-cell proliferative effects than humans, resulting in a larger clinical safety margin.

Male Sprague-Dawley rats received vehicle or 5 mg/kg LY2189265 (~58X clinical AUC) by subcutaneous injection twice weekly for up to 1 year. Groups of 20 males were sacrificed after 94, 185, 276, and 374 days of treatment. Another untreated group was euthanized on Day 3 for a baseline assessment. Approximately 3 days before scheduled sacrifice, animals received an intravenous dose of calcium chloride to induce calcitonin secretion. Thyroid glands were exhaustively sectioned through at 5 µm. A series of 6 serial sections were collected at 100 µm intervals through the entire pair of thyroid glands. Three slides with the 6 thyroid sections were stained with H&E; and an additional 3 slides with 6 thyroid sections were co-labeled with calcitonin and Ki67. Quantitative image analysis included determinations of group mean total C-cell mass, and individual C-cell mass. Image analysis software was used for thyroid morphometric analysis to determine the thyroid calcitonin-stained area.

Treated animals gained less weight than controls throughout the study, with final mean body weights being 20% less than controls. The effect on body weight generally correlated with decreased food consumption. An age-related increase in plasma calcitonin was observed in both control and treated animals. No difference CaCl-stimulated calcitonin was observed between control and treated groups. There were no noteworthy differences in the incidence or severity of C-cell hyperplasia after treatment for up to 9 months. After 1 year of treatment, an increase in incidence and severity (slight) of focal/multifocal C-cell hypertrophy/hyperplasia was noted for the treated group. No difference in incidence or severity of diffuse C-cell hyperplasia was observed between the two groups. One C-cell adenoma was observed in each of the control and treated groups. One animal from the LY2189265-treated group had a C-cell carcinoma. A higher severity (slight) for Ki67/calcitonin double staining was observed for treated animals after 1 year of treatment. However, at earlier time points, more control animals had severity scores of slight or moderate for double staining, so the significance of the finding at 1 year is uncertain. Tissue morphometery was conducted on thyroids to determine if C-cell volume or number increased over time in treated animals and if that correlated with increased plasma calcitonin levels. No statistically significant difference was noted between the two groups for percent calcitonin positive cells, volume of calcitonin positive tissue, thyroid tissue volume, or thyroid organ volume. Although there was some correlation between the number/size of calcitonin positive cells and plasma calcitonin

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values in individual animals, a consistent relationship was not observed. Histopathology findings of focal hyperplasia or carcinoma also did not correlate well with plasma calcitonin values, as it would be expected that these animals would have higher calcitonin levels, but this was not observed.

Male Cynomolgus monkeys (20/group) received vehicle or 8.15 mg/kg LY2189265 (~474X clinical AUC) by subcutaneous injection twice weekly for 1 year. Blood samples were collected before and after calcium gluconate administration on Days 88, 179, 270, and 365 to evaluate calcitonin levels before and after induction. For the thyroid, three slides, each containing one section of each thyroid lobe from one of the spatial thirds of the thyroid, were stained with H&E; an additional three slides, each containing one section of each thyroid lobe from one of the spatial thirds of the thyroid, were co-labeled for calcitonin and Ki67. Positive and negative control co-labeled slides were included. Slides were evaluated at 400x magnification and up to 200 C-cells were counted in each section on the slide.

One drug-treated monkey was sacrificed moribund around Day 30 due to body weight loss and poor condition. The treated group had an increased incidence of few or no feces, low food consumption, and thin appearance compared with the control group. Mean body weights for the LY2189265-treated group decreased from baseline by 0.3 kg between Days 1 and 29. By the end of the study, the final mean body weight for the treated group was 20% lower than the control group. Effects on body weight correlated with decreased food consumption. Mean serum calcitonin values remained below the level of quantitation for both groups throughout the study. A slight trend for higher calcitonin secretion was noted for the LY2189265-treated group compared with control at 2 and 10 minutes after calcium gluconate treatment. However, these differences were not statistically significant and did not correlate with C-cell morphometry data. No treatment-related adverse microscopic findings were noted for the thyroid or parathyroid. There was no increase in the percentage of Ki67-labeled thyroid C-cells. Histomorphometry analysis also did not reveal any treatment-related cell proliferative effects in the thyroid.

Enhanced Pancreas Analysis

Male Cynomolgus monkeys (20/group) received vehicle or 8.15 mg/kg LY2189265 (~474X clinical AUC) by subcutaneous injection twice weekly for 1 year. Pancreas slides from each animal included three sections from the head, two sections from the body, and one section from the tail. Slides were stained with H&E and an additional three slides (containing sections from the head) were labeled for Ki67. After the primary microscopic evaluation and independent peer review, the stained slides from each animal were also reviewed by an expert working group.

No treatment-related adverse microscopic findings were noted for the pancreas. A slight increase in the severity of increased pancreatic goblet cells in interlobular ducts was noted for the treated group. This finding did not correlate with increases in serum amylase or lipase. There was no increase in the percentage of Ki67-labeled pancreatic ductal cells. The conclusion of the pathologist working group was: “Of the findings

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recorded for the pancreas in the anatomic pathology report, the consensus of the PWG was that only the increases severity of "goblet cells, increased, interlobular duct" in four treated animals was considered to be treatment related. This effect was considered non-adverse because there was no evidence of an associated inflammatory or proliferative change including pancreatitis, ductal dilatation, ductal blockage, or PanIN."

To further evaluate the potential effect of LY2189265 on the exocrine pancreas, male ZDF rats were treated with twice weekly subcutaneous injections of vehicle or 0.5, 1.5, or 5 mg/kg LY2189265 for 3 months. The pancreas was dissected with the common duct and attached duodenum with papilla. The pancreas was serially trimmed entirely (head, body, tail) into cross sections of approximately 3 mm. For each block containing multiple cross sections of pancreatic tissue, 2 sets of 7 consecutive sections were collected and one slide of each set was stained with H&E and the adjacent sections were used for immunohistochemistry (Ki67 plus cytokeratin-19 and TUNEL plus cytokeratin-19).

LY2189265-treated animals lost weight during the first 2 weeks of the study. For the next 4 weeks, body weight gain was similar for all groups. However, after approximately Day 42, the control group gained significantly less weight in spite of eating significantly more food as a result of their worsening hyperglycemia. In addition to the body weight and food consumption data, mean glucose, insulin, and HbA1c values also indicated drug-related efficacy for the mid- and high-dose groups. A dose-related increase in mean serum pancreatic-specific amylase was observed for all dose groups, with the increase being statistically significant for the MD and HD groups (↑22% and 33% from controls, respectively). No increases in serum lipase were observed. Increased incidence and severity (minimal to moderate) of increased ductal epithelium was observed at all dose levels (≥3X clinical AUC). Only one control animal was noted with this finding, which was of minimal severity. Animals treated with ≥1.5 mg/kg (≥8X clinical AUC) had an increased incidence of minimal to slight acinar atrophy, with or without mixed cell inflammation. An increase in the incidence of minimal to slight neutrophilic acinar inflammation occurred at 5 mg/kg (30X clinical AUC). An increase in the severity, but not incidence, of neutrophilic periductal inflammation (minimal to moderate) and islet cell hyperplasia was observed for treated groups.

A non-statistically significant, dose-related trend for increased mean Ki-67 staining and decreased mean TUNEL staining in pancreatic duct cells was observed at ≥1.5 mg/kg, indicating an increase in ductal cell proliferation and/or a decrease in ductal cell apoptosis. With the exception of a few animals, there generally was not a good correlation for individual animals having both a high percentage of Ki-67 positive duct cells and an increased incidence/severity of increased ductal epithelium. The microscopic changes observed in this study might imply that some diabetic patients taking LY2189265 could be more susceptible to morphological changes in the exocrine pancreas. However, the observed changes were not sufficient or severe enough to classify the effects as drug-induced pancreatitis or premalignant lesions.

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Carcinogenicity

The carcinogenic potential of LY2189265 was studied in a 6-month TgHras transgenic mouse study and a 2-year rat study. Genetic toxicology studies were not conducted, as LY2189265 is a biotechnology-derived product.

Twice-weekly subcutaneous injections to transgenic mice for 26 weeks did not affect survival and there was no evidence of drug-related neoplasms at the highest dose administered (3 mg/kg; 5X clinical AUC). Postmortem non-neoplastic observations of note were limited to an increase in cytoplasmic volume of thyroid C-cells stained with calcitonin for all treated groups (≥0.3 mg/kg; ≥1X clinical AUC) and an increased incidence of extramedullary hematopoiesis in the spleen of all female treatment groups (≥0.3 mg/kg).

In rats administered LY2189265 twice weekly for two years, there were no drug-related adverse effects on survival. The study was terminated early (Week 93) as a result of low survival rates in the control groups for which no cause was ascertained. Final body weights were statistically significantly lower for all male treatment groups and the female high-dose group. Effects on body weight gain generally correlated with decreased food consumption. Animals administered ≥0.5 mg/kg (7X clinical AUC) showed a statistically significant or nearly statistically significant increase in the incidence of thyroid C-cell adenomas. A non-statistically significant, slight numerical increase in thyroid C-cell carcinomas was observed at 5 mg/kg (58X clinical AUC). An increase in the incidence of C-cell adenoma and carcinoma combined was also noted, but this was a reflection of the high incidence of adenomas. An increased incidence of diffuse (males and females) and focal (females only) thyroid C-cell hyperplasia was also evident at ≥0.5 mg/kg. The NOEL for increased C-cell tumors was 0.05 mg/kg (0.5X clinical AUC). No treatment-related neoplasms or non-neoplastic lesions were observed in other tissues. A summary of C-cell findings for male and female rats is shown in the tables below.

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Summary of Thyroid C-Cell Tumor Incidence in Male Rats

Dose (mg/kg)	Vehicle Control	0.05	0.5	1.5	5.0	Historical Control
C-cell diffuse hyperplasia	9/60 (15%)	9/59 (15%)	16/60 (27%)	19/60 (32%)	28/60 (47%)	0.2% (0-3.3%)
C-cell focal hyperplasia	39/60 (65%)	28/59 (47%)	25/60 (42%)	25/60 (42%)	15/60 (25%)	17.3% (8.3-78.5%)
C-cell adenoma	10/60† (17%) **p=0.0018**	14/59 (24%)	**25/60*** (42%) **p=0.0141**	**24/60*** (40%) **p=0.0139**	**31/60*** (52%) **p=0.0017**	12.6% (8.3-14.6%)
C-cell carcinoma	1/60 (1.7%) p=0.0399	0/59 (0%)	2/60 (3.3%)	2/60 (3.3%)	4/60 (6.7%) p=0.2503	1.3% (0-2.6%)
C-cell adenoma + carcinoma	11/60† (18%) **p=0.0010**	14/59 (24%)	**27/60*** (45%) **p=0.0100**	**24/60*** (40%) **p=0.0207**	**33/60*** (55%) **p=0.0011**	NP
Clinical Exposure Margin§		0.5X	8X	20X	46X	

Historical control data from the previous 5 years; NP = not provided.
*Statistically significant by pair-wise analysis compared with vehicle control; p≤0.01 for adenomas and p<0.05 for carcinomas.
†Statistically significant for dose response; p≤0.005 for adenomas and p≤0.025 for carcinomas.
§Exposure margins based on the clinical $AUC_{0\text{-}168h}$ value of 14.0 µg•h/mL at 1.5 mg once weekly.

Summary of Thyroid C-Cell Tumor Incidence in Female Rats

Dose (mg/kg)	Vehicle Control	0.05	0.5	1.5	5.0	Historical Control
C-cell diffuse hyperplasia	7/60 (12%)	6/59 (10%)	12/60 (20%)	11/60 (18%)	25/60 (42%)	0.4% (0-8.3%)
C-cell focal hyperplasia	7/60 (12%)	11/59 (19%)	20/60 (33%)	20/60 (33%)	20/60 (33%)	20.2% (0-80%)
C-cell adenoma	7/60† (12%) **p<0.0001**	6/59 (10%)	**19/60** (32%) p=0.0399	**19/60** (32%) p=0.0399	**32/60*** (53%) **p<0.0001**	12.9% (0-23.3%)
C-cell carcinoma	1/60† (1.7%) **p=0.0177**	0/59 (0%)	1/60 (1.7%)	1/60 (1.7%)	4/60 (6.7%) p=0.2458	1.0% (0-2.3%)
C-cell adenoma + carcinoma	8/60† (13%) **p<0.0001**	6/59 (10%)	**20/60** (33%) p=0.0499	**20/60** (33%) p=0.0499	**35/60*** (58%) **p<0.0001**	NP
Clinical Exposure Margin§		0.5X	7X	19X	70X	

Historical control data from the previous 5 years; NP = not provided.
*Statistically significant by pair-wise analysis compared with vehicle control; p≤0.01 for adenomas and p<0.05 for carcinomas.
†Statistically significant for dose response; p≤0.005 for adenomas and p≤0.025 for carcinomas.
§Exposure margins based on the clinical $AUC_{0\text{-}168h}$ value of 14.0 µg•h/mL at 1.5 mg once weekly.

Developmental and Reproductive Toxicology

Male Sprague-Dawley rats were treated with vehicle or 1.6, 4.9, or 16.3 mg/kg LY2189265 every third day by subcutaneous injection for 4 weeks prior to cohabitation and during a 2-week cohabitation period. Decreased body weight gain and decreased food consumption were noted at all dose levels. Decreased weights were noted for the testes, epididymides, seminal vesicles, and prostate, which were likely secondary effects of decreases in body weight gain. There were no direct test article effects on gross pathology or histopathology. There were no effects on male mating performance or fertility; on sperm concentration, motility, or morphology; or on early embryonic viability at doses up to 16.3 mg/kg (~130X clinical AUC).

Female Sprague-Dawley rats were treated with vehicle or 1.6, 4.9, or 16.3 mg/kg LY2189265 every third day by subcutaneous injection for 2 weeks prior to mating, during the cohabitation period until mating was confirmed, and through GD 4, 5, or 6. Rats were euthanized on GD 15 for evaluation of maternal reproductive parameters. Maternal body weight loss during the pre-mating period and a decrease in body weight gain during the gestation treatment period were noted at all dose levels, which correlated with reduced food intake. A dose-related compensatory increase in food intake and corresponding body weight gain was noted during the gestation non-treatment period for all dose levels. An increase in the number of females with prolonged diestrus was noted at ≥4.9 mg/kg (~32X clinical AUC). A dose-related decrease in the mean number of corpora lutea, implantation sites, and viable embryos was seen at all dose levels; however these decreases were only statistically significant at ≥4.9 mg/kg groups. There were no compound-related effects on embryonic survival or maternal mating, fertility, or conception. On the basis of prolonged diestrus and statistically significant decreased number of corpora lutea, implantation sites, and viable embryos noted at ≥4.9 mg/kg, the NOAEL for female reproductive performance was 1.6 mg/kg (~17X clinical AUC). These effects occurred in the presence of maternal toxicity (decreased body weight/body weight gain).

In a Segment 2 developmental toxicology study, pregnant Sprague-Dawley rats received vehicle or 0.49, 1.63, or 4.89 mg/kg LY2189265 by subcutaneous injection every third day from GD 6 through GD15. Fertility and embryonic development parameters were assessed on GD 20. One death occurred in the high dose group on GD 8 that was considered to be possibly test article-related. Clinical signs, which were mainly observed at ≥1.63 mg/kg, consisted of hunched posture, decreased defecation, hair loss on ventral abdominal and thoracic regions, and red material around the eyes, nose, mouth, and forelimbs. A significant reduction in maternal body weight gain was noted for all test article groups, with body weight loss occurring at ≥1.6 mg/kg from GD 6 to 9. The effects on body weight correlated with decreased food consumption. An increase in percent post-implantation loss and a decrease in percent viable fetuses were noted for the 4.9 mg/kg group. Fetal body weight was significantly decreased at ≥1.63 mg/kg. An increase in mean litter proportions of unossified sternebrae, reduced skull ossification, unossified hyoid, and reduced vertebral arch ossification were observed at 4.9 mg/kg. On the basis of decreased fetal body weights at ≥1.63 mg/kg and increased post-implantation loss and decreased skeletal ossification at 4.9 mg/kg,

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which occurred in the presence of decreased maternal body weight gain, the NOAEL for fetal development was 0.49 mg/kg (4X clinical AUC).

In a Segment 2 developmental toxicology study, pregnant New Zealand white rabbits received vehicle or 0.04, 0.12, or 0.41 mg/kg LY2189265 by subcutaneous injection every third day from GD 7 through GD 19. Fertility and embryonic development parameters were assessed on GD 29. Initial significant weight loss followed by decreased body weight gain occurred at ≥0.12 mg/kg; decreased body weight gain was also noted for the 0.04 mg/kg group throughout the treatment period. These effects on weight correlated with a significant decrease in food consumption at all dose levels. A compensatory increase in body weight and food consumption occurred once dosing ended, which lasted until the end of the study. There were no effects on maternal reproductive parameters. Increased fetal malformations (lung lobular agenesis [upper end of historical control range], vertebral anomaly with or without associated rib anomaly, and costal cartilage anomaly) occurred at 0.41 mg/kg. Increased fetal skeletal variations occurred at 0.41 mg/kg (increased incidence of 27 presacral vertebrae, 13th full rib, bent hyoid arches, malaligned sternebrae, and reduced ossification of the ribs. Also, a decreased incidence of having a 13th rudimentary rib was noted for kits from the 0.12 and 0.41 mg/kg litters. On the basis of the fetal malformations and increase in skeletal variations observed at 0.41 mg/kg, the NOAEL for fetal development was 0.12 mg/kg (4X clinical AUC). The adverse effects on fetal development were observed only in the presence of maternal toxicity (body weight loss).

In a Segment 3 developmental toxicology study, pregnant Sprague-Dawley rats received vehicle or 0.2, 0.49, or 1.63 mg/kg LY2189265 by subcutaneous injection every third day from GD 6 through lactation day (LD) 19. A subset of F_1 generation pups were followed through postnatal day (PND) 85, during which time pups were evaluated for locomotor activity, acoustic startle, learning and memory, sexual maturation, and reproductive performance. A dose-related decrease in mean body weight gain was noted for all F_0 treated groups. HD females lost weight between GD 6 and 9 followed by body weight gains similar to or slightly greater than the MD group, but less than the control group. During lactation, body weight gains were similar to controls. The effects on body weight correlated with decreased food consumption during the gestation and lactation periods. Treatment did not affect mean values for gestation duration, litter size, live litter size, number of implantation sites, or pup sex ratio.

Survival rates during the lactation period for F_1 pups from HD dams were lower than historical control values, but were similar to the concurrent control values. An increased incidence of pups showing small stature, paleness, or cool to touch was noted for pups from HD dams. Mean body weights for male and female pups from HD dams were statistically significantly lower than controls at all time points from LDs 1 to 21. Weights remained lower throughout the remainder of the study, with the differences being statistically significant up to PND 63 for males and up to PND 84 for females. HD males had a slightly longer mean time to balano-preputial separation (47.0 days vs. 45.6 days for control), which was likely secondary to the smaller size of the HD male pups. A decrease in forelimb and hindlimb grip was noted for male and female HD pups on PND

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20, and mean total and ambulatory motor activity was increased for male and female HD pups during a 60-minute observation period on PND 21. HD female pups also showed a slight decrease in maximum startle response on PND 22. The effects on grip strength, motor activity, and startle response were not statistically significantly different from control values and were not observed during the tests occurring from PND 60 to 62. These findings may be indicative of a slight developmental delay, possibly due to the smaller size of the pups. A statistically significant increase in mean Biel maze straight channel swim trial escape time was noted for HD female pups on PND 70 (6.8 sec vs. 5.0 sec for control). An increase in mean Biel maze memory escape time was also noted for HD female pups during trial number 12 (62.6 sec vs. 31.9 sec for control), which was associated with a greater number of memory errors during this trial. No effects in the Biel maze tests were noted for male pups.

Mean body weights remained lower for HD F_1 pregnant females during gestation, although body weight gains during gestation were similar to controls. There were no effects on F_1 generation mating, fertility, or reproductive organs (microscopic findings or organ weights). There were no effects on the mean number of F_2 pups born, live litter size, sex ratio, body weight, or postnatal survival (to PND 1). There were no external findings suggestive of a test article effect. On the basis of effects on body weight and food consumption for F_0 females, the NOAEL for maternal toxicity was considered to be 0.2 mg/kg. On the basis of effects on body weight and body weight gain of the F_1 pups, the NOAEL for neonatal toxicity was considered to be 0.49 mg/kg (4X clinical AUC). Because effects on memory were observed for F_1 females derived from HD dams, the NOAEL for developmental neurotoxicity was considered to be 0.49 mg/kg for females and 1.63 mg/kg for males (16X clinical AUC). The NOAEL for F_1 reproductive parameters was 1.63 mg/kg.

A summary of clinical exposure multiples achieved in the toxicology program is presented in the sponsor-generated table below. Note that the NOAEL for organ toxicity (does not include adverse effects on body weight and food consumption), carcinogenicity, and developmental toxicity are notated for each study. These values are consistent with this reviewer's evaluation of the toxicology studies.

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Table 2.4.5.3.1: Exposure Multiples for Twice Weekly SC Injection of Dulaglutide in Pivotal Toxicology Studies

Species Dose	$AUC_{0-\tau}$, steady-state (µg*h/mL)	C_{ave}	Exposure Multiple[a]
Human			
1.5 mg/week	14[b]	0.0833	-
Repeat-Dose Toxicity			
1-Month 001178-WT Mouse (Study 8224143)[c]			
100 mg/kg/twice weekly[d]	8696	90.6	1087
10 mg/kg/twice weekly	1145	11.9	143
1 mg/kg/twice weekly	99.3	1.03	12.4
1-Month Rat (Study R00359)[e]			
20 mg/kg/twice weekly[d]	1724	18.0	215
6 mg/kg/twice weekly	739	7.69	92.3
2 mg/kg/twice weekly	237	2.47	29.6
3-Month Rat (Study 7608-191)[f]			
16.29 mg/kg/twice weekly[d]	1181	12.3	148
4.89 mg/kg/twice weekly	277	2.89	35.0
1.63 mg/kg/twice weekly	124	1.29	15.5
6-Month Rat (Study 7608-236)[g]			
16.29 mg/kg/twice weekly[d]	1295	13.5	162
4.89 mg/kg/twice weekly	297	3.10	37.2
1.63 mg/kg/twice weekly	121	1.26	15.1
1-Month Monkey (Study P00054)[h]			
10 mg/kg/twice weekly[d]	3921	40.8	490
3 mg/kg/twice weekly	1072	11.2	134
1 mg/kg/twice weekly	360	3.75	45.0
3-Month Monkey (Study 7608-192)[i]			
8.15 mg/kg/twice weekly[d]	3034	31.6	379
1.63 mg/kg/twice weekly	682	7.10	85.2
0.41 mg/kg/twice weekly	155	1.61	19.4
9-Month Monkey (Study 7608-235)[j]			
8.15 mg/kg/twice weekly[d]	3792	39.5	474
1.63 mg/kg/twice weekly	718.9	7.49	89.9
0.41 mg/kg/twice weekly	205.5	2.14	25.7
Carcinogenicity			
6-Month Transgenic Mouse (Study 8224144)[k]			
3.0 mg/kg/twice weekly[l]	37.0	0.385	4.63
1.0 mg/kg/twice weekly	23.3	0.243	2.91
0.3 mg/kg/twice weekly	8.63	0.090	1.08
93-Week Rat (Study 8203405)[m]			
5.0 mg/kg/twice weekly	465	4.84	58.1
1.5 mg/kg/twice weekly	159	1.66	19.9
0.5 mg/kg/twice weekly	59.6	0.621	7.45
0.05 mg/kg/twice weekly[l]	4.06	0.042	0.508
Reproductive and Developmental Toxicity			
Rat Embryo/Fetal Development (Study (b) (4) 353116)[n]			
4.89 mg/kg/every 3 days	384.14	3.63	43.5
1.63 mg/kg/every 3 days	110.06	1.15	13.8
0.49 mg/kg/every 3 days[o]	35	0.365	4.38

Table 2.4.5.3.1: Exposure Multiples for Twice Weekly SC Injection of Dulaglutide in Pivotal Toxicology Studies (Concluded)

Species Dose	$AUC_{0-\tau}$, steady-state (µg*h/mL)	C_{ave}	Exposure Multiple[a]
Rabbit Embryo/Fetal Development (Study (b) (4) 53119)[p]			
0.41 mg/kg/every 3 days	77.94	1.08	13.0
0.12 mg/kg/every 3 days[o]	26.52	0.368	4.42
0.04 mg/kg/every 3 days	7.05	0.0979	1.18
Rat Pre/Postnatal Development (Study (b) (4) 353253)[q]			
1.63 mg/kg/every 3 days	131	1.365	16.4
0.49 mg/kg/every 3 days[o]	35.7	0.372	4.46
0.2 mg/kg/every 3 days	15.3	0.159	1.91
Mechanistic Studies			
ZDF Rat Pancreatic Morphology (Study 504758)[r]			
5.0 mg/kg/twice weekly	238	2.48	29.8
1.5 mg/kg/twice weekly[d]weekly	65.4	0.681	8.18
0.5 mg/kg/twice weekly	21.2	0.221	2.65

Abbreviations: AUC = area under the plasma concentration-time curve; C_{ave} = average concentration; GD = gestation day; LD = lactation day; NOAEL = no-observed-adverse-effect level; NOEL = no-observed-effect level.

a Exposure multiple = [(AUC_{0-96h} in animals)/96 h]/[(AUC_{0-168h} in humans)/168h].

b Geometric mean for AUC_{0-168h} from combined multiple-dose data with 1.5 mg/week of dulaglutide in T2DM subjects (Studies GBCT and GBCL: Section 2.7.2.5.3.2.5, Table APP.2.7.2.5.83).

c Plasma AUC determined on Day 29.

d NOAEL for target organ toxicity.

e Plasma AUC determined on Day 32.

f Plasma AUC determined on Day 92.

g Plasma AUC determined on Day 183.

h Plasma AUC determined on Day 28.

i Plasma AUC determined on Day 92.

j Plasma AUC determined on Day 274.

k Plasma AUC determined on Day 176.

l NOEL for neoplasia.

m Plasma AUC determined on Week 52.

n Plasma AUC determined on GD 15.

o NOAEL for developmental toxicity.

p Plasma AUC determined on GD 19.

q Plasma AUC determined on LD 4.

r Plasma AUC determined on Day 88.

Conclusions and Discussion

The toxicity profile of dulaglutide has been extensively evaluated in a standard toxicology program. One of the prominent treatment-related effects observed in animals is body weight loss or decreased body weight gain that correlates with decreased food and water consumption. This is an expected pharmacodynamic effect that is also observed in the clinic. During the first few weeks of initial treatment, patients often experience gastrointestinal discomfort, nausea, and/or vomiting. As with animals, these gastrointestinal effects are transient and usually subside within 2 or 3 weeks. Although high dose levels can lead to adverse degrees of body weight loss and dehydration in animals, these effects are not considered to be overly concerning for the clinical dose level that type 2 diabetics receive, many of whom are overweight or obese.

Increased heart rate and QTc intervals were observed in monkeys after single and repeated dosing. However, the QTc prolongation effects were observed at high multiples of the clinical C_{max} (≥75X), were not observed in the 9-month monkey study, and were not observed in a clinical thorough QT study. Therefore, QT prolongation does not appear to be a high risk for the clinical setting.

As with other long-acting GLP-1 receptor agonists, dulaglutide shows a risk for thyroid C-cell hyperplasia and tumorigenesis in rats. Other long-acting GLP-1 receptor agonists have also induced tumors in mice after 2 years of treatment, although generally at higher exposures than required for C-cell proliferation in rats. For dulaglutide, the mouse carcinogenicity study was conducted in the TgHRas transgenic model, which is a 6-month study and was agreed upon by the ECAC. Although this study was negative for proliferative effects on C-cells, it would be expected that an increase in the incidence of C-cell adenomas would be observed after 2 years of treatment in CD-1 mice at high dose levels, based on the other products in the drug class. Because rats appear to be more sensitive than mice, the use of a 6-month transgenic study rather than the typical 2 year study does not impact the ability to calculate clinical exposure margins for the NOEL in the most sensitive species.

The human relevance of GLP-1 receptor agonist-induced C-cell tumorigenesis in rodents is unknown. Many mechanistic studies have been conducted by sponsors of this drug class to investigate human relevance. Although none of these studies have definitively shown this to be a rodent-specific effect, the studies have also not indicated that humans, or non-human primates, are sensitive to the C-cell proliferative effects of GLP-1 receptor agonists. Compelling lines of evidence to support a rodent-specific effect include the following:

1) Rats have a higher percentage of C-cells in the thyroid and have a high baseline incidence of C-cell hyperplasia and adenomas, so it appears that GLP-1 receptor agonists accelerate a process that normally occurs in rats (Martin-Lacave et al., 1992, Cell Tis Res, 270:73; Bjerre Knudsen et al., 2010, Endocrinol, 151:1473). In contrast, medullary thyroid tumors are rare in humans, so enhancement of a background lesion is not expected to be overly relevant to humans;

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2) Research has shown that rodents have a higher density of GLP-1 receptor expression on thyroid C-cells (Korner et al., 2007, J Nucl Med, 48(5):736; Bjerre Knudsen et al., 2010, Endocrinol, 151:1473; Waser et al., 2011, Neuroendocrinol, 94(4):291). It has also been shown that the GLP-1 receptor is required for GLP-1 receptor agonist-induced C-cell proliferation in mice (Madsen et al., 2012, Endocrinol, 153:1538). Although it has not been determined that receptor density influences C-cell proliferation, a higher receptor density is an additional factor that may result in a rodent-specific effect;

3) Several studies have been conducted in monkeys and dogs for up to 1 year, longer in the case of liraglutide. Some of these studies, such as the one conducted for dulaglutide and reviewed herein, have involved extensive microscopic thyroid evaluation to assess for C-cell proliferation. Although 1 year of treatment is a much shorter exposure duration for a nonrodent in relation to its life expectancy compared with a 1 or 2 year study in rats, the fact that no indication of C-cell proliferation has been observed in monkeys or dogs after chronic administration is supportive of the hypothesis that rodents are more sensitive to GLP-1-induced C-cell proliferation than nonrodents.

4) Although the precise role of postprandial calcitonin secretion for bone homeostasis is uncertain, it has been suggested that this regulation may be more important for rodents compared with nonrodents because of continuous bone growth throughout life resulting in a higher rate of bone metabolism. This could imply a greater role for endogenous GLP-1 in postprandial calcitonin secretion in rodents. If this hypothesis is correct, it could explain the apparent higher degree in sensitivity for C-cell proliferation in rodents (Talmage et al., 1980, Proc Natl Acad Sci, 77(1):609; Wang, Lewin, and Olgaard, 2002, Eur J Clin Invest, 32:674; Bjerre Knudsen et al., 2010, Endocrinol, 151:1473).

Overall, human relevance to drug-induced C-cell tumors cannot be discounted and therefore a boxed warning and appropriate epidemiological monitoring continue to be warranted for these products. However, the totality of the available data does indicate that rodents are more sensitive to the C-cell proliferative effects than nonrodents and presumably humans. The sponsor's 1 year thyroid studies in rats and monkeys have contributed to this assessment and additional mechanistic studies from Lilly are not being requested at this time. If future patient data indicate an increased risk for medullary thyroid tumors, then additional nonclinical mechanistic data may be required from the sponsor at that time.

GLP-1 receptor agonists have been implicated in an increased risk for acute pancreatitis in type 2 diabetics that is primarily based on post-marketing reports. Studies in healthy animals, including the studies conducted with dulaglutide, have not shown that GLP-1 receptor agonists cause pancreatic injury or tumors, even when treated for up to 2 years at exposure multiples that are several fold higher than the clinical exposure. Studies in diabetic animal models have shown varying degrees of evidence for a drug-related effect on the pancreas in a background of type 2 diabetes. Extreme models of diabetes or premalignancy, such as the HIP rat and the $Kras^{G12D}$

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mouse model, respectively, have indicated an increased risk for GLP-1-based pancreatic toxicity and/or ductal proliferation (Matveyenko et al., 2009, Diabetes, 58:1604; Gier et al., 2012, Diabetes, 61:2195). Additionally, treatment with exenatide in healthy mice fed a high-fat diet was reported to exacerbate the incidence and severity of background pancreatic lesions (Rouse et al., 2014, Toxicol Appl Pharmacol, 276(2):104).

However, in studies conducted in ZDF rats, no definitive adverse effects on the pancreas were observed after treatment with three different GLP-1 based therapeutics for 3 months (Vrang et al., 2012, Am J Physiol Endocrinol Metab, 303(2):E253; Tatarkiewicz et al., 2013, Diabetes Obes Metab, 5:417; Forest et al., 2014, Endocrinology, 155(3):783). For dulaglutide, a 3-month study in ZDF rats did show evidence of treatment-related effects including increased incidence and/or severity of increased ductal epithelium and neutrophilic periductal inflammation at ≥3X clinical AUC, increased incidence of minimal to slight acinar atrophy, with or without mixed cell inflammation at ≥8X clinical AUC, and an increase in the incidence of minimal to slight neutrophilic acinar inflammation at 30X clinical AUC. Although these data did not indicate treatment-related pancreatitis or premalignant lesions, the data did differ slightly from the other ZDF studies. The sponsor stated that their study undertook a more thorough evaluation of the entire pancreas than previously reported, especially by including the head of the pancreas in their analysis. However, based on the individual animal data, the majority of increased ductal epithelium occurred in the pancreas body rather than the head. Also, at least one of the other studies evaluated the pancreas head, so it is not clear that methodological differences completely explain the observation of increased ductal epithelium while this was not observed in the other three ZDF studies. Taken together, the pancreatic changes observed in diabetes models may suggest that a subset of diabetic subjects taking GLP-1-based therapies could be more susceptible to morphological changes in the exocrine pancreas; however, the studies did not show compelling evidence for drug-induced pancreatitis or premalignant lesions. Currently, a robust animal model to further investigate a susceptible diabetic subpopulation has not been identified although additional work in the high-fat diet mouse may be useful. In the meantime, it will be important that post-marketing analyses collect thorough patient information for those who develop acute pancreatitis while taking GLP-1 receptor agonists in an effort to determine if there are characteristics that can identify a susceptible diabetic subpopulation.

Reference ID: 3527260

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/s/

BRIAN T HUMMER
06/18/2014

KAREN L DAVIS BRUNO
06/18/2014
concur-AP

BLA 125469
Dulaglutide
Eli Lilly and Company
45 Day Filing Meeting Checklist
NONCLINICAL PHARMACOLOGY/TOXICOLOGY

ITEM	YES	NO	COMMENT
1) Does this section of the BLA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed?	X		
2) Is this section of the BLA indexed and paginated in a manner to enable a timely and substantive review?	X		
3) Is this section of the BLA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)?	X		
4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this BLA? Please itemize the critical studies included and indicate any significant studies that were omitted from the BLA. (genotox, reprotox, adequate duration of chronic tox, carcinogenicity)	X		Key Toxicology Studies include: 6-month rat 9-month monkey Complete DART program Carcinogenicity studies 1-year studies to evaluate C-cell hyperplasia in rats and monkeys and effects on pancreas in monkeys 3-month study in Zucker diabetic fatty rats for effects on pancreas Genetic toxicology studies were not conducted because dulaglutide is a biotechnology-derived product
5) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?	X		

ITEM	YES	NO	COMMENT
6) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)?	X		
7) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	X		
8) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m2 or comparative serum/plasma AUC levels?	X		Sponsor has proposed boxed warning for risk of C-cell tumors
9) From a pharmacology/toxicology perspective, is this BLA fileable? If not, please state in item # 10 below why it is not.	X		
10) Reasons for refusal to file: None			

No comments to sponsor.

Reviewing Toxicologist: Tim Hummer, PhD, DABT
Supervisory Pharmacologist: Karen Davis-Bruno, PhD

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/s/

BRIAN T HUMMER
10/31/2013

KAREN L DAVIS BRUNO
11/01/2013

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 70,930
Review number: 3
Supporting document number: 164 and 199
Submit date: 04 June 2010 and 17 December 2010
Type of submission: Nonclinical information for corporate IND
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Eli Lilly and Company, Lilly Corporate center, Indianapolis, Indiana 46285.
Manufacturer for drug substance: [redacted] (b) (4)

Reviewer name: B. Timothy Hummer, Ph.D., DABT
Division name: Division of Metabolism and Endocrinology Products
HFD #: 510
Review completion date: 03 February 2011

Drug:

Trade name: NA
Generic name (list alphabetically): NA
Code name: LY2189265
Chemical name: NA
CAS registry number: NA
Mole file number: NA

Molecular formula/molecular weight: C2646H4044O836N704S18 (b) (4) [redacted] 59,672 Da [redacted] (b) (4).

Structure: Sequence of individual chain (molecule is a homodimer). The LY2189265 molecule as shown below, consists of two identical, disulfide-linked chains each containing an N-terminal GLP-1 variant sequence covalently linked to a human IgG4 heavy chain by a (GGGS)3 linker.

HGEGTFTSDVSSYLEEQAAKEFIAWLVKGGGGGGGSGGGGSGGGGSAESKYGPPCPPCPAPE

AAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQ

FNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM

TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG

NVFSCSVMHEALHNHYTQKSLSLSLG

Relevant INDs/NDAs/DMFs:

Exendin-4 analogs

NDA 22-200 / IND 67,092 - exenatide, BYDUREON, Amylin, once weekly formulation
NDA 21-773 / IND 57,725 - exenatide, BYETTA, Amylin
NDA 21-919 / IND 57,725 - exenatide, BYETTA, Amylin, monotherapy
IND (b) (4)
IND
IND
IND
IND
IND

GLP-1 analogs

NDA 22-341 / IND 61,040 - GLP-1 analog, liraglutide, Novo Nordisk
IND (b) (4)
IND
IND
IND
IND
IND
IND

Drug class: GLP-1 receptor agonist

Intended clinical population: Type 2 diabetes

Clinical formulation: LY2189265 is supplied for clinical trial use (b) (4) in a glass vial. The drug product is composed of LY2189265 and the inactive ingredients are sodium citrate, citric acid, sucrose, and polysorbate 80. (b) (4) The drug product is stable when stored in refrigerated conditions (2° to 8° C).

Ingredient	Quantity (mg/vial)	Function	Ingredient	Quantity (mg/vial)	Function
LY2189265	(b) (4)		Polysorbate 80	(b) (4)	
Trisodium Citrate Dihydrate			(b) (4)		
Citric Acid Anhydrous					

Route of administration: Subcutaneous

Proposed clinical protocol: NA

Previous clinical experience: The current clinical dose is 1.5 mg.

Table GBCH.7.5. Summary of Pharmacokinetic Parameters of LY2189265 Following Single and Multiple Doses of 1 and 3 mg LY2189265 (Excluding Concentrations >400 ng/mL)

Parameter	Geometric mean (CV%)			
	1 mg LY2189265		3 mg LY2189265	
	Day 1 (N=22)	Day 22 (N=21)	Day 1 (N=8)	Day 22 (N=6)
AUC(0-168) (ng.h/mL)	6410 [c] (34)	8670 (38)	22100 [d] (28)	21200 (97)
C_{max} (ng/mL)	56.3 (48)	87.2 (46)	194 (73)	185 (112)
t_{max} [a] (h)	47.58 (24.00 - 167.42)	47.58 (24.00 - 96.00)	59.81 (47.58 – 96.00)	35.79 (24.00 - 72.05)
$t_{½}$ [b] (h)	NC	139 [e] (99.8 - 216)	NC	116 [f] (86.1 - 157)
CL/F (L/h)	NC	0.115 (38)	NC	0.141 (97)
V_z/F (L)	NC	21.0 [e] (64)	NC	15.3 [f] (85)

Abbreviations: AUC(0-168) = area under the curve from time zero to 168 hours postdose, AUC(0-t_{last}) = area under the curve from time zero up to the last quantifiable concentration, CL/F = apparent clearance, C_{max} = maximum observed concentration, N = Number of subjects, NC = Not calculated, $t_{½}$ = half-life, t_{max} = time to maximum observed concentration, V_z/F = apparent volume of distribution during the terminal phase.

[a] Median (min - max) data; [b] Geometric mean (min – max) data; [c] N = 21; [d] N = 6; [e] N = 12; [f] N = 2

Disclaimer: Some tables, figures, and/or text were taken from the Sponsor's submission, where indicated.

Studies reviewed within this submission:

Study (b) (4) - Evaluation of the potency and efficacy of LY2189265 on glucose-dependent insulin secretion from rat and cynomolgus monkey pancreatic islets

Study 8216121 - A Repeat-Dose Non-GLP Pilot Toxicity Study in CD-1 Mice Given LY2189265 Twice Weekly by Subcutaneous Injection for 2 Weeks

Study 8224143 - A Repeat-Dose Toxicity and Toxicokinetic Study in 001178-W (wild type) Mice Given LY2189265 Twice Weekly by Subcutaneous Injection for 4 Weeks

Studies not reviewed within this submission: None

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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Mechanism of action:

LY2189265 binds to and activates the human glucagon-like peptide 1 (GLP-1) receptor. This is anticipated to enhance glucose-dependent insulin biosynthesis and secretion; inhibition of glucagon secretion; slowing of gastric emptying; reduction of appetite; possible regulation of pancreatic islet cell apoptosis and neogenesis; and possibly weight loss in humans.

Drug activity related to proposed indication:

LY2189265 has been shown to enhance glucose stimulated insulin secretion and decrease glucose levels in rats and monkeys following 4-5 weeks of treatment.

Pharmacology summary (from previous submissions)**:**

Mechanistic studies were conducted to examine the potential of LY2189265 to activate the GLP-1 receptor in vitro and the insulinotropic activity in vivo in rodent and non-human primate models of stepped glucose infusion (SGI). The results of these experiments demonstrate that:

- LY2189265 potently activated the human GLP- 1 receptor.
- LY2189265 increased insulin secretion in a dose-dependent fashion after a single subcutaneous administration in a rodent model of SGI.
- LY2189265 increased insulin secretion after a single subcutaneous administration in a nonhuman primate model of SGI.
- LY2189265 increased insulin secretion after multiple weekly subcutaneous administrations in a non-human primate model of SGI.
- LY2189265 decreased glucose levels in rats and monkeys

2.6.2.2 Primary pharmacodynamics

LY2189265 Glucose-Dependently Stimulates Insulin Secretion from Rodent and Primate Pancreatic Islets through Interaction with the GLP-1 Receptor (Study (b) (4) (text and figure taken from sponsor's study report)

Insulin secretion from isolated rat islets was potently (2.5- to 3-fold) enhanced by the inclusion of 3 or 30 nM LY2189265 in the presence of high (16.8 mM) glucose but not in the presence of low (2.8 mM) glucose (Figure 1A). Unmodified human GLP-1 (3 nM) produced a 4-fold enhancement of insulin secretion from rat islets elicited by 16.8 mM glucose. The EC_{50} of insulin secretion by LY2189265 was 4.2 ± 2.9 nM (4 individual experiments) with maximal stimulation (4-fold) occurring at 300 nM LY2189265 (Figure 1B; N=4). The inclusion of 1 μM of the GLP-1 receptor antagonist [9-39]exendin-4 reversed the glucose-dependent stimulation of insulin secretion induced by LY2189265, suggesting that LY2189265 acts through the GLP-1 receptor (Figure 1C).

In islets isolated from cynomolgus monkeys, LY2189265 also dose-dependently increased insulin secretion in the presence of a high glucose concentration (Fig. 1D).

LY2189265 also dose-dependently stimulated insulin secretion from INS-1 832/3 cells when stimulated by 16.8 mM glucose, with EC_{50} values of 8.3 and 34 nM in two individual experiments.

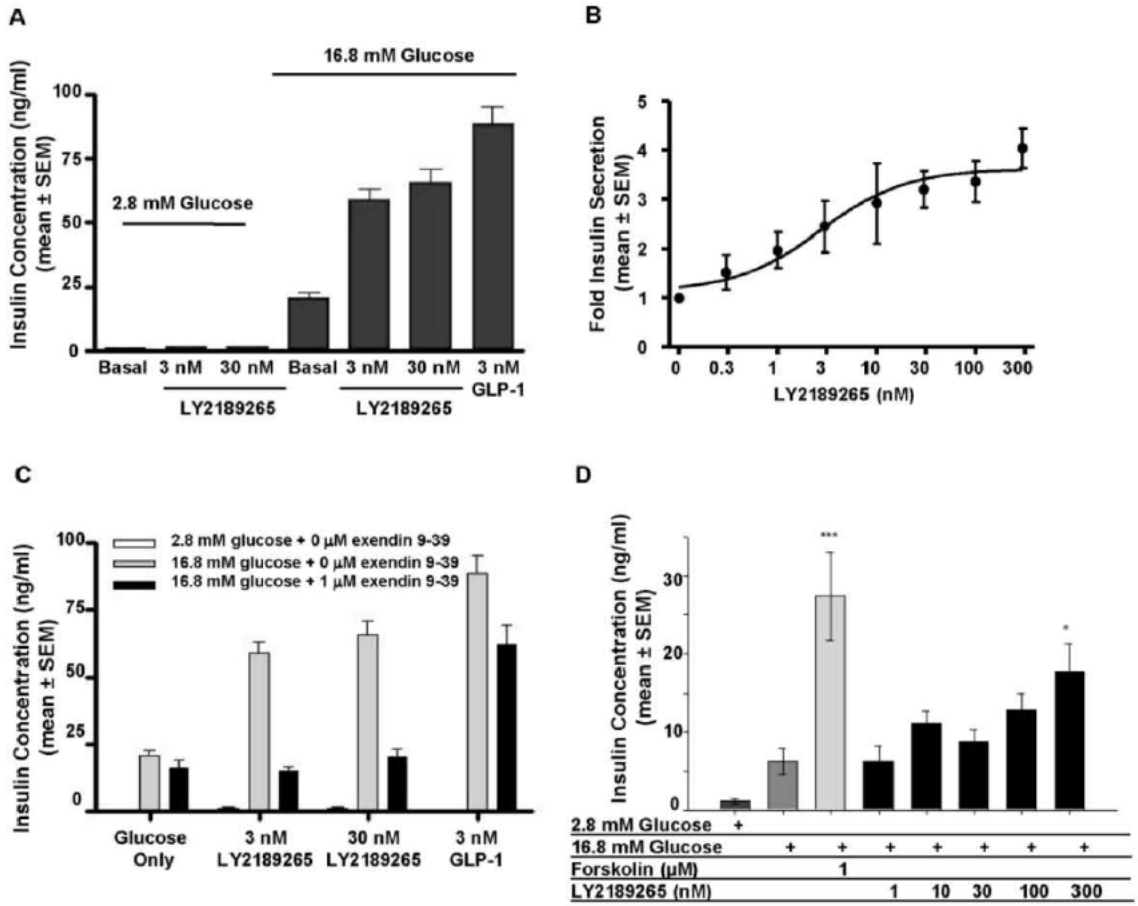


Figure 1: **LY2189265 induces glucose-dependent insulin secretion through activation of the GLP-1 receptor in rat and monkey islets.**

A: LY2189265 stimulates insulin secretion from rat islets at high (16.8 mM; right) but not at low glucose (2.8 mM; left).
B: Enhancement of insulin secretion from rat islets in the presence of 16.8 mM glucose expressed as average fold-increase for 4 individual experiments.
C: The GLP1R antagonist [9-39]exendin-4 reverses the stimulation elicited by LY2189265.
D: LY2189265 dose-dependently enhances insulin secretion in the presence of 16.7 mM glucose (*$p<0.05$ and ***$p<0.001$ vs. 16.8 mM glucose).
Values shown (panel A, C, and D) are averages ± SEM of 3-6 batches of 3 or 4 islets. The data in panels A and C are representative for two individual experiments.

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

Study title: **A Repeat-Dose Non-GLP Pilot Toxicity Study in CD-1 Mice Given LY2189265 Twice Weekly by Subcutaneous Injection for 2 Weeks** (Study #8216121)

Key Findings

- One female from the HD TK group was sacrificed moribund on Day7.
- Body weight loss occurred during the first week for MD and HD animals. Body weight gain was higher in treated animals compared with controls during the second week of treatment. Body weight effects correlated with effects on food consumption.
- A dose-related decrease in erythrocyte parameters were noted in treated animals.
- There were no adverse effects on clinical pathology parameters, organ weights, or gross pathology.
- Microscopically, an increase in **hepatocyte** mitosis and increased extramedullary hematopoiesis in the **spleen** were noted in females at all dose levels. In the **pancreas**, increased acinar cell mitosis and decreased zymogen granules were noted in MD and HD males and females. One HD male also showed minimal focal necrosis co-localized with acute inflammation. A decrease in deep dermis adipose tissue was noted at greater incidence than control at the MD and HD. Acute/subacute **subcutis** inflammation was also noted for two HD females. Two HD females also showed minimal chronic-active inflammation in the wall of the gall bladder.
- Exposures increased in a dose-related manner. Mean exposures were approximately twice as high on Day 1 compared with Day 11 for the LD and MD groups. At the HD, exposures were slightly lower on Day 11 compared with Day 1.
- Because of the moribund sacrifice of the single HD TK group female that was likely related to the severe effect on weight loss and food consumption, the NOAEL for the study is considered to be 10 mg/kg twice weekly.

Study Design:

Species/strain: Mouse/CD-1

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg LY2189265/kg)	Dose Concentration (mg LY2189265/mL)
Toxicity Animals				
1 (Control)	5	5	0	0
2 (Low)	5	5	1	0.1
3 (Mid)	5	5	10	1
4 (High)	5	5	100	10
Toxicokinetic Animals				
5 (Low)	30	30	1	0.1
6 (Mid)	30	30	10	1
7 (High)	30	30	100	10

Vehicle: (b) (4) sodium citrate (b) (4) (pH (b) (4) mannitol (w/v), and (b) (4) (w/v) polysorbate 80 prepared in Sterile Water for Injection.

Results:

Mortality and clinical signs: One compound-related mortality occurred. One TK group female given 100 mg/kg was sacrificed in a moribund condition on Day 7. This animal exhibited hunched posture, few feces, hypoactivity, squinted eyes, and thin body condition. By Day 4 of the dosing phase, the animal had lost 27.3% of its Day 1 body weight, the most of any animal given 100 mg/kg. All other animals survived to their scheduled sacrifice and no other animals exhibited adverse clinical signs.

Body weight and food consumption: As shown in the two tables below, during the first week of treatment, animals receiving the low dose gained a similar amount or slightly less weight than vehicle control animals. MD and HD animals gained no weight or lost weight. During the second week of treatment, treated animals gained more weight than control animals. The effects on weight correlated with decreased food consumption during Week 1 (especially between Days 1 and 3) and a level of food consumption that was similar to the control group by Days 11 to 14, although HD animals still had slightly lower food consumption at the end of the study (see sponsor-derived Figures 3 and 4 below). These data demonstrate that the pharmacological effect on weight and food consumption is short lived.

Body Weight Gain: Day 1 to Day 8

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Weight (g) - Day 1	34.7	25.3	33.5	25.7	34.2	25.5	32.9	25.3
Weight (g) -Day 8	35.3	26.9	34.3	26.8	32.9	25.5	29.8	23.8*
Weight gain (g)	0.6	1.6	0.8	1.1	-1.3	0	-3.1	-1.5
Diff from control (g)			0.2	-0.5	-1.9	-1.6	-5.2	-3.1
% diff from control			↑33%	↓31%	↓317%	↓100%	↓861%	↓194%

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

Body Weight Gain: Day 8 to Day 15

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Weight (g) - Day 8	35.3	26.9	34.3	26.8	32.9	25.5	29.8	23.8*
Weight (g) -Day 15	36.3	27.7	36.1	28.5	34.1	27.1	32.8	26.7
Weight gain (g)	1.0	0.8	1.8	1.7	1.2	1.6	3.0	2.9
Diff from control (g)			0.8	0.9	0.2	0.8	2.0	2.1
% diff from control			↑80%	↑113%	↑20%	↑100%	↑200%	↑263%

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

Figure 3
Mean Food Consumption Data - Males

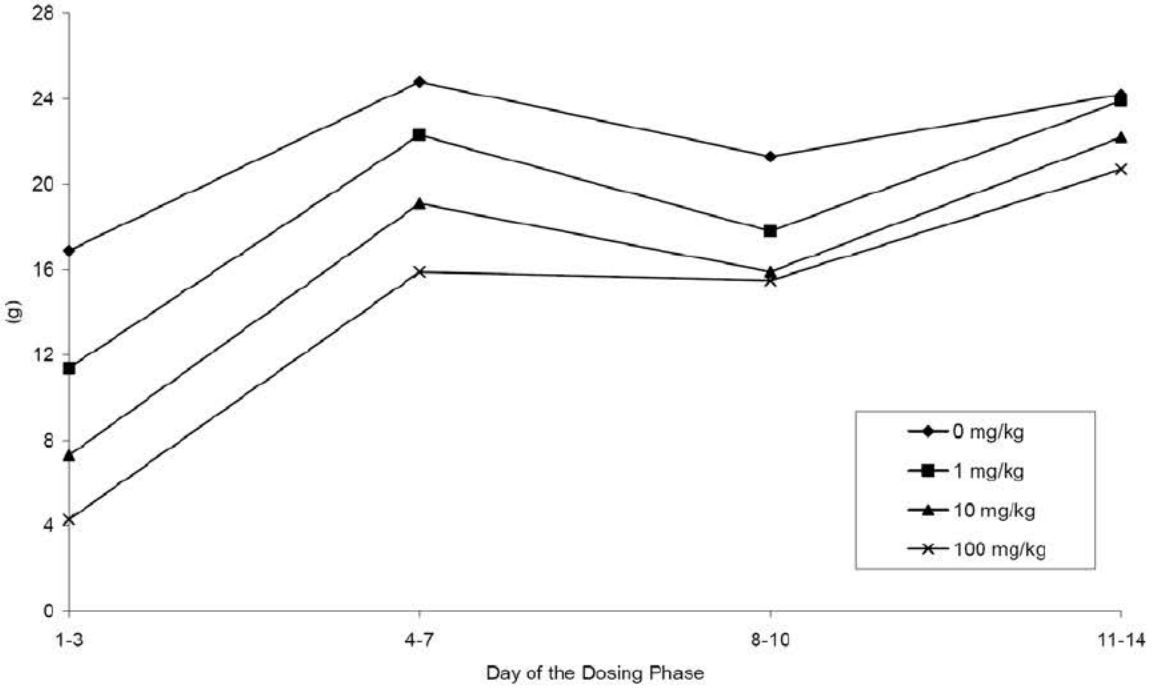
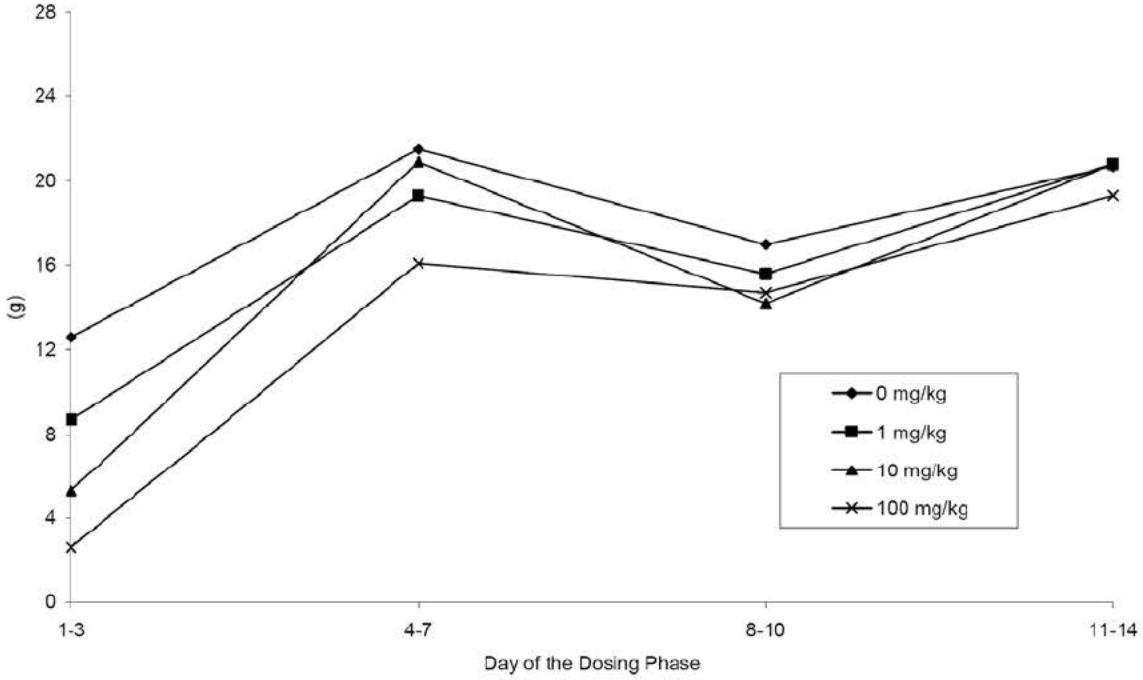


Figure 4
Mean Food Consumption Data - Females



Hematology:
A dose-related, statistically significant decrease in erythrocyte parameters (RBC, hemoglobin, hematocrit) was noted for males (up to 13%) and females (up to 18%).

Clinical chemistry:
A 23% decrease in mean glucose compared to control was noted for HD males. A similar finding was not observed for females.

Gross pathology:
There were no meaningful macroscopic findings noted.

Organ weights:
There were no meaningful changes in organ weights noted.

Histopathology: (taken from sponsor-generated table)

Controls from group(s): 1	Animal sex:	-- Animals Affected -- Males --				-- Females --			
	Dosage group:	Ctls	2	3	4	Ctls	2	3	4
Tissues With Diagnoses	No. in group:	5	5	5	5	5	5	5	5
Liver	Number examined:	5	0	0	5	5	5	5	5
	Unremarkable:	5	0	0	4	1	2	0	0
Hematopoiesis, Extramedullary		0	0	0	0	3	0	1	0
Inflammation, Acute/Subacute		0	0	0	1	0	0	0	0
Mitosis, Hepatocyte, Increased		0	0	0	0	1	3	4	5
Vacuolation, Hepatocyte, Increased		0	0	0	1	0	0	0	0
Gallbladder	Number examined:	5	0	0	5	4	5	5	5
	Unremarkable:	5	0	0	5	4	5	5	3
Inflammation, Chronic-Active		0	0	0	0	0	0	0	2
Vacuoles, Epithelium		0	0	0	0	0	0	0	1
Spleen	Number examined:	5	0	0	5	5	5	5	5
	Unremarkable:	5	0	0	5	5	4	1	2
Hematopoiesis, Extramedullary, Increased		0	0	0	0	0	1	4	3
Pancreas	Number examined:	5	5	5	5	5	5	5	5
	Unremarkable:	2	5	0	0	5	4	2	1
Hemorrhage		1	0	0	0	0	0	0	0
Infiltrate, Lymphocytes/Macrophages		2	0	0	0	0	0	0	0
Inflammation, Acute		0	0	0	1	0	0	0	0
Mitosis, Acinar Cell, Increased		1	0	4	4	0	1	2	2
Necrosis		0	0	0	1	0	0	0	0
Zymogen Granules, Decreased		0	0	5	5	0	0	3	4
Skin/Subcutis	Number examined:	5	5	5	5	5	5	5	5
	Unremarkable:	4	3	1	0	3	4	3	1
Acanthosis/Hyperkeratosis		0	0	0	0	1	0	0	0
Adipose Tissue, Decreased, Deep Dermis		1	2	4	5	1	1	2	3
Congestion/Hemorrhage		0	0	0	0	1	0	0	0
Erosion/Ulcer		0	0	0	0	1	0	0	0
Exudate		0	0	0	0	1	0	0	0
Infiltrate, Neutrophils		0	0	0	0	0	0	0	1
Inflammation, Acute/Subacute, Subcutis		0	0	0	0	0	0	0	2
Inflammation, Chronic-Active		0	0	0	0	1	0	0	0

Histopathology summary (sponsor-generated table):

Text Table 1
Incidence and Severity of Compound-Related Microscopic Findings

	LY2189265 mg/kg							
Sex	Males				Females			
Dose Level	0	1	10	100	0	1	10	100
No. of Animals	5	5	5	5	5	5	5	5
Liver								
Mitosis, Hepatocyte, Increased								
Minimal	0	0	0	0	1	1	0	1
Slight	0	0	0	0	0	2	3	2
Moderate	0	0	0	0	0	0	1	2
Spleen								
Hematopoiesis, Extramedullary, Increased								
Minimal	0	0	0	0	0	1	4	3
Pancreas								
Mitosis, Acinar Cell, Increased								
Minimal	1	0	4	4	0	1	2	2
Zymogen Granules, Decreased								
Minimal	0	0	5	5	0	0	3	4
Skin/Subcutis								
Adipose Tissue, Decreased, Deep Dermis								
Minimal	1	2	4	5	1	1	2	3

Toxicokinetics:

Mean Plasma Toxicokinetic Data Summary

	Administered Dose (mg/kg)					
	1		10		100	
Parameter Sex	M	F	M	F	M	F
Day 1						
C_{max} (µg/mL)	3.60	3.41	26.2	21.8	145	152
$AUC_{0\text{-}96\,h}$ (µg*Hours/mL)	119	112	961	1000	7340	6930
Day 11						
C_{max} (µg/mL)	1.29	1.84	15.1	22.4	165	252
$AUC_{0\text{-}96h}$ (µg*Hours/mL)	52.1	56.4	578	546	5690	6600

Abbreviations: M = male, F = female, C_{max} = maximum observed plasma concentration, h = hour, $AUC_{0\text{-}96\,h}$ = area under the plasma concentration curve.

Study title: A Repeat-Dose Toxicity and Toxicokinetic Study in 001178-W (wild type) Mice Given LY2189265 Twice Weekly by Subcutaneous Injection for 4 Weeks

Key study findings:

- There were no unscheduled deaths. One HD male and 2 HD females were noted as being hunched on Day 4.
- Mean body weight gain was statistically significantly decreased at the MD and HD. This effect was most severe during the first week of treatment, which correlated with decreased food consumption. After Week 1, weight gain was still decreased for MD and HD animals compared with controls, but not to as great of extent as during Week 1. This is consistent with food consumption being more similar to controls after Day 4.
- Slight dose-related decreases in erythrocyte parameters (up to 12%) were noted for males. A decrease in reticulocytes was noted for all treated animals, up to 47% for HD females.
- Increased mean **AST** (↑270%) and **ALT** (↑272%) were noted for HD females, which primarily due to increases for 2/5 animals. A slight, statistically significant increase ALP was noted for MD (↑23%) and HD (↑37%) males.
- Statistically significant decreases in absolute and relative **liver weights** were noted for MD and HD animals. Statistically significant decreases in absolute and relative **heart weights** were noted for MD males and females and HD females. Statistically significant decrease in absolute and relative **uterus weights** was noted for HD females.
- Microscopically, decreased vacuolation (glycogen content) was noted in the **liver** for MD and HD animals; decreased granulation of the granular secretory duct epithelium of the mandibular **salivary gland** was noted for HD females; decreased zymogen granules in the **pancreas** was noted for MD and HD males; decreased dilation and increased atrophy were noted in the **uterus** of MD and HD females; and increased inflammation and erosion/ulceration was noted at the **injection site** for MD males and females.
- Exposure increased with dose in a near linear fashion. Mean exposures were higher on Day 29 compared with Day 1 suggesting either accumulation because of the long half life or possibly because of antibody production (observed with other GLP-1R agonists). However, Ab production was not assessed because the pharmacological effect on body weight gain and food consumption was maintained throughout the dosing period.
- Most findings appeared to be secondary to decreased body weight gain, which is an expected pharmacological effect of the drug. Although increases in AST and ALT were observed, adverse microscopic findings were not identified in the liver. In the absence of definitive drug-induced systemic toxicity, the NOAEL for this study was considered to be 100 mg/kg twice weekly. This dose level resulted in mean $AUC_{0\text{-}96}$ values of 9292 µg·h/mL in males and 8100 µg·h/mL in females on Day 29.

Study no.: 8224143
Volume # and page #: NA, electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 02 June 2010
GLP compliance: Yes
QA statement: yes (X) no ( )
Drug, lot #, and % purity: LY2189265, Lot #PP0105-09 (drug substance), Lot #17804-1 (final bulk API), 87.7% pure, 40.3 mg/mL potency/content
Formulation: (b) (4) citrate (pH (b) (4) and (b) (4) polysorbate 80 (the process description for formulating the drug does not mention that mannitol was added, but it is present in the vehicle control).
Vehicle control: (b) (4) citrate (pH (b) (4)), (b) (4) mannitol (w/v), and (b) (4) polysorbate 80.

Methods

Species/strain: Mouse/ Model 001178-W, CByB6F1-Tg(HRAS)2Huc (wild type for Tg.rasH2 transgenic)

Doses levels and group numbers:

Group	Number of Animals Male	Number of Animals Female	Dose Level (mg LY2189265/kg/dose)[a]	Dose Concentration (mg LY2189265/mL)[a]
Toxicity Animals				
1 (Control)[b]	10	10	0	0
2 (Low)	10	10	1	0.1
3 (Mid)	10	10	10	1
4 (High)	10	10	100	10
Toxicokinetic Animals[c,d]				
5 (Control)[b]	8	8	0	0
6 (Low)	38	38	1	0.1
7 (Mid)	38	38	10	1
8 (High)	38	38	100	10

a The dose volume was10 mL/kg. Concentrations were corrected for lot specific test article content.
b Animals in Groups 1 and 5 received only (b) (4) sodium citrate (b) (4) (pH (b) (4) mannitol (w/v), and (b) (4) (w/v) polysorbate 80, prepared in sterile water for injection, which served as the vehicle for LY2189265 formulations.
c Toxicokinetic animals were included solely for the purpose of blood sample collections.
d Groups 6 through 8 included 2 animals/sex for immunogenicity analysis.

Number/sex/group (recovery): None
Route and regimen: subcutaneous, twice weekly
Dosing volume: 10 mL/kg
Age: 7 weeks
Weight: 21.4 to 27.2 g (males) and 17.4 to 23.6 g (females)
TK sampling times (2 mice/sex/timepoint):

Control - predose and 24 hours postdose on Days 1 and 29
Treated - predose, 4, 12, 24, 48, and 96 hours postdose on Days 1 and 29 and at 24 hours postdose on Days 8, 15, and 22.

Ab sampling times: at 96 hours postdose on Day 29 (2/sex for Groups 6, 7, and 8)
Analysis of dosing preparations: Week 1 preparations were assessed for concentration. The results showed that concentrations were within 1% of target.
Unique study design or methodology (if any):

Observation and Times:
Clinical signs: Twice daily
Detailed exams: Once weekly
Body weights: Twice weekly

Food consumption: Twice weekly (quantitatively)
Ophthalmoscopy: Predose (all animals) and during Week 5 (toxicity animals)
EKG: NA
Hematology: First 5 animals per group from nonfasted toxicity animals
Clinical chemistry: Second 5 animals per group from nonfasted toxicity animals
Urinalysis: Not conducted
Gross pathology: All toxicity animals
Organ weights: All toxicity animals; see histopathology table at end of section
Histopathology: Control and high-dose toxicity animals only, except for the following organs that were examined for all groups based on findings at the high dose; see histopathology table at end of section for tissue collection list
Females: liver and uterus
Males: liver and pancreas

Adequate Battery: yes ( ), no ( )—explain
Peer review: yes (X), no ( ), performed by sponsor on 3 mice/sex from control and high-dose groups. Additionally, liver and pancreas from all mice and ovary, uterus, cervix, and vagina from all female mice in all dose groups were examined.

Results:

Mortality: There were no unscheduled deaths.

Clinical signs: One HD male and 2 HD females were noted as being hunched on Day 4. Mice from the LD (1 male and 1 female) and the HD groups (4 males and 5 females) had sores/scabs on the dorsal abdomen (back), whereas no control or HD animals were noted as having this finding.

Body Weight Gain: Day 1 to Day 32

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Weight (g) - Day 1	24.7	19.6	24.6	20.1	24.5	19.2	24.1	19.4
Weight (g) -Day 32	27.8	23.3	27.7	23.9	25.5*	20.3*	24.5*	19.6*
Weight gain (g)	3.1	3.7	3.1	3.8	1.0*	1.1*	0.4*	0.2*
Diff from control (g)			0	0.1	-2.1	-2.6	-2.7	-3.5
% diff from control			-	↑3%	↓68%	↓70%	↓87%	↓95%

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

Body Weight Gain: Day 8 to Day 32

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Weight (g) - Day 8	25.4	20.5	25.4	21.5	24.0	19.1	22.3	18.0
Weight (g) -Day 32	27.8	23.3	27.7	23.9	25.5*	20.3*	24.5*	19.6*
Weight gain (g)	2.4	2.8	2.3	2.4	1.5	1.2	2.2	1.6
Diff from control (g)			-0.1	-0.4	-0.9	-1.6	-0.2	-1.2
% diff from control			↓4%	↓14%	↓38%	↓57%	↓8%	↓43%

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

Figure 1
Mean Body Weight - Males

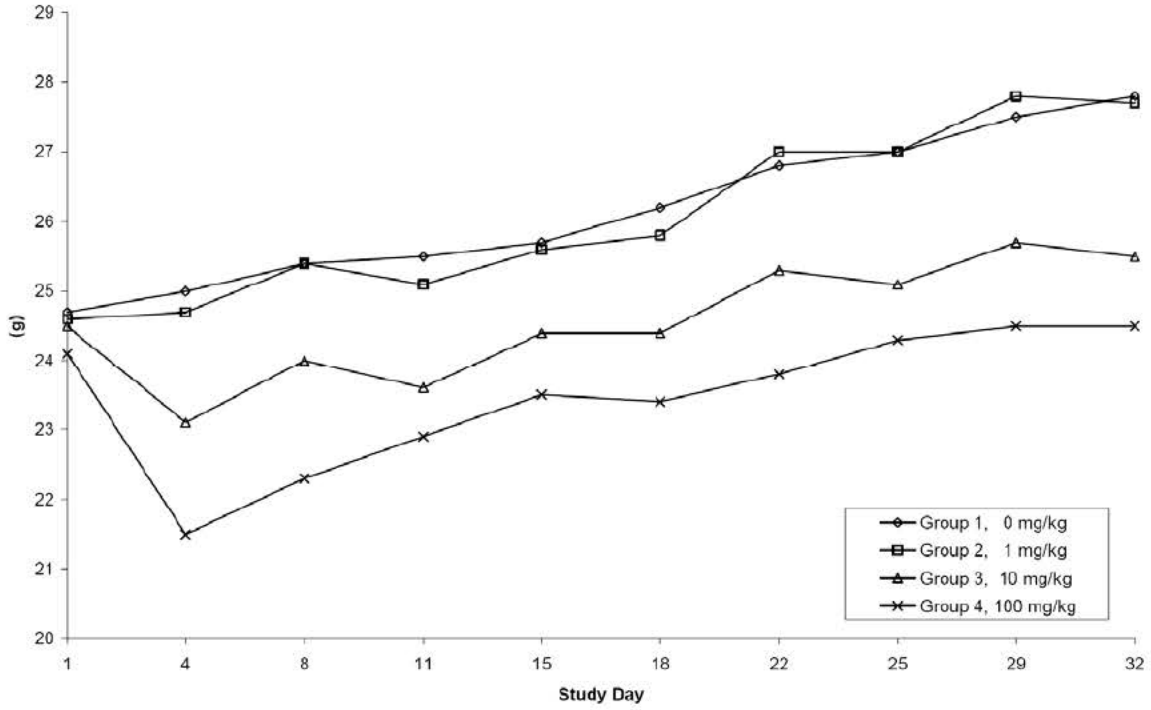


Figure 1
Mean Body Weight - Males

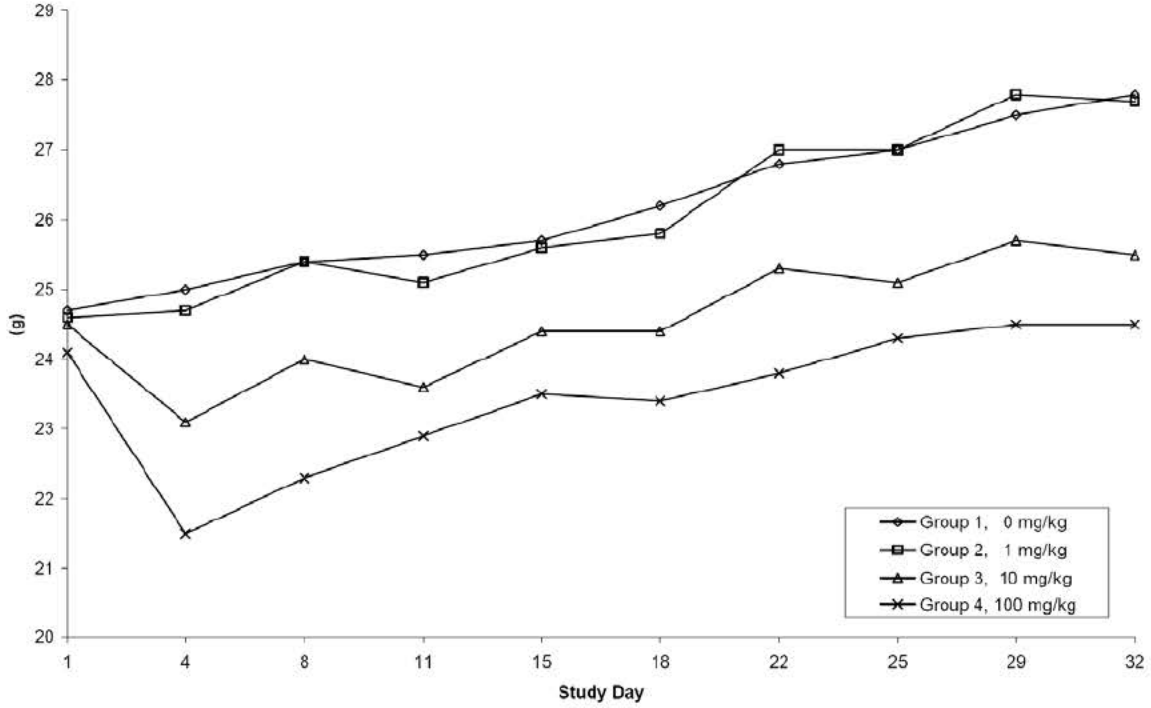
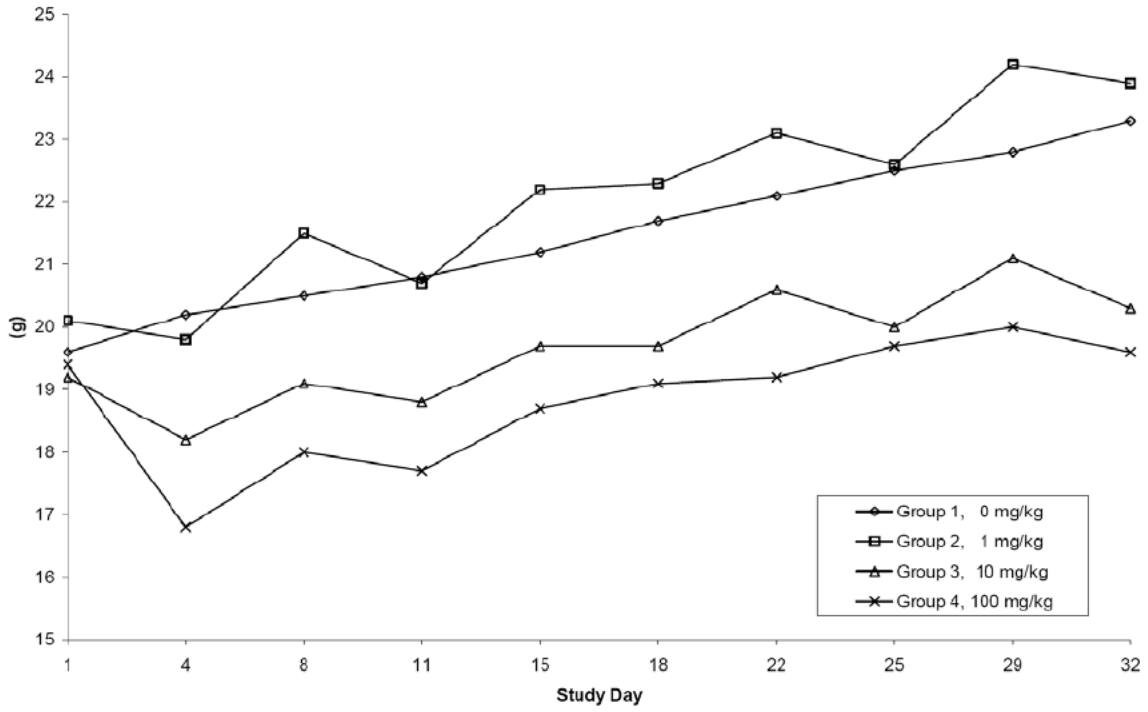


Figure 2
Mean Body Weight - Females



Food consumption: Day 1 to Day 3

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Daily intake (g/mouse)	15.9	13.5	11.9*	9.9*	9.0*	7.8*	6.4*	6.1*
Diff from control (g)			-4.0	-3.6	-6.9	-5.7	-9.5	-7.4
% diff from control			↓25%	↓27%	↓43%	↓42%	↓60%	↓55%

F = female; M = male.
*p≤0.05

Food consumption: Day 4 to Day 31

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Daily intake (g/mouse†)	18.8	17.5	18.7	18.1	18.1	16.8	16.3	14.0*
Diff from control (g)			-0.1	0.6	-0.7	-0.7	-2.5	-3.5
% diff from control			↓1%	↑3%	↓4%	↓4%	↓13%	↓20%

F = female; M = male.
*p≤0.05
†mean of the eight 3 or 4-day food measurement periods between Days 4 and 31.

Figure 3
Mean Food Consumption - Males

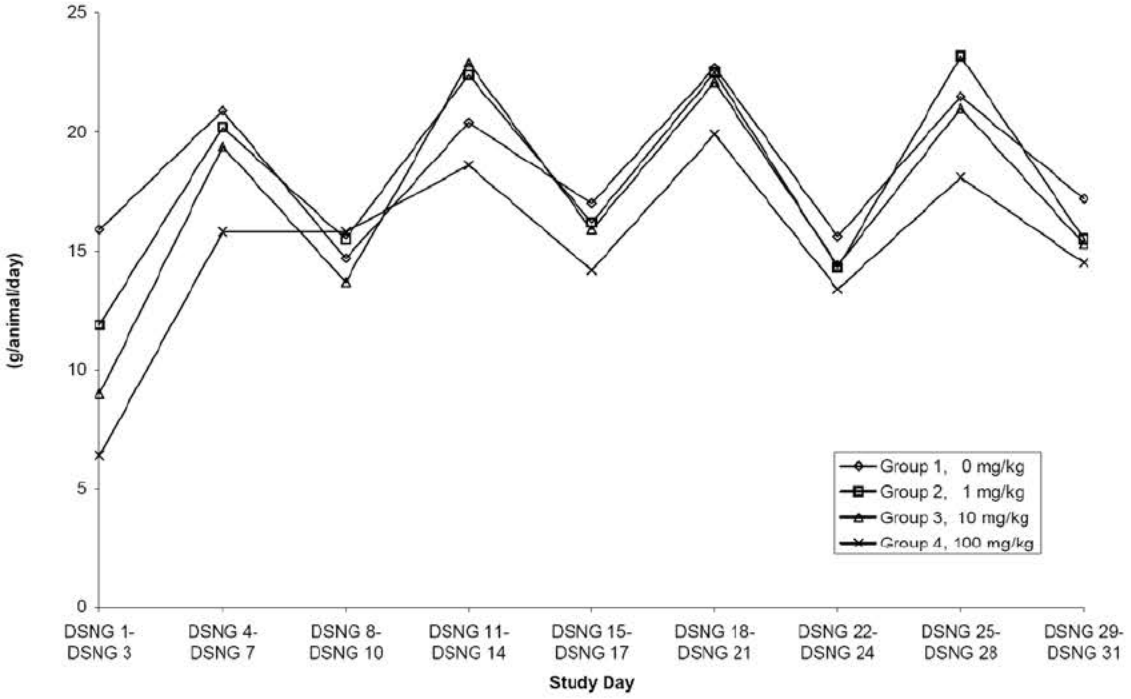
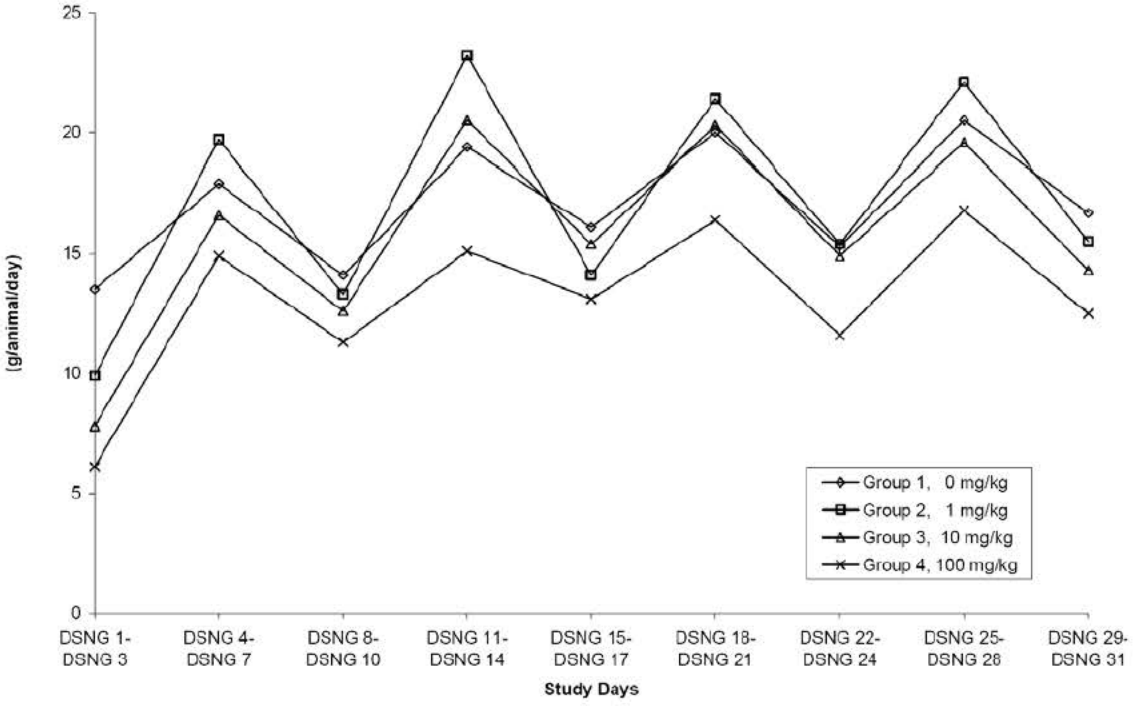


Figure 4
Mean Food Consumption – Females



Ophthalmoscopy: There were drug-related findings noted.

EKG: Not applicable

Hematology:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Red blood cells ($1\times10^6/\mu L$)	10.60	9.84	9.81*	9.27*	9.71*	9.33*	9.55* (↓10%)	9.81
Hemoglobin (g/dL)	16.0	15.3	14.8*	14.3*	14.6*	14.2*	14.1* (↓12%)	14.8
Hematocrit (%)	51.0	50.2	48.3	46.5	47.5	46.0*	46.5* (↓9%)	47.9*
Mean cell hemoglobin concentration (g/dL)	31.5	30.5	30.7	30.8	30.8	30.9	30.5 (↓3%)	31.0
Reticulocytes ($1\times10^3/\mu L$)	312.3	329.9	244.7*	250.1	248.8	201.6*	250.4 (↓20%)	176.3* (↓47%)
White blood cells ($1\times10^3/\mu L$)	3.61	1.76	3.09	2.68	2.80	4.00*	2.12 (↓41%)	2.22
Neutrophils ($1\times10^3/\mu L$)	0.46	0.28	0.59	0.39	0.42	0.66*	0.30 (↓35%)	0.42
Lymphocytes ($1\times10^3/\mu L$)	2.97	1.36	2.28	2.11	1.75*	3.03*	1.65* (↓44%)	1.63

*$p\leq0.05$; F = female; M = male.

Clinical chemistry:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Glucose (mg/dL)	190	199	140	182	138	138	160	176
AST† (U/L)	-	44	-	53	-	50	-	163*† (↑270%)
ALT† (U/L)	-	18	-	26	-	19	-	67† (↑272%)
ALP (U/L)	82	-	96	-	101* (↑23%)	-	112* (↑37%)	-

*$p<0.05$; **$p<0.01$; "-" = no noteworthy difference from control; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; F = female; M = male.
†Mean increase due to individual increase for 2/5 animals

Gross pathology:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Uterus								
-Distended		1/8		4/5		1/5		0/9
-Thickened		1/8		0/5		0/5		0/9

Organ weights:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Liver/gall bladder (g)	1.4385	1.2171	1.4532	1.2924	1.1937*	1.0562*	1.1103* (↓23%)	0.9402* (↓23%)
Rel. to BW	5.2531	5.3589	5.1962	5.2595	4.5822*	4.8827	4.4632* (↓15%)	4.7282* (↓12%)
Rel. to BrW	288.417	234.171	294.947	260.062	245.746*	219.168	233.565* (↓19%)	196.064* (↓16%)
Heart (g)	0.1832	0.1669	0.1791	0.1543	0.1512*	0.1372*	0.1646	0.1261* (↓31%)
Rel. to BW	0.6702	0.7353	0.6402	0.6306*	0.5801*	0.6389*	0.6623	0.6349* (↓14%)
Rel. to BrW	36.7373	32.0828	36.4112	31.0742	31.1364*	28.5711	34.6535	26.3539* (↓18%)
Uterus (g)		0.1876		0.2417		0.1660		0.1057* (↓44%)
Rel. to BW		0.8252		0.9823		0.7642		0.5329 (↓35%)
Rel. to BrW		36.1524		48.6480		34.5791		22.2431 (↓38%)

*p<0.05; **p<0.01; "-" = no noteworthy difference from control; F = female; M = male; NA = not applicable.

Histopathology:

Dose (mg/kg)	0		1		10		100	
Sex	M	F	M	F	M	F	M	F
Liver -Decreased vacuolation (glycogen content)	0/10	0/10	0/10	0/10	**2/10**	**2/10**	**5/10**	**3/10**
Salivary gland (mandibular) -Decreased granulation; granular secretory duct epithelium	0/10	0/10	NE	NE	NE	NE	0/10	**2/10**
Pancreas -Decreased zymogen granules	0/10	0/10	0/10	NE	**5/10**	NE	**6/10**	0/10
Uterus -Dilation -Atrophy	NA	4/10 0/10	NA	6/10 0/10	NA	1/10 2/10	NA	1/10 4/10
Subcutan site -Acute inflammation -Erosion/ulceration -Serocellular exudate	0/10 0/10 0/10	1/10 0/10 0/10	NE	NE	2/3 1/3 1/3	2/2 2/2 2/2	0/10 0/10 0/10	0/10 0/10 0/10

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

Toxicokinetics:

Parameter	Administered Dose (mg/kg)					
	1		10		100	
Sex	M	F	M	F	M	F
LY2189265						
Day 1						
Cmax (µg/mL)	2.87	2.87	27.2	24.5	180	170
$AUC_{0\text{-}96hr}$ (ug·hr/mL)	62.0	62.4	724	604	6385	5379
SEM $AUC_{0\text{-}96hr}$ (ug·hr/mL)	2.06	3.01	18.0	7.98	1359	1162
Day 29						
Cmax (µg/mL)	3.25	2.70	32.5	21.5	264	193
$AUC_{0\text{-}96hr}$ (ug·hr/mL)	118	80.6	1394	896	9292	8100
SEM $AUC_{0\text{-}96hr}$ (ug·hr/mL)	5.05	2.07	43.0	89.1	584	415

Immunogenicity:
Samples collected for the assessment of anti-drug antibodies were discarded without analysis because analysis was not warranted based on the exposure data.

Histopathology inventory for IND #70,930

Study	**8224143**
Species	**Mouse**
Adrenals	X
Aorta	X
Bone Marrow Smear	X†
Bone (femur)	X
Brain	X*
Cecum	X
Colon	X
Duodenum	X
Epididymides	X*
Esophagus	X
Eyes	X
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	
Heart	X*
Ileum	X
Injection sites	X
Jejunum	X
Kidneys	X*
Lachrymal gland	
Larynx	
Liver (weighed with gallbladder)	X*
Lungs	X
Lymph nodes, axillary	X
Lymph nodes, mesenteric	X
Mammary Gland (females only)	X
Nasal cavity	
Optic nerves	X
Ovaries	X*
Pancreas (longitudinal section)	X
Peyer's patches	X
Pharynx	
Pituitary	X
Prostate	X*
Rectum	
Salivary gland (mandibular)	X
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle (biceps femoris)	X
Skin	X
Spinal cord (cervical, thoracic, and lumbar)	X
Spleen	X*
Sternum with bone marrow	X
Stomach	X
Testes	X*
Thymus	X*
Thyroid + parathyroid	X
Tongue	X
Trachea	X
Ureters	
Urinary bladder	X
Uterus + cervix	X*
Vagina	X
Zymbal gland	

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

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:
The sponsor conducted two range-finding studies in mice. In one study, CD-1 mice were administered LY2189265 by subcutaneous injection twice weekly for 2 weeks and in the other study, wild type 001178-W mice were administered LY2189265 by subcutaneous injection twice weekly for 4 weeks to aid in the dose selection for a 6-month carcinogenicity study in Tg.rasH2 transgenic mice (001178-T hemizygous). The primary drug-related effect was body weight loss during the first week of dosing followed by decreased body weight gain that correlated with decreases in food consumption. This effect was most noteworthy during the first week of dosing. The effect on food and water consumption likely resulted in the moribund sacrifice of a TK group female receiving 100 mg/kg on Day 7 of the 2-week study and the observation of hunched posture at 100 mg/kg on Day 4 of the 4-week study. After the first week of treatment, body weight gain rebounded to a greater degree in CD-1 mice than in 001178-W mice.

In the 2-week study, minimal to moderate increased hepatocyte mitosis and minimal increased pancreatic acinar cell mitosis was noted for females treated with ≥1 mg/kg and in males and females treated with ≥10 mg/kg, respectively. These findings were not noted in the 4-week study. One HD male also showed minimal focal necrosis co-localized with acute inflammation; this finding was also not observed in the 4-week study. Because this finding only occurred in one animal, it is uncertain whether the necrosis and inflammation was drug related. In the 4-week study, mean AST and ALT were increased by nearly 4-fold in females receiving 100 mg/kg. Correlative microscopic findings were not observed and this observation was not noted in the 2-week study. The slight effect on erythrocyte parameters has also been observed with several other GLP-1R analogs and is not considered adverse. Other organ weight and microscopic observations were likely secondary to decreased body weight gain. Differences in findings between the two studies are likely due to the difference in mouse strains that were used for the two studies.

Safety Margins: (sponsor-generated table)

Table B 1: Exposure Multiples for Subcutaneous Administration of LY2189265 Based on Systemic Exposure of 001178-W (wild type) Mice for 1-Month

Species Dose	C_{max} (µg/mL)	$AUC_{0-\tau hr}$ (µg•hr/mL)	Exposure Multiple[a]
Human[b] Phase 3 clinical dose (1.5 mg)	0.08	10.6	-
Mouse[c] Lowest Dose Tested (1 mg/kg)	2.98	99.1	9.3
Mouse[c] Mid-Dose Tested (10 mg/kg)	27.0	1145	108
Mouse[c] Highest Dose Tested (100 mg/kg)	229	8696	820

Abbreviations: AUC = area under the plasma concentration versus time curve; C_{max} = maximal observed plasma concentration.

a Exposure multiple is the $AUC_{0-96\ hr}$ in animals/$AUC_{0-168\ hr}$ in humans .

b Plasma area under the concentration time curve (AUC) shown was mean $AUC_{(0-168hr)}$ during a dosing interval at steady-state for 1.5 mg estimated based on population PK model using data collected following multiple weekly doses ranging from 0.05 mg to 12 mg in men and sterile women from all available Phase 1 and Phase 2 (study GBCJ) studies.

c Mean (M+F) plasma toxicokinetics determined on Day 29 in 1-month toxicology study (8224143).

Internal comments:
The dose level of 100 mg/kg twice weekly represents the MTD due to significant effects on body weight and food consumption after 4 weeks. Although body weights do slightly recover after the first week of dosing, this dose level is considered to be too high for the carcinogenicity study because of the effect on body weight. Therefore, the mid-dose level of 10 mg/kg is recommended as the high dose for the 6-month carcinogenicity study. Additional study design recommendations for the 6-month carcinogenicity study will be discussed in the information provided to the ECAC (see attachment).

External comments (to sponsor):
Comments were conveyed to sponsor via the ECAC meeting minutes.

ATTACHMENTS

Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet

Review of Carcinogenicity Study Design/Dose Selection Proposals

Application (IND/NDA) number: 70,930
Submission date and number: 17 December 2010, #199
Division: Metabolism and Endocrinology Products
Project manager: Ray Chiang
CAS#: NA
Drug name: LY2189265
Pharmacological Classification: Long-acting GLP-1 receptor agonist
Clinical dosing regimen: Once weekly subcutaneous injection
Sponsor/Applicant: Eli Lilly
Sponsor/Applicant contact name: Sharon Myers
Sponsor/Applicant telephone and fax number: 317-276-1609 (p); 317 276-1652 (f)
Date submitted (stamp date): 17 December 2011
45-day date (from submission stamp date): 31 January 2011
P/T Reviewer(s): Tim Hummer
Date Review Completed: 14 January 2011
Date of CAC review: 25 January 2011
CAC members: David Jacobson-Kram (chair), Abby Jacobs, Paul Brown, Linda Fossom

Summary of Proposal for Review:

Species/strain: Mouse/ 001178-T (hemizygous)Tg rasH2 for carcinogenicity group
001178-W (wild type) for TK group
Number/sex/dose: 25
Route: Subcutaneous

	male	**female**
Doses proposed (mg/kg twice weekly):	0, 0.1, 1, 10	0, 0.1, 1, 10

Positive control: single IP dose of MNU at 75 mg/kg

Basis of dose selection:		
MTD	___X__	___X__
AUC ratio	_____	_____
saturation	_____	_____
MFD	_____	_____
PD	_____	_____
other	_____	_____

Kinetics submitted:	**rodent**	**human**
pharmacokinetics	___X__	___X__
metabolism	_____	_____
protein binding	___	_____

Notable design features:

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg LY2189265/kg)[a]	Dose Concentration (mg LY2189265/mL)[a]
Carcinogenicity Animals				
1 (Control)[b]	25	25	0	0
2 (Low)	25	25	0.1	0.01
3 (Mid)	25	25	1	0.1
4 (High)[c]	25	25	10	1
5 (Positive Control)[c]	25	25	75	7.5
Toxicokinetic Animals[d]				
6 (Control)[b]	12	12	0	0
7 (Low)	42	42	0.1	0.01
8 (Mid)	42	42	1	.01
9 (High)	42	42	10	1

a The dose volume will be 10 mL/kg. Concentrations will be corrected for lot specific test article content.
b Animals in Groups 1 and 6 will receive the vehicle control article only.
c Group 5 animals will be dosed with one intraperitoneal dose of MNU, on Day 1 of study at a dose level of 75 mg/kg/mouse and a dose volume of 10 mL/kg.
d Toxicokinetic animals included solely for the purpose of blood sample collections.

Vehicle: (b) (4) sodium citrate (b) (4) pH (b) (4) mannitol, (b) (4) (w/v) polysorbate 80, prepared in sterile water for injection.

Age: 7-9 weeks at initiation of dosing
Weight: 15-35 g
TK sampling times (3/sex/time point; 1 time point per mouse):
Control: Pre-dose and 24 hours post-dose (Weeks 1 and 26)
Treated: Pre-dose and 4, 12, 24, 48, and 96 hours post-dose (Weeks 1, and 26) and pre-dose and 24 hours post-dose (Week 13)
Ab sampling times: Collected, but only analyzed if warranted (Week 26 - TK group animals).
Analysis of dosing preparations: Samples to be collected during Weeks 1, 14, and 26 for concentration verification. Concentrations to be within ±10% of nominal value for acceptance.
General observations: Twice daily
Detailed observations: Day 1, weekly during study, and day of necropsy
Body weights: Before dosing on Day 1, twice weekly (before dosing) thereafter
Food consumption: Twice weekly (quantitative)
Clinical chemistry: Day of sacrifice, all carcinogenicity animals, if sufficient blood volume available
Ophthalmology: Once during pre-dose phase and during Week 26
Gross pathology: Exam of external features of the carcass; external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues (all main study animals and TK animals with unscheduled death)
Organ weights: Brain, epididymis, heart, kidney, liver (w/ gallbladder), spleen, testes
Histopathology: All carcinogenicity animals and all unscheduled sacrifices and deaths
Macroscopic lesions and thymus glands from positive control animals
Tissues to be preserved in 10% neutral buffered formalin, except where noted otherwise
Bone marrow smears to be collected from femur for possible future examination
Slides to be stained with H & E
Calcitonin immunohistochemistry of thyroid
Adequate Battery: Yes
Peer review: Yes

Summary of Recommendations to CAC

	male	**female**
Doses recommended by reviewer:	0, 0.1, 1, 10	0, 0.1, 1, 10

Basis for recommendation (details): Note: information in the cover sheet given to the ECAC prior to the meeting and in the ECAC meeting minutes sent to the sponsor state that two range-finding studies were conducted in 001178-W mice. However, the 2-week study was conducted in CD-1 mice. This error has been corrected in the text below.

A 2-week study in CD-1 mice and a 1-month range-finding study in 001178-W (wild type) mice were conducted. Animals were administered LY2189265 using twice weekly subcutaneous injections at doses of 1, 10, and 100 mg/kg. The results of the range-finding studies show that the proposed high dose for the carcinogenicity study (10 mg/kg) resulted in body weight loss during the first week of dosing that correlated with decreased food consumption. Decreased body weight gain and food consumption is a known pharmacological effect of GLP-1R agonists. After Week 1, body weight gains exceeded controls in the 2-week study (CD-1 mice) but remained lower than controls in the 4-week study (001178-W mice) with mean body weight gains being 38% and 57% less for male and female 10 mg/kg-group animals and 8% and 43% less for male and female 100 mg/kg-group animals, respectively. In the 2-week study, one TK group female was sacrificed moribund on Day 7, possibly due to excessive effects on body weight and food consumption. An increase in minimal to moderate hepatocellular mitosis in the absence of other liver findings was noted at 100 mg/kg in the 2-week study and an approximate 4-fold increase in mean ALT and AST were noted for 100 mg/kg females in the 4-week study with no correlative microscopic lesions.

It is felt that the effect on body weight at 100 mg/kg (820X clinical exposure) exceeds the acceptable limit for a carcinogenicity study. Additionally, one moribund sacrifice occurred at this dose in the 2-week study (CD-1 mouse), which was likely drug related. The effect on body weight at 10 mg/kg (~100X clinical exposure) may be slightly greater than ideal (70% less body weight gain over the 4-week period), however body weight gains did rebound slightly after the first week. The next lowest dose that was tested (1 mg/kg; ~8X clinical exposure) did not induce a sufficient pharmacological response or toxicity, and therefore is not considered to be an appropriate high dose for the carcinogenicity study. The proposed carcinogenicity study low dose of 0.1 mg/kg is near clinical exposure. Other GLP-1R agonists have shown an increase in thyroid c-cell tumors at or near clinical exposure. Therefore, this reviewer agrees with the sponsor's proposed doses because the degree of body weight effect at the high dose is considered to be at the upper limit for a carcinogenicity study and the low dose is near the clinical exposure, which will potentially help ensure that a NOEL for c-cell tumors is identified, based on data from other compounds in this class.

CAC Concurrence (y/n): No

CAC Recommendations:
The Committee felt that the extent of decreased body weight gain observed at 10 mg/kg was too great. The Committee had the following recommendations:

- The Committee recommended changing the LY2189265 doses to 0.3, 1, and 3 mg/kg by twice weekly subcutaneous injection, based on MTD (reduced body weight gain).
- The Committee recommended the addition of a saline control group in addition to the planned vehicle control group.
- Accordingly, the Committee recommended the following treatment groups: 0 (vehicle), 0 (saline), 0.3, 1, 3 mg/kg LY2189265, and positive control.

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

/s/

BRIAN T HUMMER
02/03/2011

KAREN L DAVIS BRUNO
02/04/2011

Carcinogenicity Assessment Committee (CAC/CAC-EC) Cover Sheet
Review of Carcinogenicity Study Design/Dose Selection Proposals

Application (IND/NDA) number: IND 70,930
Submit date and supporting document number: 24 June 2009, #124
Division: DMEP
Project manager: Lina AlJuburi
CAS#: NA
Drug name: LY2189265
Pharmacological Classification: GLP-1 receptor agonist (LY2189265 consists of two identical, disulfide-linked chains each containing an N-terminal GLP-1 variant sequence covalently linked to a human IgG4 heavy chain by a (GGGS)3 linker; ~60kD)
Sponsor/Applicant: Eli Lilly
Sponsor/Applicant contact name: Sharon Myers
Sponsor/Applicant telephone and fax number: (317) 276-1606 / (317) 276-1652
Date submitted (stamp date): 24 June 2009
45-day date (from submission stamp date): 08 August 2009
P/T Reviewer(s): Tim Hummer
Date Review Completed: 28 July 2009
Date of CAC review: 04 August 2009
CAC members: Drs. Todd Bourcier, Paul Brown, and David Jacobson Kram

Summary of Proposal for Review:

Species/strain: Rat/Sprague-Dawley
Number/sex/dose: 60
Route: Subcutaneous

	male	**female**
Doses proposed: (twice weekly)	0, 0.05, 0.15, 1.5, 5 mg/kg	0, 0.05, 0.15, 1.5, 5 mg/kg

(Note: clinical dosing will be once weekly; however AUC comparisons are based on $AUC_{0\text{-}96h}$ values to be consistent with the sponsor's calculations. Based on these calculations, the exposure margins are likely underestimated)

Basis of dose selection: (the sponsor stated that the high dose was justified based on both effects on body weight and predicted exposure multiples to the maximum clinical dose).

MTD	_____	_____
AUC ratio	X	X
Saturation	_____	_____
MFD	_____	_____
PD	_____	_____
Body weight	X	X

Kinetics submitted:	**rodent**	**human**
pharmacokinetics	X	X
metabolism	_____	_____
protein binding	___	_____

Notable design features:

GROUP DESIGNATION AND DOSE LEVELS

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg LY2189265/kg)[a]
1 (Control)[b]	60	60	0
2 (Low)	60	60	0.05
3 (Mid)	60	60	0.15
4 (Mid-High)c	60	60	1.5
5 (High)	60	60	5
6 (Control)[b]	6	6	0
7 (Low)	12	12	0.05
8 (Mid)	12	12	0.15
9 (Mid-High)	12	12	1.5
10 (High)	12	12	5

a The dose volume will be 1 mL/kg. Concentrations will be corrected for lot specific test article content.
b Animals in Groups 1 and 6 will receive the control article only.
c Toxicokinetic animals included solely for the purpose of blood sample collections. Three animals/sex/group will serve as replacement animals, if needed.

Age: At least 4 weeks but no more than 8 weeks at initiation of dosing
Weight: 100 to 300 g
TK sampling times (3/sex/time point; 2 time points per rat):
Control: Pre-dose and 24 hours post-dose (Week 13 and 52)
Treated: Pre-dose and 4, 12, 24, 48, and 96 hours post-dose (Week 13 and 52)
Ab sampling times: **Currently not planned**
Analysis of dosing preparations: Samples to be collected during Weeks 1, 13, 27, 40, 52, 66, 78, and 92 of the dosing phase. Concentrations to be within ±10% of nominal value (or ±15% for concentrations <0.001 mg/mL) for acceptance.
General observations: Twice daily
Detailed observations: Day 1, weekly during study, and day of necropsy
Body weights: Before dosing on Day 1, weekly thereafter, and at necropsy (fasted)
Food consumption: Weekly (quantitative)
Clinical pathology: Limited to blood films made as part of the necropsy procedure
Gross pathology: Exam of external features of the carcass; external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues (all main study animals and TK animals with unscheduled death)
Organ weights: Not planned
Histopathology: Unscheduled and scheduled sacrifices and deaths from main study groups
Tissues to be preserved in 10% neutral buffered formalin, except where noted otherwise
Bone marrow smears to be collected from femur for possible future examination
Slides to be stained with H & E
Adequate Battery: Yes
Peer review: Yes

Summary of Recommendations to CAC

	male	**female**
Doses recommended by reviewer:	0, (0.05), 0.15, 1.5, 5 mg/kg	0, (0.05), 0.15, 1.5, 5 mg/kg

Note 1: low dose is optional - the sponsor has proposed a fourth treatment group.

Note 2: In the 3- and 6-month study reports, the dose levels are listed as 0, 2, 6, and 20 mg/kg. However, the sponsor has since determined that the actual dose levels were 0, 1.63, 4.89, and 16.89 mg/kg. For simplicity and consistency, dose levels for those studies will be discussed as 0, 2, 5, and 17 mg/kg.

Basis for recommendation (details):
Dose levels for the rat carcinogenicity study were considered based on both treatment-related decreased body weights as well as AUC ratios between the exposures achieved in the rat and human studies. At the end of the 6-month treatment period, body weights were 14% and 16% less than controls for males and females treated with 5 mg/kg twice weekly, respectively. Weight effects with GLP-1 receptor agonists typically are most noteworthy during the first 3 to 4 weeks, after which time weight gain decrements tend to stabilize. The proposed high-dose level also provides an exposure margin that is greater than 25 times higher than the anticipated human exposure for the 1.5 mg dose. Because of the difference in dosing regimen (twice weekly for rats and once weekly for humans), the $AUC_{0\text{-}96h}$ comparison underestimates the true exposure margin. The sponsor states that 1.5 mg has been selected as the highest clinical dose for their Phase 3 studies. Exposure margins for the 3 mg dose are also included to show that a 25-fold exposure margin also exists for this dose level (b) (4)

Summaries of the key toxicology findings in the 3- and 6-month rat studies are presented below.

General Toxicology Studies (reviews are attached)

3-Month Study in Sprague-Dawley Rats
LY2189265 was administered twice weekly by subcutaneous injection to Sprague-Dawley rats for 3 months (27 total doses) at doses of 2, 5, and 17 mg/kg. The key study findings are summarized below.

- There were no test article-related deaths.
- Decreased feces, thinness, and rough haircoat were noted for HD males and females.
- Body weights were statistically significantly decreased from control values at all dose levels for both males (↓15% to 21%) and females (↓10% to 13%). The effects on body weight were generally associated with decreased food consumption.
- A statistically significant decrease in reticulocytes (up to 30% decrease) was noted for males at all doses. A slight decrease was also noted for HD females.
- Absolute and relative mean testis weights were slightly decreased by 4% to 5% in HD males.
- Microscopically, minimal to slight degeneration of the seminiferous tubules (2/10), slight inflammation of the epididymis (1/10), and minimal hypospermia (1/10) were noted for HD males.
- Exposure increased in an approximate dose-proportional manner and no significant accumulation was noted after repeated dosing.

- Because no overt toxicity was observed, the NOAEL was determined to be the high dose level of 20 µg/kg twice weekly; at this dose level, the mean C_{max} and $AUC_{0\text{-last}}$ at Day 92 were 32.6 µg/mL and 1450 µg·h/mL, respectively.

6-Month Study in Sprague-Dawley Rats

LY2189265 was administered twice weekly by subcutaneous injection to Sprague-Dawley rats for 6 months (with 1 month recovery) at doses of 2, 5, and 17 mg/kg. The key study findings are summarized below.

- One mid-dose female was sacrificed moribund on Day 78. Pathology findings indicated that the moribund condition was due to urinary inflammation/calculi and renal pyelonephritis, which was considered an incidental finding.
- Abnormal clinical signs were limited to few feces, thin, and rough haircoat, which were more pronounced at the mid- and high-dose levels.
- A dose related decrease in body weight was noted for all treated animals that correlated with decreased food consumption, an expected pharmacological effect. Decreases in mean body weight ranged from 7% to 18% for males and 16% to 19% for females. Once dosing was halted, high-dose recovery animals gained considerably more weight than controls during the recovery period, demonstrating both reversibility of the weight effects and a durable pharmacodynamic effect during the dosing period.
- Slight decreases in mean reticulocyte counts were noted for all male dose groups, high dose females, and the high-dose female recovery group.
- Slight decreases in mean glucose (high-dose males), cholesterol (high-dose females), and triglycerides (high-dose males and females) were noted.
- Dose-dependent decreases in organ weights were considered a secondary effect of the decrease in body weights.
- Microscopic findings noted at the high dose included a slight increase in focal congestion/hemorrhage of the stomach (2/15 males, 1/15 females) and dilatation of the uterus (6/15). An increase in the number of animals with bile duct proliferation was noted for the high-dose recovery males (2/10).
- Although Ab production was not assessed in this study, the fact that exposure data were the same or greater on Day 183 compared with Day 1 and signs of pharmacological activity (decreased mean body weights and decreased mean food consumption) were noted during the last week of the study suggests that sufficient drug exposure was maintained throughout the study.
- Because decreased body weight and food consumption are expected pharmacological effects and did not produce adverse outcomes with regard to overall animal health, the NOAEL of this study was considered to be 20 mg/kg. At the NOAEL, the mean C_{max} and $AUC_{0\text{-last}}$ at Day 183 were 43.7 µg/mL and 1590 µg·h/mL, respectively.

Treatment-related decreased body weight associated with decreased food consumption was the key toxicological finding for the toxicology studies. A summary of treatment-related body weight effects is shown in the table below.

Treatment-Related Effects on Body Weight in Rats

Dose (mg/kg/d)	0		2		6		20	
Sex	M	F	M	F	M	F	M	F
3-month study								
Body weight on Day 92 (g)	614	313	519*	282*	516*	287*	485*	271*
% difference from control			↓15%	↓10%	↓16%	↓8%	↓21%	↓13%
6-month study								
Body weight on Day 183 (g)	642	358	597*	299*	555*	299*	527*	290*
% difference from control			↓7%	↓16%	↓14%	↓16%	↓18%	↓19%

* $p \leq 0.05$ (ANOVA)

Note: body weight vs. time figures can be found in the individual study reviews attached at the end of this document.

Margins between Rat and Human Exposures (key exposure margins are bolded for emphasis)

Dose (mg/kg/d)	2	6	20
Sex	M + F	M + F	M + F
Clinical Dose Level: 1.5 mg ($AUC_{0\text{-}96h}$ = ~6.11 µg·h/mL)			
3-month study			
Day 92 $AUC_{0\text{-}last}$ (µg·h/mL)	**152**	**340**	1450
Exposure Margin*	**25X**	**56X**	237X
6-month study			
Day 183 $AUC_{0\text{-}last}$ (µg·h/mL)	**148**	**365**	1590
Exposure Margin*	**24X**	**60X**	260X
Clinical Dose Level: 3 mg ($AUC_{0\text{-}96h}$ = ~14.4 µg·h/mL)			
3-month study			
Day 92 $AUC_{0\text{-}last}$ (µg·h/mL)	152	340	1450
Exposure Margin*	11X	24X	101X
6-month study			
Day 183 $AUC_{0\text{-}last}$ (µg·h/mL)	148	365	1590
Exposure Margin*	10X	25X	110X

*Based on mean $AUC_{0\text{-}96h}$ values. The sponsor estimated that 63% of the steady state $AUC_{0\text{-}168h}$ value (~9.96 µg·h/mL for the 1.5 mg dose level and 22.9 µg·h/mL for the 3 mg dose level) represents the $AUC_{0\text{-}96h}$ value. Although 3 mg has been extensively studied in the clinic, the sponsor has stated that 1.5 mg will be used for all upcoming Phase 3 studies. Because of the difference in dosing regimen, exposure margins are likely underestimated.

Sponsor's Estimated Exposure Multiples for the Proposed Carcinogenicity Study Dose Levels

Table B 2: Predicted Exposure Multiples for Subcutaneous Administration of Proposed Doses of LY2189265 Based on Systemic Exposure

Species Dose	$AUC_{0\text{-}96\ hr}$[a] (ng*hr/mL)	Exposure Multiple[b]
Human[c] Phase 3 clinical dose (1.5 mg)	6109	-
Rat Lowest Proposed Dose (0.05 mg/kg)	3709	0.6
Rat Low-Mid Proposed Dose (0.15 mg/kg)	12090	1.9
Rat Mid-High Proposed Dose (1.5 mg/kg)	120900	20
Rat Highest Proposed Dose (5.0 mg/kg)	297440	48

Abbreviations: AUC = area under the plasma concentration x time curve;

[a] Predicted AUC values based on linearity of exposure and previous data from Study 7608-236 (6-month repeat-dose study in rats).

[b] Predicted exposure multiple is the predicted $AUC_{0\text{-}96\ hr}$ in animals/$AUC_{0\text{-}96\ hr}$ in humans.

[c] Plasma pharmacokinetics shown were dose-proportionality model estimated and adjusted to steady-state AUC(0-96hr) (approximately 63% of steady-state AUC(0-168hr)) based on data collected following multiple weekly doses ranging from 0.05 mg to 8 mg in men and sterile women (Study GBCD). Dose shown is the highest proposed clinical dose for Phase 3 trials.

Antibody Formation:
The sponsor did not evaluate whether anti-LY2189265 antibodies were produced in the toxicology studies performed to date. Instead, the sponsor has relied upon sustained markers of pharmacological activity (i.e., decreased food consumption and decreased body weight gain) to indicate the absence of neutralizing activity. Dose-dependent compound-related changes in food and body weights occurred throughout the 6-month study with changes in body weight following a distinct biweekly pattern of decreased weight gain or weight loss in males and females for the three-day interval following administration of the second of the two weekly doses. Body weight gains in treated animals for the four-day interval following the first weekly dose were generally comparable or greater than controls. Moreover, the 6-month data show a clear rebound in body weight gain during the recovery period for the high-dose group indicating that the pharmacological activity of LY2189265 was not being neutralized at 6 months.

Overview of LY2189265 Pharmacology, ADME, and Genotoxicity

Mechanism of action: LY2189265 is a modified GLP-1 molecule covalently linked to a human IgG4 heavy chain. LY2189265 binds to and activates the GLP-1 receptor. Receptor binding with endogenous GLP-1 enhances glucose dependent insulin biosynthesis and secretion, inhibition of glucagon secretion, slowing of gastric emptying, reduction of appetite, and the possible regulation of pancreatic islet cell apoptosis and neogenesis. LY2189265 is anticipated to have the similar pharmacodynamic activity as endogenous GLP-1.

Drug activity related to proposed indication:
LY2189265 has been shown to enhance glucose-stimulated insulin secretion in rats and monkeys.

Metabolism:
Because LY2189265 is a biotechnology-derived protein product, classical biotransformation studies have not been conducted. The elimination of LY2189265 is expected to be through the degradation into small peptides and individual amino acids.

Protein binding:
Protein binding has not been determined.

Genotoxicity:
Because LY2189265 is a biotechnology-derived product, genetic toxicology studies were not conducted per the ICH S6 guidance document.

Reviewer Questions for ECAC :

1. Because the sponsor relied on pharmacodynamic endpoints in the 3- and 6-month studies, is it acceptable for them to use these endpoints for the carcinogenicity study in lieu of assessing for neutralizing antibodies?

ECAC response: A pharmacodynamic endpoint demonstrating sustained drug activity is actually preferred to a neutralizing antibody assay.

2. Does the committee agree that a second carcinogenicity study is justified for this product?

ECAC response: Yes

Sponsor Questions for the Division:
Sponsor Question #1
Does FDA agree with the design and dose selection of the 2-year carcinogenicity study in rats with LY2189265?

Proposed FDA Response: Yes, with the following recommendations.

- If the sponsor chooses to rely on pharmacodynamic endpoints as an indication for non-neutralizing activity, it is recommended that body weights be taken twice weekly as conducted in the 6-month study so that the durability of the distinct biweekly pattern of decreased weight gain/weight loss can be tracked throughout the study. If this approach is taken it is recommended that blood samples be stored for future antibody analysis, if needed, in case it is found that the pharmacodynamic activity diminishes over the course of the study.

Sponsor Question #2
In accordance with the ICH S6 guideline "Nonclinical safety evaluation of biotechnology-derived pharmaceuticals" and as agreed with FDA during the 17 November 2004 pre-IND meeting with Lilly, Lilly is planning to conduct a carcinogenicity study in a single species (rat) to support

registration of LY2189265. Lilly believes a rat carcinogenicity study is sufficient to evaluate the potential of LY2189265 to produce tumors. Lilly is aware of benign and malignant thyroid c-cell tumors in rodents administered another long-acting GLP-1 analog in the 2-year carcinogenicity studies. Rats appeared to be more sensitive since these findings occurred at low multiples of human exposure while in mice these occurred at somewhat higher exposures. Furthermore, in the 2-year carcinogenicity studies with the GLP-1 analog Byetta®, benign thyroid c-cell adenomas were observed in female rats at all doses while there were no effects in mice.
Does FDA agree with the plan to conduct a single carcinogenicity study in rats to support registration with LY2189265?

Proposed FDA Response: No, as the sponsor indicated in their dose selection package, other long-acting GLP-1 receptor agonists have induced thyroid C-cell adenomas and carcinomas in both rats and mice, for which the relevance to humans is currently unknown. This potential risk to humans was not known at the time of the pre-IND meeting. Although it appeared that rats were more sensitive than mice based on exposure for one particular GLP-1 receptor agonist, this assumption should not be made for all compounds in this class. For LY2189265, the GLP-1 molecule has been significantly modified through the recombinant addition of a human IgG4 heavy chain. Therefore, it should not be assumed that the tumor-inducing properties, with regard to potency, species selectivity, or target tissue, will be the same as for other compounds in this class. Accordingly, a carcinogenicity study in mice will be required to fully assess the tumorigenic potential of LY2189265.

ECAC Concurrence (y/n): The ECAC agreed with the proposed high dose, but did make other study design recommendations.

ECAC Recommendations: In addition to the reviewer's recommendation above, the Committee also had the following comments:

- The Committee recommends doses of 0, 0.05, 0.5, 1.5, and 5 mg/kg by twice weekly subcutaneous injection, based on AUC ratios, raising the sponsor's proposed low mid-dose from 0.15 to 0.5 mg/kg.
- The Committee notes that if the clinical dose changes such that the ratio is no longer at least 25 fold above the clinical exposure, the study may not be acceptable.
- The Committee notes that if the sponsor prefers to use only three dose groups that the 0.05 mg/kg group may be dropped.
- The Committee recommends the addition of a saline control group in addition to the planned vehicle control group.

Comments:
Also refer to the ECAC meeting minutes that were sent to the sponsor.

ATTACHMENTS:
The reviews for the 3- and 6-month rat studies are attached below.

Study title: A repeat-dose toxicology and toxicokinetic study in Sprague Dawley rats given LY2189265 twice weekly by subcutaneous injection for a total of 27 doses

Key study findings:

- There were no test article-related deaths.
- Decreased feces, thinness, and rough haircoat were noted for HD males and females.
- Body weights were statistically significantly decreased from control values at all dose levels for both males (↓15% to 21%) and females (↓10% to 13%). The effects on body weight generally associated with decreased food consumption.
- A statistically significant decrease in reticulocytes (up to 30% decrease) was noted for males at all doses. A slight decrease was also noted for HD females.
- Absolute and relative mean testis weights were slightly decreased by 4% to 5% in HD males.
- Microscopically, minimal to slight degeneration of the seminiferous tubules (2/10), slight inflammation of the epididymis (1/10), and minimal hypospermia (1/10) were noted for HD males.
- Exposure increased in an approximate dose-proportional manner and no significant accumulation was noted after repeated dosing.
- Because no overt toxicity was observed, the NOAEL was determined to be the high dose level of 20 µg/kg twice weekly; at this dose level, the mean C_{max} and $AUC_{0\text{-last}}$ at Day 92 were 32.6 µg/mL and 1450 µg·h/mL, respectively.

Study no.: 7608-191
Volume #, and page #: Vol. 1 (SN 029); page 1
Conducting laboratory and location: (b) (4)
Date of study initiation: 19 June 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: LY2189265, Lot # (b) (4) C1301-78, 57.6 mg/mL
Formulation/vehicle: Trisodium citrate dehydrate, citric acid anhydrous, sodium chloride, and polysorbate 80.

Group Designations and Dose Levels

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg LY2189265/kg/dose)[a]	Dose Concentration (mg LY2189265/mL)[a]
Toxicity Animals				
1 (Control)[b]	10	10	0	0
2 (Low)	10	10	2	2
3 (Mid)	10	10	6	6
4 (High)	10	10	20	20
Toxicokinetic Animals[c]				
5 (Control)[b]	5	5	0	0
6 (Low)	11	11	2	2
7 (Mid)	11	11	6	6
8 (High)	11	11	20	20

a The dose volume will be 1 mL/kg.
b Animals in Groups 1 and 5 will receive the control article only.
c Toxicokinetic animals included solely for the purpose of blood sample collections. Two animals/sex/group will serve as replacement animals, if needed.

Methods

Doses: 0 (vehicle), 2, 6, and 20 mg/kg (actual doses were later determined to be 1.63, 4.89, and 16.89 mg/kg)
Species/strain: Rat / Sprague Dawley
Number/sex/group (main study): 10/sex/group
Route and volume: Subcutaneous twice weekly; 1 mL/kg
Satellite groups used for recovery: No Recovery Groups
Satellite groups used for toxicokinetics: 3/sex/group (control) or 9/sex/group
TK sampling times: Days 1 and 92 – predose (Day 92 only) and 4, 12, 24, 48, and 96 hours postdose.
Ab sampling times: **Immunogenicity not assessed**
Age: 6 to 7 weeks
Weight: 218 to 279 g (males); 161 to 213 g (females)
Unique study design or methodology (if any):

Observation and Times:

Clinical signs: Cage side - twice daily; Detailed observations – predosing, Day 1, and once weekly thereafter.
Body weights: Predosing and before each dose administration
Food consumption: Twice weekly
Ophthalmoscopy: Pre-dose and once during the final week of dosing
EKG: Not conducted

Hematology: Weeks 4 and 13

Hematology

red blood cell (erythrocyte) count
hemoglobin
hematocrit
mean corpuscular volume
mean corpuscular hemoglobin
mean corpuscular hemoglobin concentration
platelet count
white blood cell (leukocyte) count
differential blood cell count
blood cell morphology
reticulocyte count

Coagulation (scheduled collections only)

prothrombin time
activated partial thromboplastin time

Clinical chemistry: Weeks 4 and 13

Clinical Chemistry

glucose
urea nitrogen
creatinine
total protein
albumin
globulin
albumin/globulin ratio
cholesterol
triglycerides
total bilirubin
alanine aminotransferase
alkaline phosphatase
gamma glutamyltransferase
aspartate aminotransferase
creatine kinase
calcium
inorganic phosphorus
sodium
potassium
chloride

Urinalysis: Weeks 4 and 13

Urinalysis

appearance/color
volume
specific gravity
pH
protein
glucose
ketones
bilirubin
blood
microscopic examination of sediment
urobilinogen

Gross pathology: Conducted on all main study animals

Organ weights: Conducted on all main study animals

adrenal (2)
brain
heart
kidney (2)
liver
ovary (2)
pituitary gland
prostate
spleen
testis (2)
thymus
thyroid (2 lobes) with parathyroid
uterus

Histopathology: Conducted on all main study animals
Adequate Battery: yes (X), no ( )—explain - typical organs not evaluated included: lachrymal gland and rectum.
Peer review: yes (X), no ( ) - all tissues from the 1st 3 animals of both sexes from control and high dose groups.

adrenal (2)
aorta
brain
cecum
cervix
colon
duodenum
epididymis (2)[a]
esophagus
eye (2)[a]
femur with bone marrow (articular surface of the distal end)
Harderian gland[a]
heart
ileum
injection site(s)
jejunum
kidney (2)
lesions
liver
lung with large bronchi
lymph node (mandibular)
lymph node (mesenteric)
mammary gland
ovary (2)
optic nerve (2)[a]
pancreas
pituitary gland
prostate
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle
skeletal muscle (thigh)
skin/subcutis
spinal cord (cervical, thoracic and lumbar)
spleen
sternum with bone marrow
stomach
testis (2)[a]
thymus
thyroid (2 lobes) with parathyroid
tongue
trachea
urinary bladder
uterus
vagina

a Preserved in modified Davidson's fixative.

Results:

Mortality: Two deaths occurred in the high-dose female group during or following blood collection during week 4, which were not considered to be test article-related because they coincided with clinical pathology blood sampling. There were no significant macroscopic or microscopic findings noted in these 2 animals.

Clinical signs: Decreased feces (10/10 M; 10/10 F), thin (1/10 M; 9/10 F), and rough haircoat (4/10 M; 3/10 F) were noted in high dose animals and decreased feces (2/10 F) was noted at the mid dose.

Body weights: Decreased body weight gain was noted in all test article-treated groups.

Dose (mg/kg)	0		2		6		20	
Sex	M	F	M	F	M	F	M	F
Day 1 (g)	244	181	244	181	243	183	244	179
Day 92 (g)	614	313	519*	282*	516*	287*	485*	271*
Weight gain (g)	370	132	275*	101*	273*	104*	241*	92*
Final weight - diff from control (g)	-	-	-95	-31	-98	-26	-129	-42
Final weight - % diff from control	-	-	↓15%	↓10%	↓16%	↓8%	↓21%	↓13%

* $p \leq 0.05$ (ANOVA)

Figure 1: Mean body weight data - males.

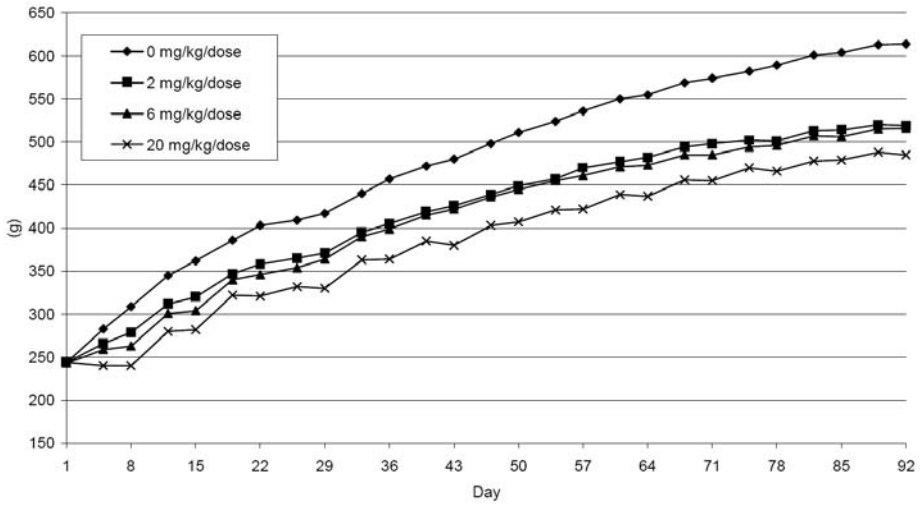
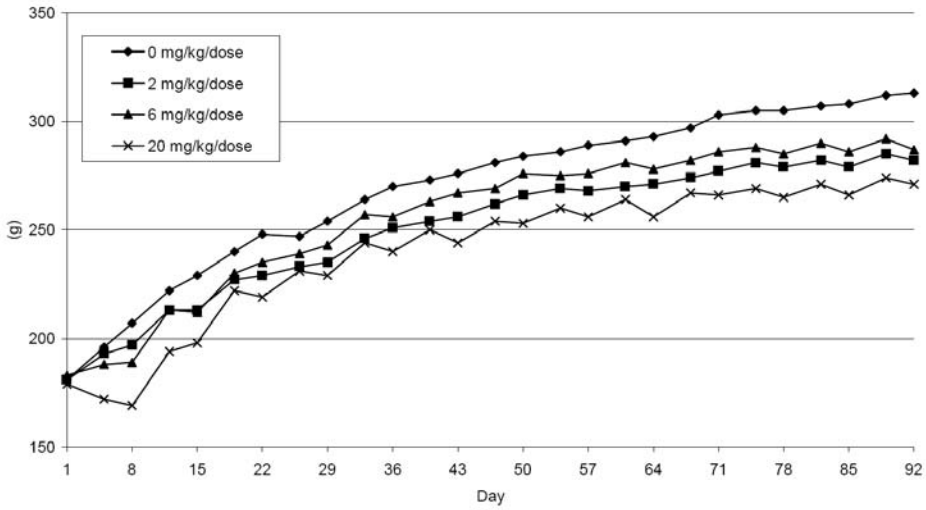


Figure 2: Mean body weight data - females.



Food consumption: Decreased food consumption was observed in all test article-treated groups.

	% Change from Control					
	Males			Females		
mg LY2189265/kg/dose	2	6	20	2	6	20
Days 1-4 (4-day interval)	↓23*	↓30*	↓49*	↓21*	↓33*	↓52*
Days 5-7 (4-day interval)	↓25*	↓37*	↓51*	↓21*	↓32*	↓51*
Days 85-88 (3-day interval)	↓12*	↓12*	↓13*	NS	NS	NS
Days 89-91 (4-day interval)	↓18*	↓15*	↓17*	NS	NS	↓14*

Abbreviations: ↓ = decrease; NS = not significant.
* = $p \leq 0.05$; statistical significance based on actual data, not on percent change from control.

Ophthalmoscopy: No treatment-related effects noted.

EKG: Not conducted.

Hematology: Day 96 values

Dose (mg/kg)	0		2		6		20	
Sex	M	F	M	F	M	F	M	F
RBC ($1 \times 10^6/\mu L$)	9.15	-	8.85	-	8.81	-	8.57* (↓6%)	-
Hgb (g/dL)	16.6	-	16.1	-	16.1	-	15.9 (↓4%)	-
Hct (%)	49.8	-	47.7	-	48.2	-	47.3 (↓5%)	-
Reticulocytes ($1 \times 10^3/\mu L$)	229.3	196.8	180.7* (↓21%)	190.9 (↓3%)	183.3* (↓20%)	182.9 (↓7%)	161.6* (↓30%)	169.7 (↓14%)
Reticulocytes (%)	2.5	2.4	2.0* (↓20%)	2.3 (↓4%)	2.1* (↓16%)	2.2 (↓8%)	1.9* (↓24%)	2.0 (↓17%)

*$p \leq 0.05$ (ANOVA); Hct = hematocrit; Hgb = hemoglobin; RBC = red blood cells.

Clinical chemistry: Day 96 values

Dose (mg/kg)	0		2		6		20	
Sex	M	F	M	F	M	F	M	F
Glucose (mg/dL)	108	102	90* (↓17%)	93 (↓9%)	93* (↓14%)	89 (↓13%)	88* (↓19%)	93 (↓9%)
Total Protein (g/dL)	7.3	8.1	6.8* (↓7%)	7.5* (↓7%)	6.9 (↓5%)	7.4* (↓9%)	6.9 (↓5%)	7.1* (↓12%)
Cholesterol (mg/dL)	88	88	59* (↓33%)	61* (↓31%)	62* (↓30%)	55* (↓38%)	66* (↓25%)	54* (↓39%)
Triglycerides (mg/dL)	62	-	47 (↓24%)	-	37* (↓40%)	-	39 (↓37%)	-
Phosphorus (mg/dL)	-	6.2	-	6.6	-	7.1	-	7.4 (↑19%)
Potassium (mmol/L)	-	5.3	-	5.4	-	5.5	-	5.7 (↑8%)

Urinalysis: No treatment-related urinalysis findings were observed.

Gross pathology: No treatment-related macroscopic findings were noted at necropsy.

Organ weights: There were dose-dependent decreases in weights of several tissues due to treatment-related decreases in body weight. A slight decrease in testis weight may have occurred independent of the effects on body weight.

Dose (mg/kg)	**0**		**2**		**6**		**20**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Testes (g)	3.647	NA	3.575	NA	3.679	NA	3.452 (↓5%)	NA
Testes (%; relative to brain)	166.71	NA	165.47	NA	169.31	NA	159.64 (↓4%)	NA

NA = not applicable

Histopathology:

Dose (mg/kg)	**0**		**2**		**6**		**20**	
Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Testes (Number of animals affected)	0	NA	0	NA	0	NA	2	NA
Degeneration, seminiferous tubules – minimal to slight	0	NA	0	NA	0	NA	2	NA
Epididymis (Number of animals affected)	0	NA	0	NA	0	NA	2	NA
Inflammation - slight	0	NA	0	NA	0	NA	1	NA
Cellular debris, lumen - slight	0	NA	0	NA	0	NA	1	NA
Hypospermia - minimal	0	NA	0	NA	0	NA	1	NA

NA = not applicable.

Toxicokinetics:

Text Table 1: Summary Mean Exposure Parameters

Group (Dose)	Study Day	Sex	C_{max} (mcg/mL)	T_{max} (h)	$AUC_{0\text{-last}}$ (mcg*h/mL)
6	1	M	2.33	24.0	91.4
(2 mg/kg)		F	2.39	24.0	106
		M + F	2.36	24.0	98.9
	92	M	3.58	24.0	140
		F	2.96	24.0	165
		M + F	3.27	24.0	152
7	1	M	7.92	12.0	279
(6 mg/kg)		F	14.24	12.0	386
		M + F	11.08	12.0	333
	92	M	9.94	24.0	368
		F	9.53	24.0	312
		M + F	9.74	24.0	340
8	1	M	56.34	12.0	1140
(20 mg/kg)		F	26.49	12.0	874
		M + F	41.42	12.0	1000
	92	M	27.39	12.0	1280
		F	37.9	24.0	1620
		M + F	32.6	24.0	1450

Abbreviations: $AUC_{0\text{-last}}$ = area under the plasma concentration curve from 0 to last quantifiable time point, C_{max} = maximum observed plasma concentration, F = Female, M = Male, M + F = Males and Females combined, T_{max} = time to achieve C_{max}.

Antibody production:

Anti-LY2189265 antibodies were not measured. Expected pharmacodynamic effects of decreased body weight gain and decreased food consumption were noted throughout the study. TK data show a slight increase in mean exposure on Day 92 compared to Day 1 at the low and high dose levels. This apparent slight accumulation was likely due to the long half-life of the drug. Overall, the data suggest that even if antibodies were produced, they did not have a significant effect on exposure or a neutralizing effect.

Study title: A repeat-dose toxicology and toxicokinetic study in rats given LY2189265 twice weekly by subcutaneous injection for 6 months with a 1 month recovery

Key study findings:

- One mid-dose female was sacrificed moribund on Day 78. Pathology findings indicated that the moribund condition was due to urinary inflammation/calculi and renal pyelonephritis, which was considered an incidental finding.
- Abnormal clinical signs were limited to few feces, thin, and rough haircoat, which were more pronounced at the mid- and high-dose levels.
- Body weights were statistically significantly decreased from control values at all dose levels for both males (↓7% to 18%) and females (↓16% to 19%). The effects on body weight generally associated with decreased food consumption. Decreases in body weight and food consumption are expected pharmacological effects of GLP-1 receptor agonists.
- Slight decreases in mean reticulocyte counts were noted for all male dose groups, high dose females, and the high-dose female recovery group.
- Slight decreases in mean glucose (high-dose males), cholesterol (high-dose females), and triglycerides (high-dose males and females) were noted.
- Dose-dependent decreases in organ weights were considered a secondary effect of the decrease in body weights.
- Microscopic findings noted at the high dose included a slight increase in focal congestion/hemorrhage of the stomach (2/15 males, 1/15 females) and dilatation of the uterus (6/15). An increase in the number of animals with bile duct proliferation was noted for the high-dose recovery males (2/10).
- Although Ab production was not assessed in this study, the fact that exposure data were the same or greater on Day 183 compared with Day 1 and signs of pharmacological activity (decreased mean body weights and decreased mean food consumption) were noted during the last week of the study suggests that sufficient drug exposure was maintained throughout the study.
- Because decreased body weight and food consumption are expected pharmacological effects and did not produce adverse outcomes with regard to overall animal health, the NOAEL of this study was considered to be 20 mg/kg. At the NOAEL, the mean C_{max} and $AUC_{0\text{-last}}$ at Day 183 were 43.7 µg/mL and 1590 µg·h/mL, respectively.

Study no.: 7608-236
Volume #, and page #: Vol. 1 (SN 041); page 1
Conducting laboratory and location: (b) (4)
Date of study initiation: 14 August 2006
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: LY2189265, Lot # (b) (4) C1301-78, 57.6 mg/mL
Formulation/vehicle:
Diluent A: (b) (4) citrate buffer and (b) (4) (w/v) polysorbate-80 with (b) (4) sodium chloride in sterile water for injection, USP.
Control article (diluent B): (b) (4) citrate buffer (pH (b) (4) and (b) (4) (w/v) polysorbate-80 with (b) (4) sodium chloride in sterile water for injection, USP.

Methods

Doses: 0 (vehicle), 2, 6, and 20 mg/kg/d (actual doses were later determined to be 1.63, 4.89, and 16.89 mg/kg)
Species/strain: Rat / Sprague Dawley
Number/sex/group (main study): 15/sex/group
Route and volume: Subcutaneous; 1 mL/kg
Satellite groups used for recovery: 10/sex/group (control and 20 mg/kg/d only)
Satellite groups used for toxicokinetics: 6/sex/group (control) or 12/sex/group
3/sex/group/timepoint
TK sampling times:
Control - Days 1 and 183 – predose (Day 183 only) and ~24 hours postdose
Test article – Days 1 and 183 - predose (Day 183 only) and 4, 12, 24, 48, and 96 hours postdose
Ab sampling times: Samples collected on recovery phase sacrifice day only;
Immunogenicity samples discarded without analysis
Age: 6 to 8 weeks
Weight: 179 to 268 g (males); 153 to 221 g (females)

Observation and Times:

Clinical signs: Cage side - twice daily; Detailed observations – predosing, Day 1, and once weekly thereafter.
Body weights: Predosing and before each dose administration
Food consumption: Twice weekly (quantitatively)
Ophthalmoscopy: Pre-dose and once during the final week of dosing
EKG: Not conducted

Hematology:

red blood cell (erythrocyte) count
hemoglobin
hematocrit
mean corpuscular volume
mean corpuscular hemoglobin
mean corpuscular hemoglobin concentration
platelet count
white blood cell (leukocyte) count
differential blood cell count
blood cell morphology
reticulocyte count
prothrombin time
activated partial thromboplastin time

Clinical chemistry:

glucose
urea nitrogen
creatinine
total protein
albumin
globulin
albumin/globulin ratio
cholesterol
triglycerides
total bilirubin
alanine aminotransferase
alkaline phosphatase
gamma glutamyltransferase
aspartate aminotransferase
creatine kinase
calcium
inorganic phosphorus
sodium
potassium
chloride

Urinalysis:

appearance/color
volume
specific gravity
pH
protein
glucose
ketones
bilirubin
blood
microscopic examination of sediment
urobilinogen

sodium
potassium
chloride
creatinine
sodium excretion
potassium excretion
chloride excretion
creatinine excretion

Gross pathology: Conducted on all main study group animals

Organ weights: Conducted on all main study group animals

adrenal (2)
brain
heart
kidney (2)
liver
ovary (2)
pituitary gland
prostate
spleen
testis (2)
thymus
thyroid (2 lobes) with parathyroid
uterus

Histopathology: Conducted on all main study group animals

Tissue Preservation

The following tissues (when present) from each animal will be preserved in 10% neutral-buffered formalin, unless otherwise indicated below.

adrenal (2)	ovary (2)
aorta	optic nerve (2)[a]
brain	pancreas
cecum	pituitary gland
cervix	prostate
colon	salivary gland [mandibular (2)]
duodenum	sciatic nerve
epididymis (2)[a]	seminal vesicle
esophagus	skeletal muscle (thigh)
eye (2)[a]	skin/subcutis
femur with bone marrow (articular surface of the distal end)	spinal cord (cervical, thoracic and lumbar)
	spleen
heart	sternum with bone marrow
ileum	stomach
injection site(s)	testis (2)[a]
jejunum	thymus
kidney (2)	thyroid (2 lobes) with parathyroid
lesions	tongue
liver	trachea
lung with large bronchi	urinary bladder
lymph node (mandibular)	uterus
lymph node (mesenteric)	vagina
mammary gland	

a Preserved in modified Davidson's fixative.

Adequate Battery: yes (X), no ( )

Peer review: yes (X), no ( ) performed by sponsor - all tissues from the 1st 3 animals of both sexes from control and high dose groups. Additionally, all tissues from the Group 3 female that was sacrificed moribund were examined.

Results:

Mortality: One mid-dose female was sacrificed moribund on Day 78. Prior to sacrifice, the animal was debilitated (i.e., thin, recumbent, genitals that were red in color, and cold to touch). Pathology results indicated that the moribund condition was due to urinary inflammation/calculi and renal pyelonephritis, which was considered an incidental finding. One low-dose TK male animal was found dead on Day 96; the cause of death was not determined. Neither event was considered test article-related.

Clinical signs: Treatment-related clinical signs are shown in Sponsor-generated Text Table 2.

Text Table 2: Clinical Sign Incidence in Dosing Phase

	Few Feces		Thin		Rough Haircoat	
mg LY2189265/kg/dose	Male	Female	Male	Female	Male	Female
0	(0) 2	(0) 4	(0) 0	(0) 0	(0) 0	(0) 15
2	(0) 0	(0) 0	(0) 0	(0) 0	(0) 7	(0) 16
6	(0) 0	(11) 11	(0) 0	(5) 7	(2) 15	(0) 29
20	(39) 39	(35) 45	(6) 6	(20) 30	(14) 33	(0) 27

Parentheses indicate occurrence within the first 8 weeks of the study.

Body weights: Decreased body weights were noted in all test article-treated groups. As shown in the sponsor-generated mean body weight figures, high-dose recovery animals gained more weight than controls during the recovery period.

Dose (mg/kg)	0		2		6		20	
Sex	M	F	M	F	M	F	M	F
Day 1 (g)	224	184	223	181	222	182	222	180
Day 183 (g)	642	358	597*	299*	555*	299*	527*	290*
Weight gain (g)	418	174	374*	118*	333*	117*	305*	110*
Final weight - diff from control (g)	-	-	-45	-59	-87	-59	-115	-68
Final weight - % diff from control	-	-	↓7%	↓16%	↓14%	↓16%	↓18%	↓19%

* $p \leq 0.05$ (ANOVA)

(Sponsor-generated figures)

Figure 1: Mean body weight data - males.

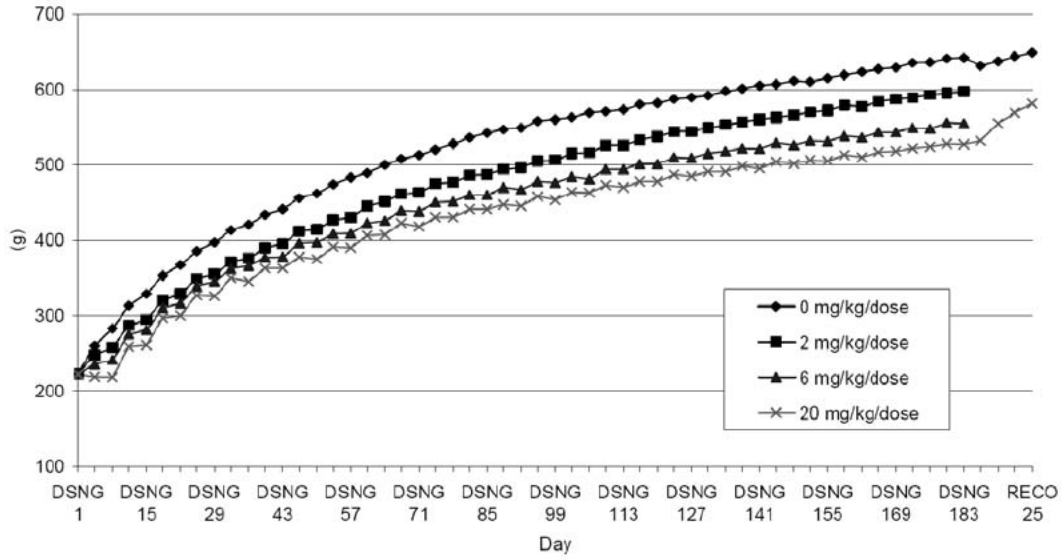
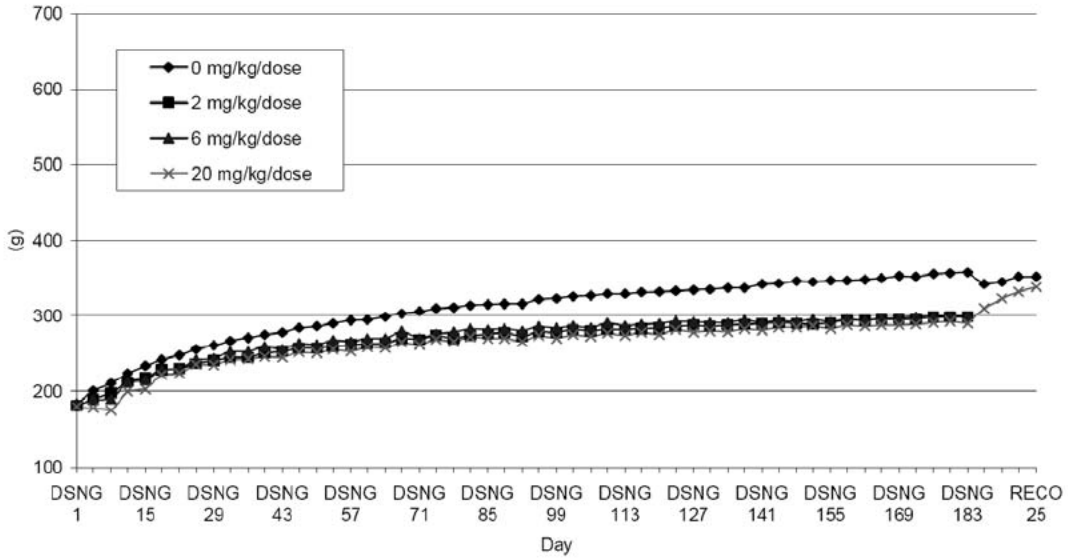


Figure 2: Mean body weight data - females.



Food consumption: Decreased food consumption was observed in all test article-treated groups.

Ophthalmoscopy: No treatment-related effects noted.

EKG: Not conducted.

Hematology:

Dose (mg/kg)	0		2		6		20	
Sex	M	F	M	F	M	F	M	F
Reticulocytes ($1 \times 10^3/\mu L$)	180.4	133.0	146.9*	119.2	144.2*	119.9	151.4* (↓16%)	142.6 (↓7%) (±118)
Reticulocytes (%)	2.0	1.6	1.6*	1.5	1.6*	1.5	1.7	1.8
	Recovery							
Reticulocytes ($1 \times 10^3/\mu L$)	194.1	144.3					175.3	112.7* (↓22%)
Reticulocytes (%)	2.1	1.8					2.0	1.3* (↓28%)

*$p \leq 0.05$ (ANOVA)

Clinical chemistry:

Dose (mg/kg)	0		2		6		20	
Sex	M	F	M	F	M	F	M	F
Glucose (mg/dL)	100	102	98	96	94	91*	87* (↓13%)	95
Total Protein (g/dL)	7.1	7.8	7.0	7.5	6.8*	7.5	6.7* (↓6%)	7.3
Cholesterol (mg/dL)	-	87	-	66	-	63	-	57* (↓33%)
Triglycerides (mg/dL)	74	43	49*	31*	43*	28*	42* (↓43%)	29* (↓33%)
ALP (U/L)	-	29	-	34	-	32	-	39* (↑34%)

"-" = no change from control value; ALP = alkaline phosphatase.

Urinalysis: No treatment-related urinalysis findings were observed.

Gross pathology: No treatment-related macroscopic findings were noted at necropsy.

Organ weights: There were no organ weight changes directly attributed to test article. There were dose-dependent decreases in weights of several tissues due to treatment-related decreases in body weight.

Histopathology:

Dose (mg/kg)	0		2		6		20	
Sex	M	F	M	F	M	F	M	F
Stomach - focal congestion/hemorrhage	0	0	0	0	0	0	2/15	1/15
Uterus - dilatation	NA	1/15	NA	1/15	NA	0	NA	6/15
Liver - bile duct proliferation	0	0	0	0	0	0	0	0
Recovery								
Liver - bile duct proliferation	0	0	NA	NA	NA	NA	2/10	0

NA = not applicable.

Toxicokinetics: There were no apparent differences in PK data between genders. Slight accumulation was noted at the mid and high doses.

Table 2: Mean Plasma Toxicokinetic Data Summary of LY2189265 in Rats Following Twice Weekly Subcutaneous Injections of 2, 6, or 20 mg/kg (Groups 6 through 8, respectively) LY2189265 on Study Days 1 and 183

Group (Dose)	Study Day	Sex	C_{max} (µg/mL)	T_{max} (h)	$AUC_{0\text{-last}}$ (µg*h/mL)
6 (2 mg/kg)	1	M	3.34	12.0	106.12
		F	4.20	12.0	140.82
		M + F	3.77	12.0	123.32
	183	M	4.39	24.0	168.28
		F	3.02	12.0	128.78
		M + F	3.71*	24.0/12.0	148.42
7 (6 mg/kg)	1	M	8.10	12.0	244.68
		F	8.46	12.0	311.07
		M + F	8.28	12.0	277.98
	183	M	11.97	24.0	369.86
		F	16.15	12.0	360.76
		M + F	14.06*	24.0/12.0	365.30
8 (20 mg/kg)	1	M	17.49	12.0	554.61
		F	25.25	12.0	793.31
		M + F	21.37	12.0	673.99
	183	M	46.10	24.0	1477.50
		F	41.20	24.0	1702.30
		M + F	43.65	24.0	1590.20

Abbreviations: $AUC_{0\text{-last}}$ = area under the plasma concentration curve from 0 to last quantifiable time point, C_{max} = maximum observed plasma concentration, F = Female, M = Male, M + F = Males and Females combined, T_{max} = time to achieve C_{max}.

*Replaced Watson generated values which were generated by using the highest overall (Male + Female) mean plasma concentrations of both sexes instead of the mean C_{max} plasma values at the actual T_{max} for each sex.

Antibody measurements:
Anti-LY2189265 antibody levels were not measured. Although antibody production was not assessed in this study, the fact that exposure data were the same or greater on Day 183 compared with Day 1 and signs of pharmacological activity (decreased mean body weights and decreased mean food consumption) were noted during the last week of the study suggests that sufficient drug exposure was maintained throughout the study. Additionally, the high-dose group gained considerably more weight during the recovery period compared with controls indicating that treatment-related weight suppression was maintained throughout the dosing period.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
IND 70930	ORIG 1		LY2189265 FOR INJECTION
IND 70930	ORIG 1		LY2189265 FOR INJECTION

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/s/

BRIAN T HUMMER
08/13/2009

KAREN L DAVIS BRUNO
08/13/2009