CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205858Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Date: May 1, 2014
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDAs: 206545 and 205858
Drug: ZYDELIG (idelalisib)
Indications: Indolent non-Hodgkin lymphoma (iNHL) and chronic lymphocytic leukemia (CLL); see the label for detailed information
Applicant: Gilead Sciences, Inc.

Idelalisib is a small molecule PI3K inhibitor with selectivity toward PI3Kδ. NDA 205858 was submitted in September 2013 for the NHL indication; NDA 206545 was submitted in December 2013 for the CLL indication. The nonclinical studies submitted to and reviewed under NDA 205858 cover both indications.

For detailed pharmacology/toxicology findings, see the primary review by Drs. Ramadevi Gudi and Natalie Simpson and for an overview of the nonclinical package see my Team Leader Memorandum, both archived under NDA 205858.

Recommendation: From a pharmacology/toxicology perspective, ZYDELIG may be approved for the proposed indications. There are no outstanding pharmacology/ toxicology issues. Also see Dr. John Leighton's concurrence, archived under NDA 205858.

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/s/

HALEH SABER
05/01/2014

MEMORANDUM

Zydelig (idelalisib)

Date: April 18, 2014
To: File for NDA 205858
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Zydelig conducted by Drs. Gudi and Simpson, and secondary memorandum and labeling provided by Dr. Saber. I concur with Dr. Saber's conclusion that Zydelig may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
04/18/2014

MEMORANDUM

Date: April 17, 2014
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 205858
Drug: ZYDELIG (idelalisib)
Indications: Treatment of patients with refractory indolent B-cell Non-Hodgkin Lymphoma (iNHL) and chronic lymphocytic leukemia (CLL)
Applicant: Gilead Sciences, Inc.

Idelalisib is a small molecule PI3K inhibitor with selectivity toward PI3Kδ. PI3Kδ is a kinase downstream of several receptors such as B-cell receptor (BCR), CD40 receptor, chemokine receptors CXCR4/5, IL-6 receptor, and integrins. These pathways are involved in B-cell proliferation, mobilization, and in homing to and maintenance of the tumor microenvironment. Idelalisib induced apoptosis and inhibited proliferation in cell lines derived from malignant B cells.

Pharmacology, safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism and excretion), and toxicology studies were conducted in *in vitro* systems and/or in animal species. Animal toxicology studies were conducted in appropriate species, using the administration route and dosing regimens that adequately addressed safety concerns in humans. Idelalisib-related toxicities in rats and dogs included findings in the following organs: liver (increased ALT, AST, and GGT, inflammation, and necrosis), heart (cardiomyopathy, inflammation, and fibrosis), pancreas (inflammation and low incidence acinar degeneration), lung (infiltration, alveolar macrophages), lymphoid tissues (depletion of lymphocytes), GI tract including the tongue (ulceration, hemorrhage, and inflammation), and male reproductive organs (spermatid depletion, testicular seminiferous tubule degeneration). Hemorrhage was occasionally observed, those included hemorrhage in the GI tract, thymus, and brain. Several of the toxicities reported (e.g. inflammation, cardiomyopathy, pancreatic acinar degeneration) may be due to the inhibition of CXCR4/5 pathways. Of note, CXCR5 is upstream from Bruton's tyrosine kinase (BTK). Inhibition of the BTK pathway may be associated with multi-organ inflammation and pancreatic acinar cell degeneration.

In pigmented Long-Evans rats, skin and eye uvea showed higher concentrations of idelalisib than that observed in Sprague-Dawley rats, suggesting that idelalisib or idelalisib-related materials (e.g. metabolites) bind to melanin. Clinical signs of, skin erythema and swelling have been reported in animals in the toxicology studies with low incidence of mononuclear infiltration.

In patients treated with ZYDELIG, liver toxicity (characterized by increased ALT, AST, and GGT), GI disturbance, and skin rash have been reported.

Idelalisib was not genotoxic in the bacterial mutagenesis (Ames) assay or *in vitro* chromosome aberration assay using human peripheral blood lymphocytes. Idelalisib was genotoxic in male rats in the *in vivo* micronucleus study; however, only at a high dose of 2000 mg/kg.

Two separate fertility studies were conducted. In one of the studies, male rats treated with idelalisib were mated with untreated females. Idelalisib caused decreased weight in epididymis and testis; however, there were no adverse effects on fertility parameters. In the second study, female rats given idelalisib were mated with untreated males. There were no adverse effects on fertility parameters in this study; however, there was a decrease in the number of live embryos at the highest dose tested. In an embryo-fetal developmental study, idelalisib caused malformations in rats when given to pregnant animals during the period of organogenesis at maternally toxic doses. Therefore, pregnancy category D is recommended. In the NDA review, the exposures used for animal-to-human AUC ratios were representative of different time-points; AUC_{0-12} for humans and AUC_{0-24} for animals. During the labeling review, it was determined that comparisons based on the same AUC time-point (i.e. AUC_{0-24} in humans or 20.2 μg.h/mL) was most appropriate. As a result, the exposure ratios in the label differ from those presented in the NDA review.

The nonclinical studies were reviewed by Drs. Ramadevi Gudi and Natalie Simpson. The nonclinical findings are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

Recommendation: I concur with Drs. Gudi and Simpson that from a nonclinical perspective, ZYDELIG may be approved and that no additional nonclinical studies are needed to support approval of ZYDELIG for the proposed indications.

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/s/

HALEH SABER
04/17/2014

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	205858
Supporting document/s:	1
Applicant's letter date:	September 10, 2013
CDER stamp date:	September 11, 2013
Product:	Zydelig (Idelalisib)
Indication:	Treatment of patients with refractory indolent B-cell Non-Hodgkin Lymphoma (iNHL) and chronic lymphocytic leukemia (CLL)
Applicant:	Gilead Sciences, Inc.
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer:	Natalie E. Simpson, Ph.D. Ramadevi Gudi, Ph.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John Leighton, Ph.D., DABT (DHOT) Ann Farrell, M.D. (DHP)
Project Manager:	Mara B. Miller

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 205858 are owned by Gilead Sciences, Inc. or are data for which Gilead Sciences, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 205858 that Gilead Sciences, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 205858.

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1 Executive Summary

1.1 Introduction

NDA 205858 has been submitted for idelalisib for the treatment of patients with refractory indolent B-cell non-Hodgkin lymphoma (iNHL) and chronic lymphocytic leukemia (CLL). Idelalisib is a kinase inhibitor with selectivity toward phosphatidylinositol 3-kinase p110δ (PI3Kδ) catalytic domain. The proposed clinical dose of 150 mg is administered orally as a tablet twice a day (BID). Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of idelalisib for the proposed indications.

1.2 Brief Discussion of Nonclinical Findings

Idelalisib (IDELA; GS-1101; CAL-101), is a kinase inhibitor with selectivity toward PI3Kδ. PI3Kδ is a downstream signal transducer for several receptors including B-cell receptor (BCR), CD40 receptor, chemokine receptor CXCR5, IL-6 receptor, and integrins. These pathways may be involved in B-cell proliferation, motility, and in homing to and maintenance of the tumor microenvironment in B-cell malignancies. The primary human metabolite of idelalisib, GS-563117, inhibits lymphocyte-oriented kinase (LOK) and Ste20-like kinase (SLK). According to the information at Cancer Genome Anatomy Project (CGAP), LOK is involved in lymphocyte migration and SLK is involved in apoptosis. GS-563117 is present in the plasma of healthy volunteers at higher levels (62% of drug-related material) than the parent drug (38% of drug-related material) following single oral dosing of idelalisib at 150 mg.

Idelalisib inhibits PI3K by binding to the ATP binding site of the catalytic subunit p110δ. P110δ is over-expressed in cell lines derived from patients with follicular lymphoma (small cleaved cell lymphoma or NHL). The (IC_{50}) of idelalisib for PI3Kδ was 19 nM in an *in vitro* assay and the EC_{50} was 8.9 nM in a cell-based assay. The primary human metabolite, GS-563117, is an inhibitor of LOK and SLK kinases with IC_{50} values of 0.11 µM and 0.05 µM, respectively. Idelalisib inhibited cell viability and induced apoptosis in malignant B-cells. Idelalisib exhibited higher sensitivity for acute lymphoblastic leukemia (B-ALL) and CLL cells compared with acute myeloid leukemia (AML) and myeloproliferative neoplasm (MPN) cells, suggesting a greater activity potential for B-cell malignancies. Idelalisib inhibited CXCR4 and CXCR5 signaling and chemotaxis in CLL cells, as well as BCR signaling and chemokine secretion and CLL cell migration in an *in vitro* simulated tumor microenvironment.

In *in vivo* and *in vitro* safety pharmacology studies conducted, no clear drug-related effects were observed for idelalisib on neurological, cardiovascular, or respiratory function. However, drug-related cardiomyopathy and increased in the heart weight were observed in the rat in the repeat-dose toxicology studies (see below).

Orally administered idelalisib was absorbed rapidly (T_{max} 0.5 to 2 hours), with bioavailability less than 50% in the animals tested. Idelalisib was localized to most

tissues in the rat, but was relatively excluded from bone, brain, spinal cord, and eye lens. In Long-Evans rats, pigmented skin and eye uvea showed higher concentrations of idelalisib than that observed for the similar tissues in Sprague-Dawley rats, suggesting some association of drug-derived radioactivity with melanin. The pharmacokinetics of idelalisib are similar between rats and dogs and humans, however, the plasma level of the metabolite GS-563117 was lower in dogs (34%) and rats (1.4%) than humans (62%). Idelalisib was the most abundant analyte in plasma of rats and dogs (~90% in rats and ~60% in dogs). Idelalisib exhibited moderately high plasma protein binding in all species with the average free fraction values of 19%, 21%, and 16% for rat, dog, and human respectively. The hepatobiliary route was the primary route of excretion within 24 to 48 hours in rats and dogs, with the majority of idelalisib being detected in the feces and 6% or less in the urine.

Toxicities following repeated dosing of idelalisib in rats and/or dogs included findings in the following tissues/organs:

- hematopoietic/lymphoid system (lymphoid depletion, reduced weight of spleen and thymus, thymic hemorrhage and necrosis, myeloid and granulopoeitic hyperplasia),
- liver (increased liver enzymes, increased liver weight, inflammation, hepatocellular necrosis);
- gastrointestinal (GI) tract including the tongue (infiltration, hemorrhage, ulceration),
- heart; seen in rats only (myocardium infiltrate, fibrosis, increased heart weight);
- male reproductive systems (testicular seminiferous tubule degeneration, reduced testicular weight).

Inflammation was observed in several tissues (e.g. in the GI tract, pancreas, lungs, heart, and liver) and may be related to the inhibition of the CXCR5 pathway, involved in homing of B-cells. Skin may be also a target of idelalisib toxicity. In pigmented rats, idelalisib-related radioactivity was present in the eyes and skin at higher concentrations than what were reported for non-pigmented rats. Skin erythema, dryness, swelling, and redness have been observed in animals in toxicology studies.

An *in vitro* photo-toxicity study was conducted in the embryonic murine fibroblast BALB/c 3T3 cell line using Neutral Red uptake as a marker of cellular viability in the presence and absence of ultraviolet A (UVA) light exposure. The study was not reviewed by the Agency, however, based on the summary provided by the Applicant, results for idelalisib were inconclusive, while the primary human metabolite, GS-563117, induced photo-toxicity in the presence of UVA exposure.

General toxicology studies were done by the oral route and included 28-day studies in rats and dogs, a 13-week study in rats, a 6-month study in rats, and a 9-month study in dogs. There were mortalities in all rat studies with cause of deaths undetermined or related to liver toxicity. Cardiomyopathy was observed in unscheduled sacrifices and surviving rats in the 13-week and 6-month studies, with an increase in heart weight

observed in the 13-week study. In the 9-month dog study, mortality was attributed to systemic inflammation, with no signs of hepatotoxicity in this study.

Idelalisib did not induce mutations in the bacterial mutagenesis (Ames) assay and, was not clastogenic in the *in vitro* chromosome aberration assay using human peripheral blood lymphocytes. Idelalisib was genotoxic in males in the *in vivo* rat micronucleus study at a high dose of 2000 mg/kg. Carcinogenicity studies have not been conducted and are not necessary for the proposed indications.

Reproductive and developmental toxicology studies were conducted in rats to assess the effects of idelalisib on fertility and embryo-fetal development. In a fertility and early embryonic development study, idelalisib-treated male rats were mated to untreated female rats. In this study, idelalisib had no effect on reproductive function or fertility, despite decreased testis and epididymis weights, and reduced sperm counts. When idelalisib-treated female rats were paired with untreated male rats, there was an increase in pre-implantation and post-implantation loss, and early embryolethality, resulting in a 20% decrease in the number of live embryos at the high dose of 100 mg/kg (600 mg/m^2).

The embryo-fetal development effects of idelalisib were studied in the rat. Idelalisib produced post-implantation loss and was teratogenic. Idelalisib was maternally toxic based on reductions in net body weight gain > 10% at the mid and high doses (75 and 150 mg/kg/day; 450 and 900 mg/m^2/day) and clinical signs of maternal toxicity, most evident at the high dose. Adverse embryo-fetal findings at doses ≥ 75 mg/kg/day (450 mg/m^2/day) included decreased fetal weights, external malformations (short tail) and skeletal variations (delayed ossifications and/or unossification of the skull, vertebrae, and sternebrae). At 150 mg/kg/day (900 mg/m^2/day) dose, idelalisib resulted in spontaneous abortion (urogenital loss, complete resorption, increased post-implantation loss, and reduced mean litter size) and malformations (vertebral agenesis with anury, microphthalmia/anophthalmia, and hydrocephaly) in live fetuses. The dose of 75 and 150 mg/kg/day of idelalisib in rats resulted in exposures (AUC) of approximately 25 and 60 times, respectively, the human exposure at the recommended dose of 150 mg BID.

As a kinase inhibitor, the teratogenic effects of idelalisib were expected and observed in rats at the mid and high doses tested. Based on teratogenicity findings, an embryo-fetal developmental study in a second species is not needed and pregnancy category D is recommended.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, approval of Zydelig is recommended for the proposed indications.

1.3.2 Additional Non Clinical Recommendations

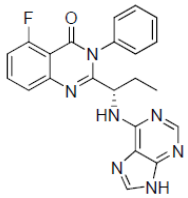
None. From a Pharmacology/Toxicology perspective, no additional post-marketing commitments (PMCs) or post-marketing requirements (PMRs) are recommended for these indications.

1.3.3 Labeling

The recommendations to the Applicant's proposed labeling were discussed internally and communicated to the Applicant. Information in the nonclinical sections of the label reflects findings of studies reviewed within this document.

2 Drug Information

2.1 Drug

CAS Registry Number	870281-82-6
Generic Name	Idelalisib
Code Name	IDELA, GS-1101, CAL-101
Chemical Name	5-Fluoro-3-phenyl-2-[(1S)-1-(9H-purin-6 ylamino)propyl]quinazolin-4(3H)-one
Molecular Formula/ Molecular Weight	$C_{22}H_{18}FN_7O$ / 415.42 g/mol
Structure or Biochemical Description	
Pharmacologic class	kinase inhibitor (Mechanism of action: PIK3δ inhibition)

2.2 Relevant INDs, NDAs, BLAs and DMFs

The IND 101254 was the original submission for CAL-101 by Calistoga Pharmaceuticals.

2.3 Drug Formulation

Idelalisib (IDELA) tablets, 100 mg and 150 mg, are orange (100 mg) or pink (150 mg), oval-shaped, film-coated tablets, debossed with "GSI" on one side and "100" for 100 mg tablets or "150" for 150 mg tablets on the on the other side.

Table 1: Quantitative Composition of Idelalisib Tablets, 100 mg and 150 mg

(Excerpted from the Applicant's submission)

Components / Tablet Strength	% w/w	Unit Formula (mg/tablet) 100 mg	150 mg	Quality Standard	Function
Idelalisib	(b) (4)	100.0	150.0	In-house (b) (4)	Active Ingredient
Microcrystalline Cellulose				NF, Ph. Eur.	(b) (4)
Hydroxypropyl Cellulose				NF, Ph. Eur.	
Sodium Starch Glycolate				NF, Ph. Eur.	
Croscarmellose Sodium				NF, Ph. Eur.	
Magnesium Stearate				NF, Ph. Eur.	
(b) (4)				USP, Ph. Eur.	
Total (tablet (b) (4)					
		Film Coating Suspension			
(b) (4) Orange (b) (4)			(b) (4)	In-house (b) (4)	
(b) (4) Pink (b) (4)				In-house (b) (4)	
(b) (4)				USP, Ph. Eur.	
Total				--	

(b) (4)

2.4 Comments on Novel Excipients

(b) (4)

The Applicant's specification of (b) (4) of (b) (4) in the drug substance is acceptable. The Applicant states that the permitted daily exposure (PDE) of (b) (4) is (b) (4) We agree with the PDE of (b) (4) and the proposed specification of (b) (4) or (b) (4) Using the data available at NTP and the PDE equation available in ICH Q3C, we obtained PDEs comparable to what the Applicant provided.

2.5 Comments on Impurities/Degradants of Concern

The proposed specifications in the drug substance are NMT (b) (4) % for impurity (b) (4) NMT (b) (4) % for impurity (b) (4) and NMT (b) (4) % for impurities (b) (4) and (b) (4) which are above the ICH qualification threshold of 0.15%. With a clinical dose of 150 mg BID (300 mg/day; 185 mg/m^2/day), the dose of (b) (4) is (b) (4), the dose of (b) (4) is (b) (4) and the dose of (b) (4) and (b) (4) is (b) (4) (Table 2). In order to qualify the impurities above the ICH qualification threshold, a one-month toxicology study in rats (Study # TX-312-2012) was conducted comparing two batches of idelalisib - one with lower levels of impurities (b) (4)

(b) (4) Lot # (b) (4) and one with higher levels of impurity (b) (4) Lot# (b) (4) (Table 81). In this study, there were no differences in toxicities observed in rats treated with low and high levels of impurities; and, toxicities were comparable to the rat general toxicology studies. Therefore, these impurities are considered qualified (Table 2).

Table 2: Dose Based on Body Surface Area (mg/m^2)

Impurity	NDA 205858 Proposed Specifications in humans (300 mg; 185 mg/m^2 BID)		Impurity Qualification study in rats (TX-312-2012) Lot # (b) (4) (90 mg/kg; 540 mg/m^2 dose)		Qualification Determination
	%	Dose(mg/m^2)	%	Dose(mg/m^2)	
(b) (4)					Qualified
					Qualified
					Qualified
					Qualified

Additionally, there are two genotoxic impurities. The specifications proposed by the Applicant are NMT (b) (4) for (b) (4) and NMT (b) (4) for (b) (4). Based on the patient population, these specifications are acceptable from a pharmacology/ toxicology perspective and meet the requirements set forth by ICH Q3A and Q3B.

2.6 Proposed Clinical Population and Dosing Regimen

Idelalisib will be administered orally at a dose of 150 mg twice a day (BID) to patients with indolent B-cell non-Hodgkin lymphoma (iNHL) or chronic lymphocytic leukemia (CLL). Indolent NHL is comprised of 4 indolent lymphomas (follicular lymphoma [FL], small lymphocytic lymphoma [SLL], lymphoplasmacytic lymphoma with or without Waldenstrom macroglobulinemia [LPL/WM], and marginal zone lymphoma [MZL]).

2.7 Regulatory Background

Calistoga Pharmaceuticals submitted their original application for CAL-101 under IND 101254 in 2008. In April 2011, Calistoga was acquired by Gilead Sciences, Inc. (Gilead). Gilead continued the development of CAL-101 and assigned the compound a Gilead identification number GS-1101. The NDA 205858 was received from Gilead on September 11, 2013 and filed on November 10, 2013.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

Study#	Title	Module
PC-312-2002	GS-1101, a p110δ Selective Phosphatidylinositol-3-Kinase (PI3K) Inhibitor for the Treatment of B Cell Malignancies Inhibits PI3K Signaling and Cellular Viability	4.2.1.1
PC-312-2003	The Phosphoinositide 3'-Kinase Delta Inhibitor, GS-1101, Inhibits B-Cell Receptor Signaling and Chemokine Networks in Chronic Lymphocytic Leukemia	4.2.1.1
PC-312-2006	GS-1101 (CAL-101) is a Potent Selective Inhibitor of PI3Kδ: Isoform Selective Cell-Based	4.2.1.1
PC-312-2007	Structure Determination of GS-1101 Bound to PI3Kδ	4.2.1.1
PC-312-2008	Biochemical Assay to Access PI3Kδ *in vitro* Potency and Isoform Selectivity	4.2.1.1
PC-312-2010	Effect of IDELA on Stimulated P-AKT Level in Malignant B-Cells Prepared from Biopsy Specimens from Patients with Follicular Lymphoma	4.2.1.1
PC-312-2011	The Effect of GS-1101 on Viability, Proliferation and Apoptosis of Two Follicular Lymphoma-Derived Cell Lines	4.2.1.1
PC-312-2012	Evaluation of GS-563117 for Inhibition of Class I PI3K Isoforms in a Biochemical Assay	4.2.1.1

Secondary Pharmacology

Study#	Title	Module
2408	Evaluation of Compounds on LOK and SLK Kinase Activities	4.2.1.2
CAL001-01-p-00001	Kinase Selectivity Profile for IDELA	4.2.1.2
CAL007-01-p-00001	Kinase Selectivity Profile of GS-563117	4.2.1.2

Safety Pharmacology

Study #	Title	Module
BHR00004	hERG Channel Inhibition by Test Compounds CAL-101 and CAL-102 Using a Rubidium Flux Method	4.2.1.3
BHR00041	A Cardiovascular and Respiratory Safety Pharmacology Study of CAL-101 Administered Orally to Telemetered Beagle Dogs	4.2.1.3
BHR00042	CAL-101: Single-Dose Safety Pharmacology Neurofunctional Assessment in Sprague-Dawley Rats	4.2.1.3

Pharmacokinetics

Study #	Title	Module
Absorption		
BHR00011	Pharmacokinetics and Bioavailability of CAL-101 Following Crossover Administration to Female Beagle Dogs	4.2.2.2
BHR00012	Pharmacokinetics and Bioavailability of CAL-101 Following Intravenous and Oral Administration to Female Sprague-Dawley Rats	4.2.2.2
Distribution		
1503-058	Tissue Distribution of Radioactivity by Quantitative Whole-Body Autoradiography in Sprague Dawley and Long Evans Rats Following a Single Oral Dose of 14C-CAL-101	4.2.2.3
AD-312-2009	Plasma Protein Binding of Idelalisib and GS-563117	4.2.2.3
Metabolism		

AD-312-2002	Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Dogs after Oral Administration of 14C-GS-1101	4.2.2.4
AD-312-2004	Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of 14C-GS-1101	4.2.2.4
Excretion		
AD-312-2003	Pharmacokinetics, Absorption, and Excretion of 14C-GS-1101 Following a Single Oral Administration to Rats	4.2.2.5

Repeat-Dose Toxicology

Study#	Title	Module
TX-312-2006	A 9-Month Oral (Capsule) Toxicity Study of GS-1101 with a 12-Week Recovery Period in Beagle Dogs	4.2.3.2
BHR00008	CAL-101: 28-Day Oral Toxicity Study with Toxicokinetics in Beagle Dogs with Recovery	4.2.3.2
TX-312-2005	A 6-Month Oral (Gavage) Toxicity Study of GS-1101 with a 12-Week Recovery Period in Sprague Dawley Rats	4.2.3.2
BHR00009	CAL-101: 28-Day Oral Toxicity Study with Toxicokinetics in Sprague-Dawley Rats with Recovery	4.2.3.2
TX-312-2001	GS-1101: A 13-Week Oral Toxicity Study in Rats with a 4-Week Recovery Period	4.2.3.2

Genetic Toxicology

Study#	Title	Module
961805	CAL-101 Bacterial Mutation Test	4.2.3.3.1
961806	CAL-101 Chromosome Aberration Test	4.2.3.3.1
961807	CAL-101 Rat Micronucleus Test	4.2.3.3.2

Reproductive Toxicology

Study#	Title	Module
TX-312-2014	Evaluation of Orally (by Gavage) Administered GS-1101 on Fertility in Male Rats	4.2.3.5.1
TX-312-2016	Evaluation of Orally (by Gavage) Administered GS-1101 on Fertility and Early Embryonic Development to Implantation in Female Rats	4.2.3.5.1
TX-312-2008	An Oral (Gavage) Embryo/Fetal Development Study of GS-1101 in Rats	4.2.3.5.2

Special Toxicology

Study#	Title	Module
TX-312-2012	A 28-Day Oral Gavage Impurity Qualification Study of GS-1101 in the Sprague Dawley Rat	4.2.3.7.6

3.2 Studies Not Reviewed

Please refer to the Appendix for the list of studies submitted to the NDA that were not reviewed.

3.3 Previous Reviews Referenced

Study BHR00008 “CAL-101: 28-Day Oral Toxicity Study with Toxicokinetics in Beagle Dogs with Recovery”, study BHR00009 “CAL-101: 28-Day Oral Toxicity Study with Toxicokinetics in Sprague-Dawley Rats with Recovery”, study 961805 “CAL-101

Bacterial Mutation Test", study 961806 "CAL-101 Chromosome Aberration Test", and study 961807 "CAL-101 Rat Micronucleus Test" was reviewed under IND 101254 by Dr. Timothy Kropp. The reviews were modified to maintain format consistency for this NDA review.

4 Pharmacology

4.1 Primary Pharmacology

Idelalisib is a selective PI3Kδ, Class I phosphatidylinositide 3-kinase (PI3K) inhibitor that competes with ATP for binding to the kinase domain of PI3Kδ. Upon activation via cell surface receptor-ligand interactions, PI3Kδ phosphorylates the second messenger phosphatidylinositol to generate phosphatidylinositol 3,4,5, trisphosphate (PIP3). PIP3 enables the transmission of cell surface receptor signaling by acting as a scaffold for the recruitment and activation of numerous intracellular signaling enzymes including the serine/threonine protein kinase Akt. Akt is an initiator of specific pathways that ultimately mediate positive pleotropic effects on cell survival, proliferation, growth, and metabolism.

PI3Kδ is a signaling molecule in normal and malignant B lymphocytes[1,2]. PI3Kδ is involved in several signaling pathways such as the B-cell receptor (BCR), CD40, B-cell activating factor receptor (BAFFR), chemokine receptors CXCR4 and CXCR5, IL-6 receptor, and integrins (Figure 1). These pathways may be involved in B-cell proliferation, motility, and in homing to and maintenance of the tumor microenvironment in B-cell malignancies[3]. Hyperactivity and deregulation of the PI3K/Akt pathway is observed in human B-cell malignancies[4,5].

[1] Puri KD, Gold MR. Selective inhibitors of phosphoinositide 3-kinase delta: modulators of B-cell function with potential for treating autoimmune inflammatorydiseases and B-cell malignancies. Frontiers in immunology 2012;3:256.

[2] Young RM, Staudt LM. Targeting pathological B cell receptor signalling in lymphoid malignancies. Nat Rev Drug Discov 2013;12 (3):229-43.

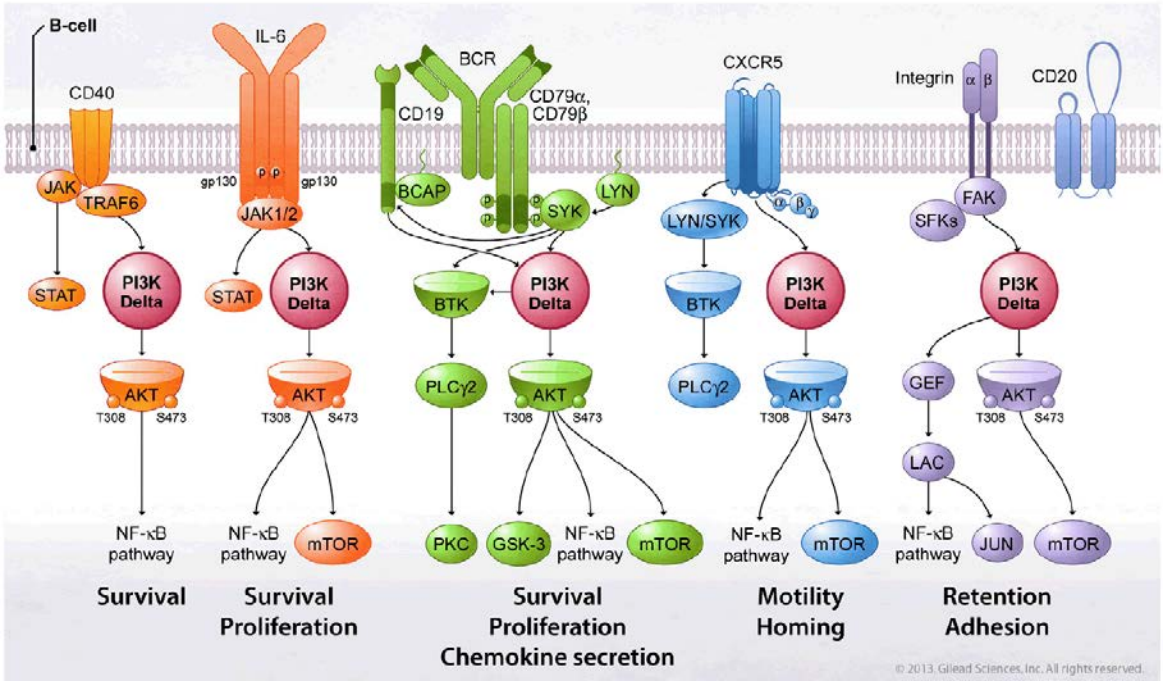
[3] Schrottner P, Leick M, Burger M. The role of chemokines in B cell chronic lymphocytic leukaemia: pathophysiological aspects and clinical impact. Ann Hematol 2010;89 (5):437-46.

[4] Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nature reviews. Cancer 2009;9 (8):550-62.

[5] Witzig TE, Kaufmann SH. Inh bition of the phosphatidylinositol 3- kinase/mammalian target of rapamycin pathway in hematologic malignancies. Current treatment options in oncology 2006;7 (4):285-94.

Figure 1: PI3Kδ Signaling Pathways Targeted by Idelalisib in B-Cell Malignancies Mediate Survival, Proliferation, and Homing

(Excerpted from the Applicant's submission)



In the primary and secondary pharmacology sections of this review, the potency, selectivity, and *in vitro* pharmacological activity of idelalisib are described. Key findings are listed below, followed by summaries of methods and results from reviewed studies.

Key Findings:

- Idelalisib has anticancer activity in cells derived from patients with B-cell malignancies.
- Idelalisib (≥ 1 μM) significantly reduced the induction and secretion of chemokines (CCL3 and CCL4) in primary CLL cells stimulated by anti-IgM or by co-culture with Nurse like cells (NLCs) at concentrations ≥ 0.5 μM.
- Idelalisib inhibited Akt and extracellular signal-regulated kinase phosphorylation in response to anti-IgM stimulation and in response to CXCL12 or CXCL13.
- In a cell-based assay, GS-1101 inhibited PI3Kδ signaling with an EC_{50} of 8.9 nM. The IC_{50} was 19 nM in *in vitro* assays using recombinant protein.
- Idelalisib caused dose-dependent apoptosis in malignant B-cell lines.
- Idelalisib inhibits PI3Kδ by binding to the ATP binding site of the catalytic subunit P110δ.
- P110δ is highly expressed in WSU-FSCCL and WSU-NHL, two cell lines derived from follicular lymphoma patients. GS-1101 treatment inhibited cell viability in both cell lines.

- Idelalisib, at a concentration of 0.1 μM, inhibited P-AKT (Ser473) an average of 86% (range 55-97%) in malignant B-cells prepared from biopsy specimens of 7 patients with follicular lymphoma.
- In a kinase screen, idelalisib inhibited other Class I PI3K isoforms between 83 and 97.3%; however, *in vitro* assays demonstrate that GS-1101 is between 110- and 453-fold more selective for inhibition of PI3Kδ.
- The primary human metabolite of GS-1101, GS-563117 (~62% in human plasma), is an inhibitor of LOK and SLK kinases with IC_{50} values of 0.11 μM and 0.05 μM, respectively.

Study title: GS-1101, a p110δ Selective Phosphatidylinositol-3-Kinase (PI3K) Inhibitor for the Treatment of B Cell Malignancies Inhibits PI3K Signaling and Cellular Viability

Study No.:	PC-312-2002
Report Date:	June 12, 2013
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Gilead Sciences, Inc.
GLP:	No

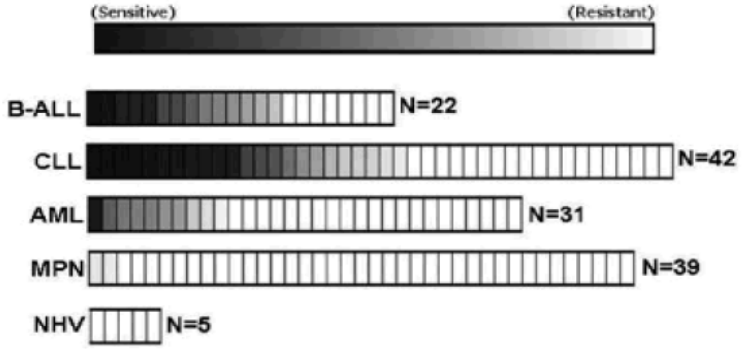
PI3Kδ inhibition by GS-1101 was assessed in a panel of malignant B-cell tumor lines and primary tumor cells from patients with CLL, mantle cell lymphoma (MCL) and B-ALL.

Cell Viability

A total of 134 leukemia patients and 5 control PBMC samples from normal healthy volunteers were tested for cell viability using a 3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium assay. Significant sensitivity (defined as an EC_{50} less than 1μM) to GS-1101 was observed in 23% of acute lymphoblastic Leukemia (B-ALL) samples (5/22), 26% of CLL samples (11/42), 3% of acute myeloid Leukemia (AML) samples (1/31), and 0% of Myeloproliferative Neoplasm (MPN) samples (0/39) suggesting a greater drug activity in patients with B-cell malignancies (Figure 2). Normal PBMCs showed no sensitivity to GS-1101.

Figure 2: Screening of Primary Malignant B-cells from Patients with ALL, CLL, Acute Myeloid Leukemia (AML), or Myeloproliferative Neoplasm (MPN) or from Normal Healthy Volunteers (NHV)

(Excerpted from the Applicant's submission)



Inhibition of Constitutive PI3K Signaling and Induction of Apoptosis

In SU-DHL-5/10, KARPAS, and CCRF-SB malignant B-cell lines from patients with diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and B-ALL, respectively, GS-1101 (CAL-101) reduced phosphorylated RAC-alpha serine/threonine protein kinase (p-Akt^{S473}) and produced a concentration-dependent reduction in phosphorylated RAC-alpha serine/threonine protein kinase (pAktT308) and the downstream target S6 ribosomal protein with an EC_{50} of 0.1-1.0 μM as measured using Western or ELISA analysis (Figure 3)

Figure 3: GS-1101 Inhibits PI3Kδ Signaling in Malignant B-cell Lines and Primary Patient Tumor Cells

(Excerpted from the Applicant's submission)

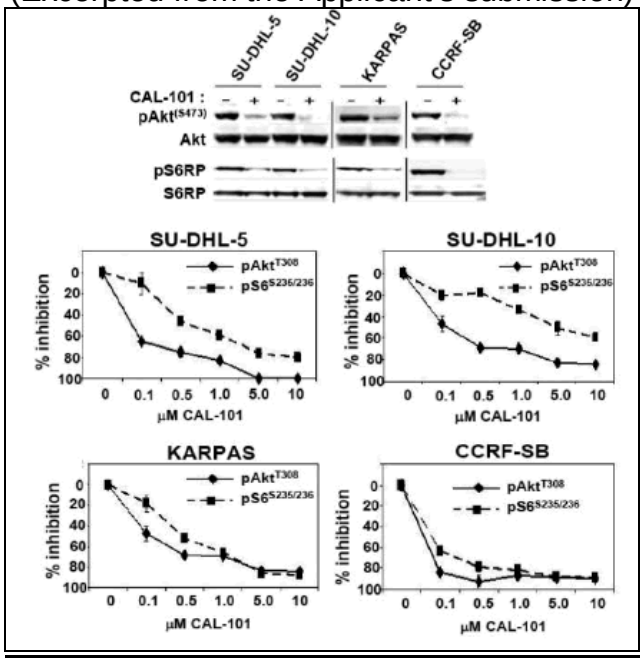


Figure 3: Serum-starved cells were incubated with 1μM GS-1101 (CAL-101), and total cell lysates were subjected to Western blot analysis using anti–phospho-AktS473, anti-Akt, anti-phospho S6S235/236, and anti-S6 antibodies (supplemental data). Starved cells were incubated with vehicle or serial dilution of GS-1101 for 1 hour, and pAktT308, pS6S235/236, total Akt, and total S6 were detected by PathScan sandwich ELISA.

FACS analysis was used to measure Akt phosphorylation in cells derived from patients with CLL or MCL. GS-1101 at 100 nM resulted in a 60% reduction in constitutive levels of $pAkt^{T308}$ (EC_{50}<100 nM) compared to untreated samples.

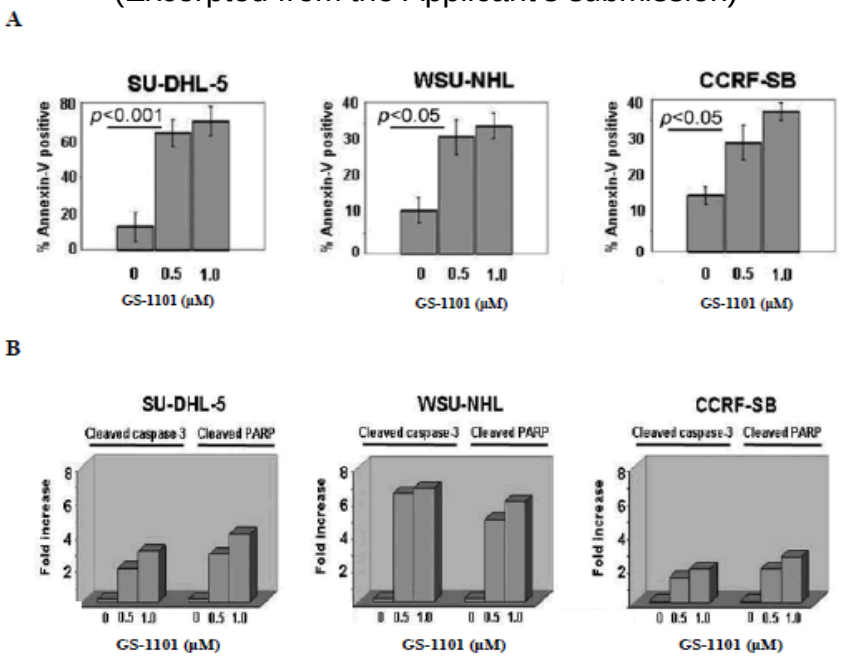
Phosphorylation of S473 in addition to T308 is required for full kinase activity and can occur through signals from the tumor microenvironment. The following tumor microenvironment signals caused rapid induction of $pAkt^{S473}$ that could be inhibited by GS-1101:

- CD40 activation in primary CLL cells and BCR activation in primary MCL cells and the Jeko MCL cell line (complete inhibition by GS-1101 at 1μM)
- CXCR5 and CXCR4 activation (~80% inhibition by GS-1101 at 1μM)
- Activation of B-cell activating factor (BAFF) which is involved in the proliferation and differentiation of B cells (~80% inhibition by GS-1101 at 1μM)

GS-1101 treatment (0.5-1μM) resulted in apoptosis induction and a dose-dependent, 2- to 8-fold increase in both caspase 3 and PARP cleavage in SU-DHL-5, WSU-NHL (FL) and CCRF-SB malignant B-cell lines compared to vehicle controls (Figure 4).

Figure 4: GS-1101 Induces Apoptosis in Malignant B-cell Lines

(Excerpted from the Applicant's submission)



A. CAL-101 induces apoptosis in diffuse large B-cell lymphoma, follicular lymphoma, and B-ALL cell lines. Cells were treated with vehicle or 0.5 or 1.0μM GS-1101 for 24 hours. The percentage of apoptotic cells was determined by annexin V–FITC/7-AAD staining followed by 2-color flow cytometric analysis.

Percentages represent both annexin V-FITC/7-AAD negative and annexin V–FITC/7-AAD double-positive. Results are expressed as mean ± SD. Statistically significant differences between means were determined using a one-way analysis of variance.

B. Cells were cultured in RPMI/10% fetal bovine serum with GS-1101 or vehicle alone for 24 hours, and cells were lysed and analyzed by PathScan Sandwich 96-well ELISA for the detection of cleaved caspase-3 and cleaved poly(ADP-ribose) polymerase (PARP).

Data are expressed as the fold change and are representative of 3 separate experiments.

Study title: The Phosphoinositide 3′-kinase Delta Inhibitor, GS-1101, Inhibits B-cell Receptor Signaling and Chemokine Networks in Chronic Lymphocytic Leukemia

Study No.:	PC-312-2003
Report Date:	May 28, 2013
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Gilead Sciences, Inc.
GLP:	No

Several *in vitro* assays were conducted that model the interactions of malignant B-cells and tumor environment which are thought to occur in patients with CLL. Chemotaxis assay, Migration assay, measurement of chemokine concentrations and immunoblotting assays are reviewed below. In these assays, CLL cells were stimulated through the B-cell receptors (BCR) with anti-IgM or through interactions induced by co-culture with Nurse-Like Cell (NLCs).

Chemotaxis Assay:

Chemotaxis assays across polycarbonate Transwell inserts were performed to determine the effect of GS-1101 on CLL cells. In the presence of 5 µM of GS-1101, CLL cell chemotaxis was significantly reduced when allowed to migrate towards 200 ng/mL CXCL12 or 1 g/mL CXCL13 compared to control cells without chemokine. The mean (± SEM) number of cells migrating toward CXCL12 decreased from 7022 (± 1420) to 4044 (± 918), while the mean (± SEM) number of cells migrating toward CXCL13 decreased from 6179 (± 1742) to 2276 (± 470) compared to control cultures (See figure below excerpted from Applicant's NDA).

Figure 5: GS-1101 Inhibits CLL Cell Chemotaxis Toward CXCL12 and CXCL13

(Excerpted from the Applicant's submission)

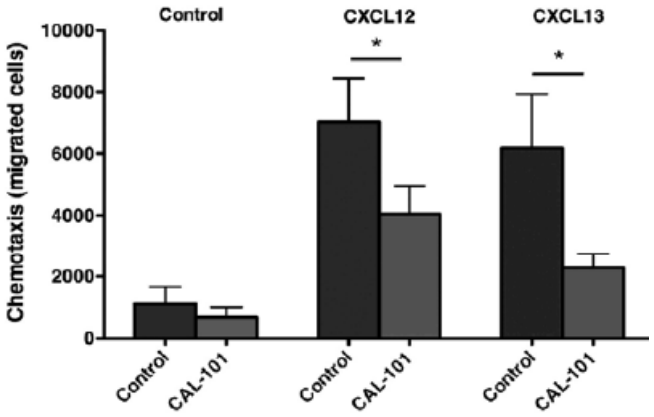


Figure 5: The bar diagram represents the mean chemotaxis (±SEM) of CLL cells from 10 different patients in the presence or absence of GS-1101 (CAL-101). Chemotaxis toward both CXCL12 and CXCL13 was significantly inhibited by GS-1101, with $P \leq 0.05$, as indicated by the asterisks.

Migration Assay Evaluating CLL Pseudoemperipolesis

CLL cell (stimulated with anti-IgM) migration beneath murine stromal cells (9-15C and TSt-4), seeded onto collagen coated plates, was quantified when incubated with and without 5 µM GS-11-1101. GS-1101 significantly reduced CLL cell migration beneath marrow stromal cells to 39.1% (± 4.6%) when 9-15C stromal cells were used, and from 191.1% (± 50.3) to 52.3% (± 7.3%) when TSt-4 stromal cells were used (See Figure 6).

Figure 6: GS-1101 Inhibits Migration Beneath TSt-4 or 9-15C Stromal Cells When CLL Were Stimulated with Anti-IgM

(Excerpted from the Applicant's submission)

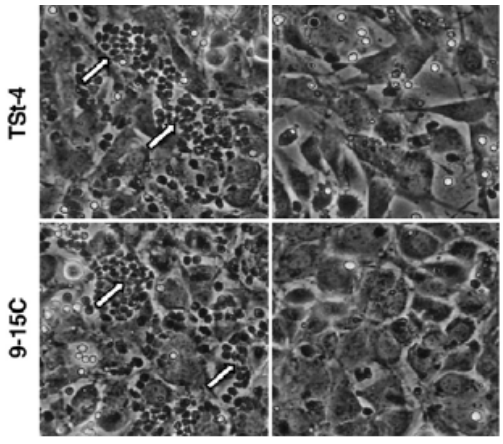


Figure 6: Representative phase-contrast photomicrographs displaying CLL cell migration beneath TSt-4 or 9-15C stromal cells. Pseudoemperipolesis is characterized by the dark appearance of CLL cells that

have migrated into the same focal plane as the stromal cells. There are numerous migrated CLL cells in the control wells (on the left), as indicated by the arrows, but only a few such cells in wells containing CLL cells pretreated with GS-1101 (CAL-101) (on the right).

Figure 7: The Mean Pseudoemperipolesis (±SEM) of CLL Cells from 9 Different Patients Beneath Each of the 2 Types of Stromal Cells in the Presence or Absence of GS-1101

(Excerpted from the Applicant's submission)

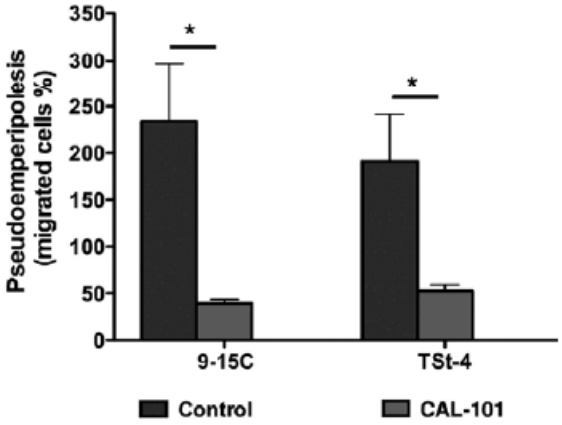


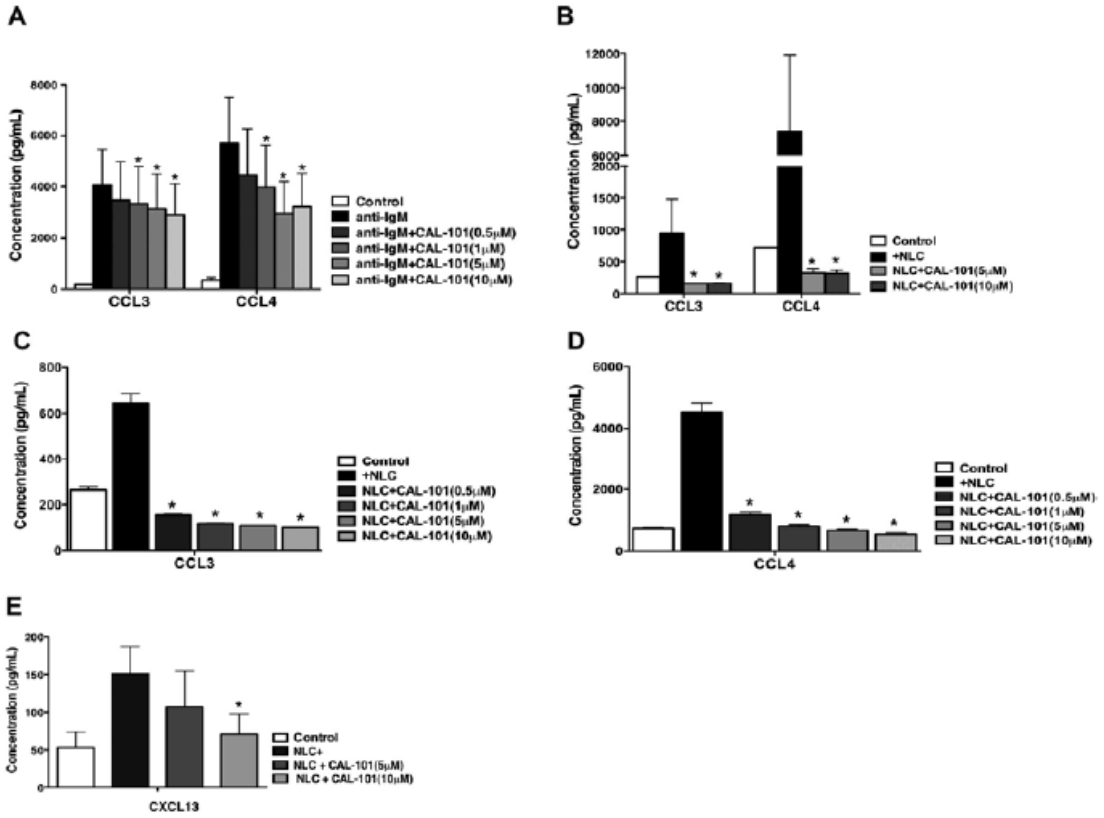
Figure 7: Pseudoemperipolesis beneath TSt-4 or 9-15C stromal cells was significantly inhibited by GS-1101 (CAL-101), with $P<0.05$, as indicated by the asterisks.

Measurement of Chemokine Concentrations using ELISA:

The effect of GS-1101 on chemokine ligand 3 (CCL3) and chemokine ligand 4 (CCL4) protein secretion after stimulation of CLL cells with anti-IgM (10 µg/mL) or with NLCs was measured. Stimulation of the BCR with anti-IgM resulted in a marked increase in secreted levels of the chemokines CCL3 and CCL4 by CLL cells. This effect was significantly inhibited by GS-1101 at concentrations of 1 µM or greater. Co-culture with NLCs also similarly induced CLL cells to secrete high concentrations of CCL3 and CCL4 into the supernatants and this secretion was significantly inhibited by GS-1101 at concentrations of 0.5 µM or greater (See Figure 8).'

Figure 8: NLC- and BCR-induced Secretion of the Chemokines, CCL3, CCL4, and CXCL13 by CLL Cells is Inhibited by GS-1101

(Excerpted from the Applicant's submission)



Legend for Figure 8:

A) The bar diagram represents the mean supernatant concentrations of CLL3 and CCL4 from CLL cells cultured in complete medium (control), medium supplemented with 10 µg/mL of anti-IgM, or anti-IgM and various concentrations of GS-1101 (CAL-101). The secretion of CCL3 and CCL4 from CLL cells in the presence of anti-IgM mAbs was significantly inhibited by GS-1101, with $P < 0.05$ (*) compared to the results from the culture containing anti-IgM alone.

B) The bar diagram represents the mean CLL cell supernatant concentrations for CCL3 and CCL4 from CLL cells cocultured with or without (controls) NLCs. The secretion of CCL3 and CCL4 from CLL cells was significantly inhibited by GS-1101, with $P < 0.05$ (*) compared to the results from the control culture.

C) Lower concentrations of GS-1101, which are indicated next to each bar diagram and depicted by different shades of gray, also significantly reduced CCL3,

D) And CCL4 from CLL cells cocultured with or without (controls) NLCs.

E) The level of CXCL13 in NLC cocultures after treatment with GS-1101. The secretion of CXCL13 was reduced by GS-1101, with $P \leq 0.05$ (*) to the results from control cultures.

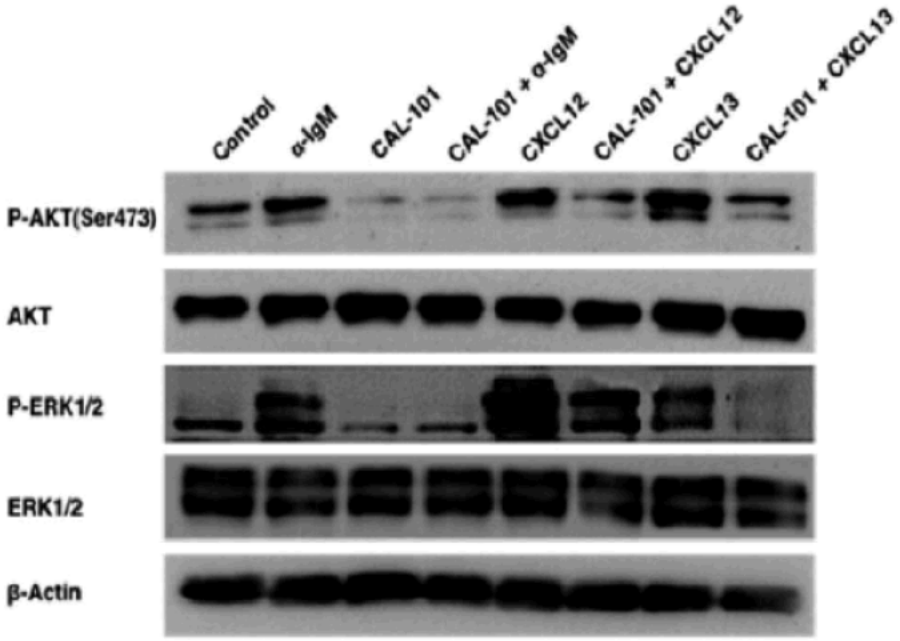
Immunoblotting:

CLL cells were activated with anti-IgM, or CXCL12, or CXCL13 stimulation by stromal cells via CXCR4 or CXCR5 chemokine receptors in the presence or absence of GS-1101. CLL cell lysates were probed with phospho-specific antibodies to AKT and ERK1/2, and antibodies for total AKT, ERK1/2, and actin. GS-1101 inhibited

phosphorylation of the downstream signaling kinases AKT and ERK in response to anti-IgM stimulation, and in response to CXCL12 or CXCL13, respectively (See Figure 9).

Figure 9: GS-1101 (CAL-101) Inhibits Signalling Downstream of the BCR, CXCR4, and CXCR5

(Excerpted from the Applicant's submission)



Study title: GS-1101 (CAL-101) is a Potent Selective Inhibitor of PI3Kδ: Isoform Selective Cell-Based

Study No.:	PC-312-2006
Report Date:	June 6, 2013
Study report location:	eCTD 4.2.1.1.
Conducting Laboratory:	Gilead Sciences, Inc.
GLP:	No

The cellular potency of GS-1101 (CAL-101) against all four of the Class I PI3K isoforms (PI3Kα, PI3Kβ, PI3Kδ, and PI3Kγ) was determined.

For the analysis of PI3Kα and PI3Kβ signaling, primary murine embryonic fibroblasts (MEFs) were stimulated for 10 minutes with platelet-derived growth factor (PDGF) or lysophosphatidic acid (LPA) prior to protein extraction and Western analysis to detect Akt and $pAkt^{S473}$ levels. EC_{50} values are based on a 50% reduction in relative phospho-Akt to total Akt levels compared to vehicle control.

For the analysis of GS-1101 on PI3Kδ and PI3Kγ signaling, basophil activation was measured in isolated human leukocytes (WBCs) using the Flow2 CAST® kit (b) (4)

following manufacturer's instructions. Activated basophils are CCR3 and CD63 positive and were measured using flow cytometry. EC_{50} values are based on CCR3/CD63+ Basophils (50% reduction relative to control).

Table 3: Summary of GS-1101 Potency in PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ Isoform-specific Cell-based Assays

(Excerpted from the Applicant's submission)

PI3K Isoform	Cell-based Assay and Stimulus	Primary Endpoint	EC_{50}[a] (nM)	Cell-based Delta Selectivity (fold)
PI3Kδ	Basophil and Anti- FcεRI	CD63 expression	8.9 n=27	1
PI3Kα	Murine Embryonic Fibroblast and PDGF	pAKT	> 10,000 N=3	> 1,124
PI3Kβ	Murine Embryonic Fibroblast and LPA	pAKT	1,419 N=4	159
PI3Kγ	Basophil and fMLP	CD63 expression	2,500 n=14	281

a EC_{50} data are geometric mean values

In these primary cell-based *in vitro* assays, GS-1101 inhibited PI3Kδ with an EC_{50} of 8.9 nM. There was more than a 100-fold difference in the EC_{50} for PI3Kδ and PI3Kγ, PI3Kβ and PI3Kα isoforms, indicating selectivity toward PI3Kδ in this assay.

Study title: Structure Determination of GS-1101 Bound to PI3Kδ

Study No.: PC-312-2007
Report Date: January 15, 2013
Study report location: eCTD 4.2.1.1.
Conducting Laboratory: Gilead Sciences, Inc.
GLP: No

The crystal structure of GS-1101 bound to PI3Kδ revealed that GS-1101 inhibits PI3Kδ by binding to the ATP binding site of the kinase domain.

Study title: Biochemical Assay to Access PI3Kδ *in vitro* Potency and Isoform Selectivity

Study No.: PC-312-2008
Report Date: May 28, 2013
Study report location: eCTD 4.2.1.1.
Conducting Laboratory: Gilead Sciences, Inc.
GLP: No

Enzymatic activity of the class I PI3K isoforms, expressed and purified as heterodimeric recombinant proteins, was measured using a time resolved fluorescence resonance energy transfer (TR-FRET) assay. The formation of 3,4,5-inositol triphosphate (PIP3)

molecule, in the presence of PI3K, ATP, and PIP2, is measured by its ability to compete with and displace fluorescently labeled PIP3 for binding to the GRP-1 pleckstrin homology domain protein, thereby decreasing TR-FRET. Therefore, inhibition of PI3K is measured as an increase in TR-FRET. Data are normalized based on a positive (1 μM wortmanin) and negative (DMSO) controls and IC_{50} values were calculated from the fit of the dose−response curves to a four-parameter equation.

The TR-FRET assay is generally more sensitive than the radioactivity format because it utilizes pM concentration of enzyme rather than nM and can accurately determine IC_{50} values below 1 nM. For PI3Kα,β,δ, between 25-50 picomolar (pM) was used in the assay and for PI3Kγ, 2 nanomolar (nM) was used. The ATP Km values determined using the TR-FRET assay for each PI3K isoform were different (Table 4) and the Applicant decided to determine IC_{50}'s at 2 X Km ATP for each PI3K isoform in order to accurately compare compound selectivity between all four isoforms.

Table 4: ATP Km for PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ from TR-FRET Assay

(Excerpted from the Applicant's submission)

	TR-FRET ATP Km
PI3Kα	48 μM
PI3Kβ	279 μM
PI3Kδ	118 μM
PI3Kγ	37 μM

Table 5: IC_{50} values GS-1101 for PI3Kα, PI3Kβ, PI3Kδ and PI3Kγ

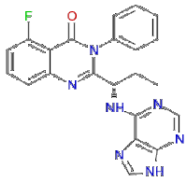
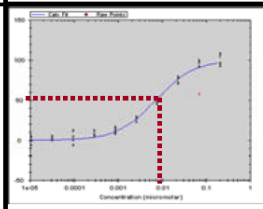
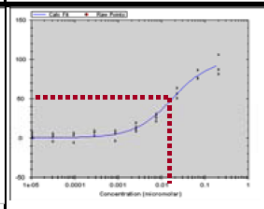
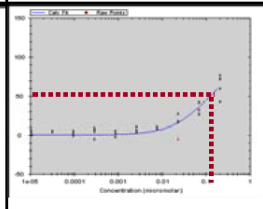
(Excerpted from the Applicant's submission)

	IC_{50} Values Determined at 2XKm ATP
PI3Kα	8600 nM
PI3Kβ	4000 nM
PI3Kδ	19 nM
PI3Kγ	2100 nM

GS-1101 is 453-fold more selective for PI3Kδ relative to PI3Kα, 210-fold more selective relative to PI3Kβ, and 110-fold more selective over PI3Kγ (Table 5).

Table 6: Effect of ATP concentration on the IC_{50} value of GS-1101 on PI3Kδ enzymatic activity

(Excerpted from the Applicant's submission)

		IC50 (nM) at varying ATP (μM)		
GS ID	**Structure**	**20**	**200**	**2000**
GS-001101		 **7 nM**	 **19nM**	 **122nM**

Increasing ATP concentrations from the concentration that was used in historical radioactive assays (20 μM) to 200 μM (2 X KmATP) results in weaker (less potent) IC_{50} values for GS-1101 against PI3Kδ (Table 6), which along with the crystal structure demonstrating that GS-1101 binds to the ATP binding site of PI3Kδ (Study No. PC-312-2007) is consistent with a competitive mode of inhibition.

Study title: Effect of IDELA on Stimulated P-AKT Level in Malignant B-Cells Prepared from Biopsy Specimens from Patients with Follicular Lymphoma

Study No.: PC-312-2010
Report Date: May 30, 2013
Study report location: eCTD 4.2.1.1.
Conducting Laboratory: Gilead Sciences, Inc.
GLP: No

IDELA (GS-1101), at a concentration of 0.1 μM for 60 minutes, inhibited P-AKT (Ser473) an average of 86% (range 55-97%) in malignant B-cells prepared from biopsy specimens of 7 patients with follicular lymphoma and stimulated by crosslinking the BCR (Table 7, last column). P-AKT (Ser473) was measured by flow cytometry and anti-IgM/IgG was used for BCR stimulation for 15 minutes after treatment with GS-1101 or vehicle control. The percentage of the maximum inhibition of the BCR-stimulated signal that can be achieved by GS-1101 is calculated with the formula (% BCR stim control - % BCR stim + 0.1 μM GS-1101) / (% BCR stim control – basal signal + 0.1 μM GS-1101) x 100.

Table 7: Normalized Median Fluorescence Intensity (MFI) Signal for P-AKT (Ser473) Alexa Fluor 488 Staining in CD20+/CD3- Follicular Lymphoma Cells Prepared from Fine Needle Biopsies of 7 Patients

(Excerpted from the Applicant's submission)

	Basal Signal Control[a]	Basal Signal +0.1μM GS-1101	% Inhibition of Basal Signal	BCR stim Control	BCR stim +0.1μM GS-1101	% Inhibition of Maximal Inhibition[b]
Patient 13	100	99	1	200	114	85
Patient 14	100	71	29	138	73	97
Patient 15	100	84	16	178	92	91
Patient 16	100	74	26	311	180	55
Patient 17	100	94	6	211	98	97
Patient 18	100	95	5	146	97	96
Patient 19	100	78	22	450	138	84

a Data normalized for each patient unstimulated untreated MFI = 100%

b Calculated with the formula (% BCR stim control - % BCR stim + 0.1μM GS-1101) / (% BCR stim control – basal signal + 0.1μM GS-1101) x 100

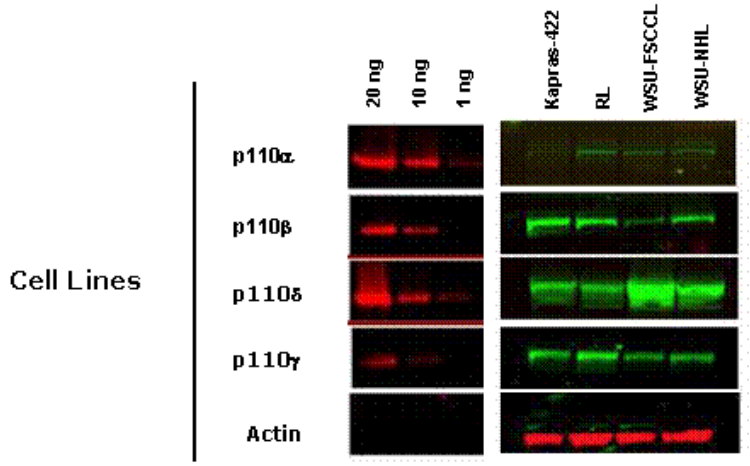
Study title: The Effect of GS-1101 on Viability, Proliferation and Apoptosis of Two Follicular Lymphoma-Derived Cell Lines

Study No.: PC-312-2011
Report Date: May 29, 2013
Study report location: eCTD 4.2.1.1.
Conducting Laboratory: Gilead Sciences, Inc.
GLP: No

Western Blot analysis demonstrated that P110δ (the catalytic subunit of PI3Kδ with kinase activity, containing the ATP binding site) was highly expressed in WSU-FSCCL and WSU-NHL, two cell lines derived from follicular lymphoma patients (Figure 10). These cell lines were selected for further evaluation of the activity of GS-1101 on viability, proliferation, and apoptosis

Figure 10: Expression by Western Blotting of PI3K Type I Catalytic Sub-units in WSU-NHL and WSU-FSCCL Cell Lines Derived from FL Patients and Karpas-422 and RL Derived from Diffuse Large B-cell Lymphomas

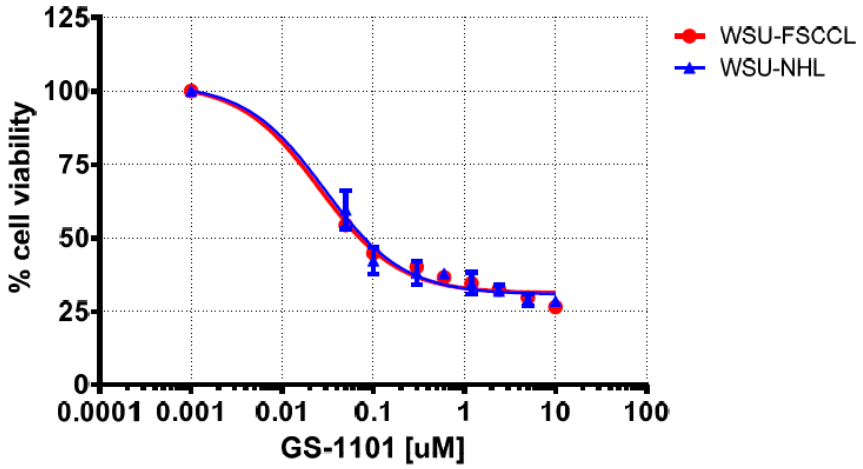
(Excerpted from the Applicant's submission)



The concentration of GS-1101 eliciting a 50 % effect on viability was 0.03μM both for WSU-NHL and WSU-FSCCL at 72h (Figure 11).

Figure 11: Dose-dependent Inhibition of Viability in WSU-NHL and WSU-FSCCL Cells after 72 Hours of Treatment with GS-1101 Measured by alamarBlue Assay

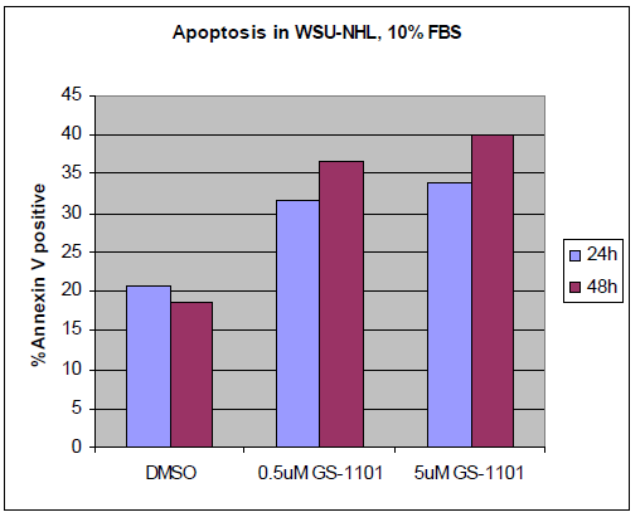
(Excerpted from the Applicant's submission)



Treatment of WSU-FSCCL cells with 0.5 and 5 μM GS-1101 for 24 hours resulted in an increased proportion of cells in the G1 phase of the cell cycle and a corresponding decrease in cells in S and G2/M (as measured by propidium iodide labeling of DNA and fluorescent flow cytometry). This was not observed in WSU-NHL cells, where there was no change in the proportion of cells in G1, S or G2/M phases of the cell cycle, but there was a 5-fold increase in the proportion of cells with a sub-G1 content of DNA, indicating apoptosis was observed in WSU-NHL cells. Consistent with this observation, treatment of WSU-NHL cells with 0.5 or 5 μM GS-1101 for 24h or 48h led to an increase in the proportion of AnnexinV positive cells (at 24h: DMSO: 20%, 0.5μM GS-1101: 31%) (Figure 12).

Figure 12: Analysis of the Proportion of WSU-NHL Cells Undergoing Apoptosis after 24 and 48 hours of GS-1101 Treatment Measured by AnnexinV Staining

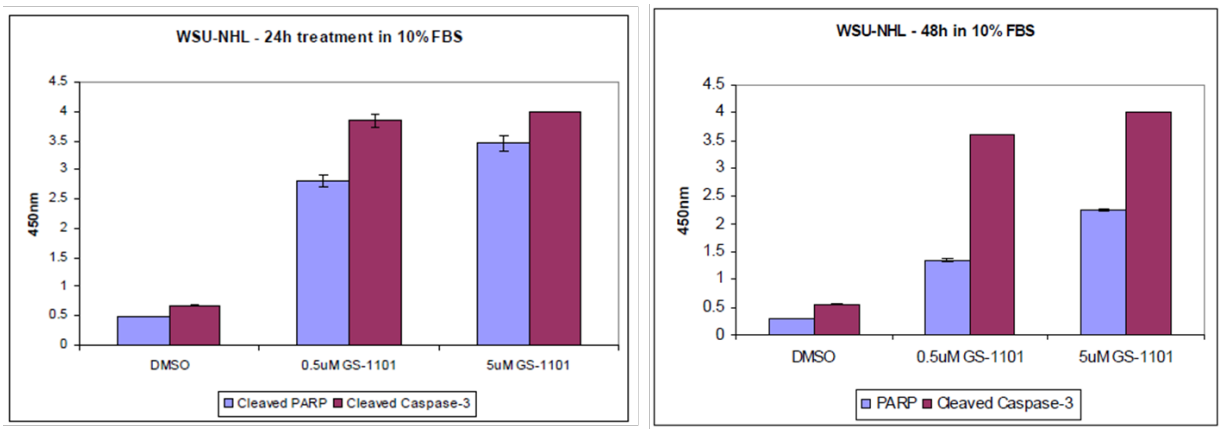
(Excerpted from the Applicant's submission)



Similarly, the presence of cleaved caspase 3 or cleaved PARP was increased by 5.6- and 5.8-fold respectively after 24h treatment of WSU-NHL cells with 0.5 µM GS-1101 (Figure 13).

Figure 13: Analysis of the Proportion of WSU-NHL Cells Undergoing Apoptosis after 24 and 48 hours of GS-1101 Treatment Measured by Cleaved Caspase 3

(Excerpted from the Applicant's submission)



Study title: Evaluation of GS-563117 for Inhibition of Class I PI3K Isoforms in a Biochemical Assay

Study No.: PC-312-2012
Report Date: June 24, 2013
Study report location: eCTD 4.2.1.1.

Conducting Laboratory: Gilead Sciences, Inc.
GLP: No

The major metabolite of GS-1101, GS-563117 (also called CAL-244), was evaluated for its potential to inhibit PI3Kδ and other Class I isoforms using the TR-FRET method (described in study number PC-312-2008).

The IC_{50} values of GS-563117 for PI3Kδ, PI3Kα, PI3Kβ, and PI3Kγ were all greater than 10 µM in this assay, indicating that GS-563117 has substantially less inhibitory activity than GS-1101 against PI3K isoforms. The IC50 of GS-1101 was 19 nM for PI3Kδ (see Study number PC-312-2008)

4.2 Secondary Pharmacology

Study title: Evaluation of Compounds on LOK and SLK Kinase Activities

Study No.: 2408
Report Date: December 22, 2010
Study report location: eCTD 4.2.1.2.
Conducting Laboratory: Calistoga Pharma, Inc.
GLP: No

The phosphotransferase activity of LOK and SLK protein kinase targets (~50-100 ng/assay) in the presence of CAL-101 (0.03-100µM) or the primary human metabolite of CAL-101, CAL-244 (GS-563117), was performed using radioisotope labeling of ATP (10 µM ^{33}P-ATP). 1µM staurosporine was used as a positive control for LOK and SLK kinase inhibition.

At the highest concentration of 100 µM, CAL-244 (the human metabolite, GS-563117) completely inhibited the activity of LOK and SLK, whereas CAL-101 only inhibited LOK and SLK by 33% and 27%, respectively, compared to the untreated control. The IC_{50} values for CAL-244 inhibition of LOK and SLK were 0.11 µM and 0.05 µM, respectively. Therefore, CAL-244 was a stronger inhibitor than the parent drug and CAL-101 was a weak inhibitor of LOK and SLK protein kinases.

Study title: Kinase Selectivity Profile for IDELA

Study No.: CAL001-01-p-00001
Report Date: January 25, 2008
Study report location: eCTD 4.2.1.2.
Conducting Laboratory: Calistoga Pharma, Inc.
GLP: No

Six compounds at 10µM, including IDELA (CAL-101), were screened against panel of 353 kinases[6]. Assays were performed in duplicate. The cutoff for being a positive hit

[6] Fabian et al. (2005) Nature Biotechnology, vol. 23, p.329

was greater than 35% of the vehicle control, or greater than 65% kinase inhibition. Any inhibition below the cutoff for a positive hit was listed as "no hit" in the study report and actual values for % kinase inhibition were not provided.

CAL-101 tested positive for inhibiting PIK3CA (PI3Kα), PIK3CA (E545K), PIK3CB (PI3Kβ), PIK3CD (PI3Kδ), and PIK3CG (PI3Kγ) to 17%, 18%, 0%, 0.35% and 2.7%, respectively, of the DMSO vehicle-treated control.

Study title: Kinase Selectivity Profile of GS-563117

Study No.:	CAL007-01-p-00001
Report Date:	October 26, 2010
Study report location:	eCTD 4.2.1.2.
Conducting Laboratory:	Gilead Sciences, Inc.
GLP:	No

The primary human metabolite of GS-1101, CAL-244 (GS-563117, Lot # 805-DJA-28K) at 10μM, was screened against panel of 442 kinases using KINOMEscan™ *in vitro* competitive binding assay by (b) (4) developed from the same technology used to screen the parent compound[7]. The ability of GS-563117 to compete with immobilized small molecule ligands for binding to a DNA-tagged kinase is measured via quantitative PCR of the DNA tag.

GS-563117 inhibits 9 kinases similar to or greater than PI3Kδ and is therefore not selective for PI3Kδ.

Table 8: Selectivity of GS-563117 using KINOMEscan™ *in vitro* competitive binding assay

Ambit Gene Symbol	% Vehicle Control at 10μM
ABL1(H396P)-nonphosphorylated	18
LCK	27
LIMK2	26
LOK	0.1
MAP3K1	4.2
PIK3CD (PI3Kδ)	27
QSK	20
SIK2	34
SLK	1
TNNI3K	19

The following kinases were also inhibited to < 50% activity compared to the vehicle control by GS-563117: ABL1(H396P)-phosphorylated (43%), ABL1(M351T)-phosphorylated (37%), ABL1(T315I)-phosphorylated (40%), BRAF(V600E) (41%), DDR1 (44%), LIMK1 (47%), MKNK2 (44%), PIK3C2G (46%), PIK3CB (41%), and SIK (46%).

[7] Fabian et al. (2005) Nature Biotechnology, vol. 23, p.329

4.3 Safety Pharmacology

Study title: HERG Channel Inhibition by Test Compounds CAL 101 and CAL 102 Using a Rubidium Flux Method

Study no:	BHR00004
Study report location:	4.2.1.3.
Conducting laboratory and location:	(b) (4)
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	CAL-101, Lot # 22033-34, and CAL-102 (a structurally related compound to Cal -101, Lot# 946-24-1); purity information was not provided. Both test articles are GS-1101 with different molecular weights.

Key study findings:

- Under the conditions tested, CAL-101 (idelalisib) and CAL-102 (a structurally related compound to CAL -101 before the lead compound was chosen) have no inhibitory effect on hERG channel at concentrations up to 50 μM. The IC_{50} of CAL-101 and CAL-102 on the hERG potassium channels was greater than 50μM.

Methods:

Strains/species/cell line:	Human HEK293 cells
Controls:	Vehicle: DMSO
Negative Control	Lidocaine
Positive control	Astemizole
Concentrations:	10 concentrations ranging from 0.0977 to 50 μM
Test system:	Rubidium flux-based method

Results:

Figure 14: CAL-101 and CAL-102 Inhibition of HERG Channel Activity

(Excerpted from the Applicant's submission)

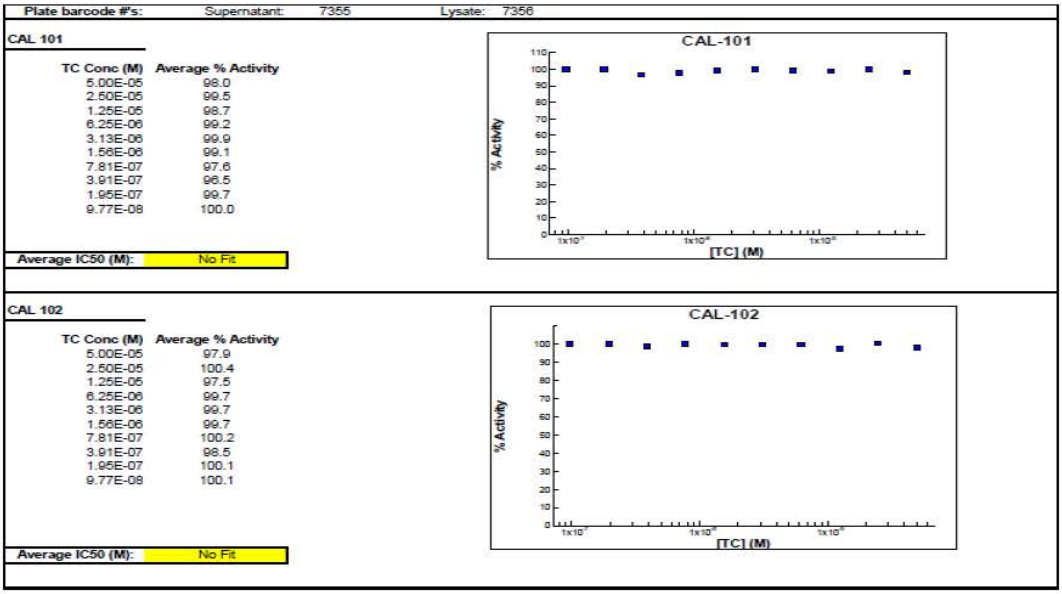


Plate barcode #'s: Supernatant: 7355 Lysate: 7356

CAL 101

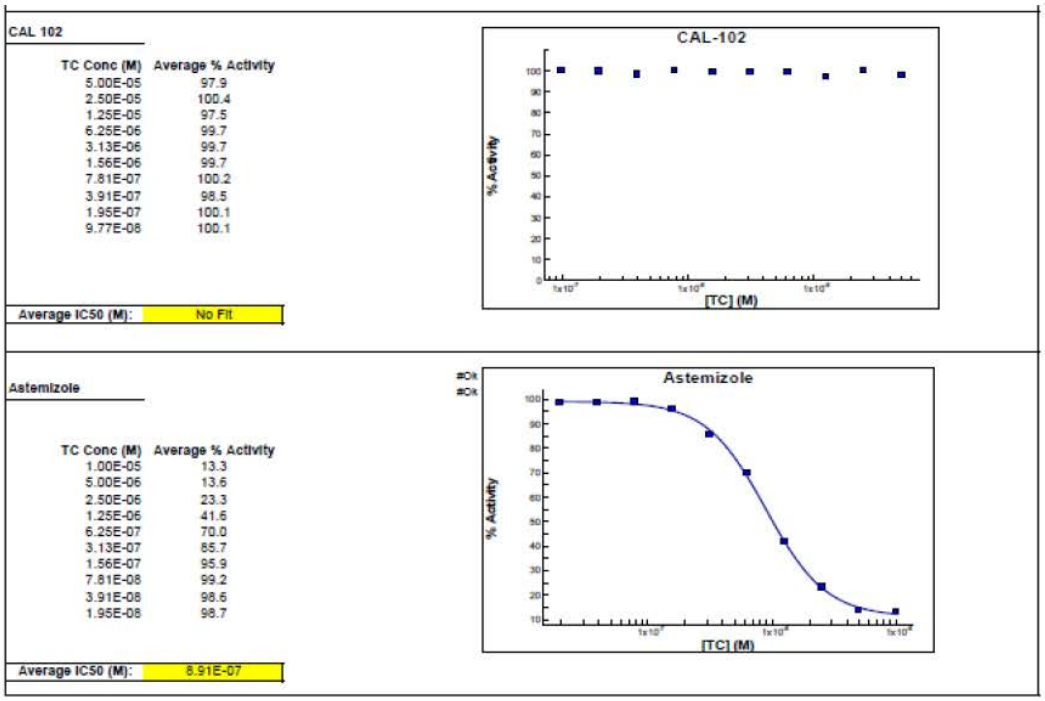
TC Conc (M)	Average % Activity
5.00E-05	98.0
2.50E-05	99.5
1.25E-05	98.7
6.25E-06	99.2
3.13E-06	99.9
1.56E-06	99.1
7.81E-07	97.6
3.91E-07	96.5
1.95E-07	99.7
9.77E-08	100.0

Average IC50 (M): No Fit

CAL 102

TC Conc (M)	Average % Activity
5.00E-05	97.9
2.50E-05	100.4
1.25E-05	97.5
6.25E-06	99.7
3.13E-06	99.7
1.56E-06	99.7
7.81E-07	100.2
3.91E-07	98.5
1.95E-07	100.1
9.77E-08	100.1

Average IC50 (M): No Fit



CAL 102

TC Conc (M)	Average % Activity
5.00E-05	97.9
2.50E-05	100.4
1.25E-05	97.5
6.25E-06	99.7
3.13E-06	99.7
1.56E-06	99.7
7.81E-07	100.2
3.91E-07	98.5
1.95E-07	100.1
9.77E-08	100.1

Average IC50 (M): No Fit

Astemizole

TC Conc (M)	Average % Activity
1.00E-05	13.3
5.00E-06	13.6
2.50E-06	23.3
1.25E-06	41.6
6.25E-07	70.0
3.13E-07	85.7
1.56E-07	95.9
7.81E-08	99.2
3.91E-08	98.6
1.95E-08	98.7

Average IC50 (M): 8.91E-07

Study title: A Cardiovascular and Respiratory Safety Pharmacology Study of CAL-101 Administered Orally to Telemetered Beagle Dogs

Study no:	BHR00041
Study report location:	4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 4, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CAL-101, Lot # 990547-31-07-07-01, 10% w/w in Lactose Monohydrate

Key study findings:

- No toxicologically significant CAL-101 induced effects were seen on heart rate, arterial pressure, body temperature, or electrocardiographic intervals, under the conditions tested.

- Slight increases in systolic arterial pressure (SAP; increased up to 6%) and mean arterial pressure (MAP; increased up to 4%) were observed 60 to 90 minutes after the 5- and 20-mg/kg doses of CAL-101. These changes were small and transient and hence, not considered adverse.

Methods:

Doses:	0, 1, 5, 20 mg/kg/day
Frequency of dosing:	Single dose with monitoring up to 24 hours post-dose
Route of administration:	Orally via capsule
Dose volume:	NA
Formulation/Vehicle:	Lactose monohydrate at an amount equivalent to the amount of blend needed to dose
Species/Strain:	Dog / Beagle (telemetered non-naïve male dogs)
Number/Sex/Group:	4 males
Age:	>9 months old
Weight:	7 and 15 kg
Satellite groups:	None

Dose justification: Based on previous studies including 28-day study in dogs.
-The lowest dose represented a projected no-effect dose level
-The highest dose was not expected to cause toxicity that would interfere with collection of the cardiovascular or respiratory data.
-The intermediate dose was present to assess potential dose-response relationships.

Experimental Design (excerpted from the Applicant's Submission)

Treatment Number	Number of Animals Males[a]	Test Material	Dose Level (mg/kg)[b]	Monitoring Period
1	4	Control[c]	0	2 hours before dosing through 24 hours after dosing
2		CAL-101	1	
3			5	
4			20	

[a] The same 4 animals were dosed in each dose session.
[b] Animals were administered between 1 and 5 capsules; actual number of capsules administered was recorded in the raw data.
[c] Control animals were dosed with lactose monohydrate at an amount equivalent to the amount of blend needed to dose Treatment 4 (corrected for the absence of CAL-101).

Schedule of Treatments

Animal Number	Session 1	Session 2	Session 3	Session 4
1001	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1002	Treatment 2	Treatment 4	Treatment 1	Treatment 3
1003	Treatment 3	Treatment 1	Treatment 4	Treatment 2
1004	Treatment 4	Treatment 3	Treatment 2	Treatment 1

Session 1 = Study Day (D)1; Session 2 = D3; Session 3 = D8; Session 4 = D13

Observations and Results:

Mortality: No mortality occurred in the study.

Heart rate: unremarkable

Mean arterial pressure (MAP): MAP was increased by 3% to 4% approximately 60 to 90 minutes after dosing with both the 5- and 20-mg/kg doses of CAL-101. This change was transient and not considered adverse.

Figure 15: MAP Changes after Oral Administration of CAL-101 to Dogs

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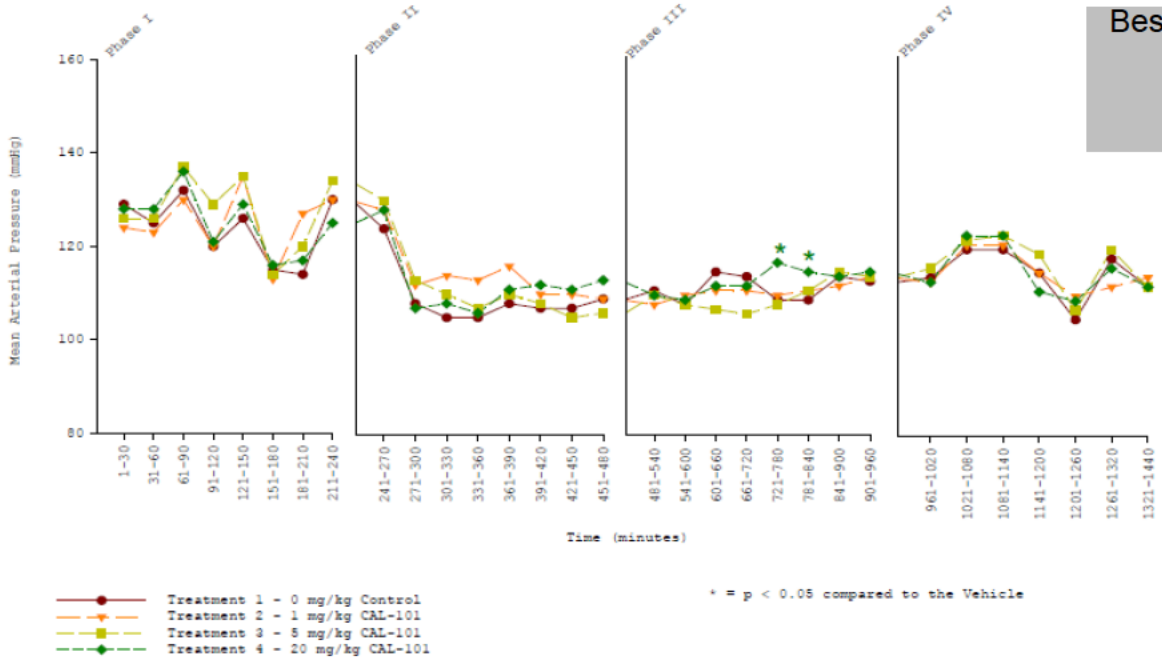
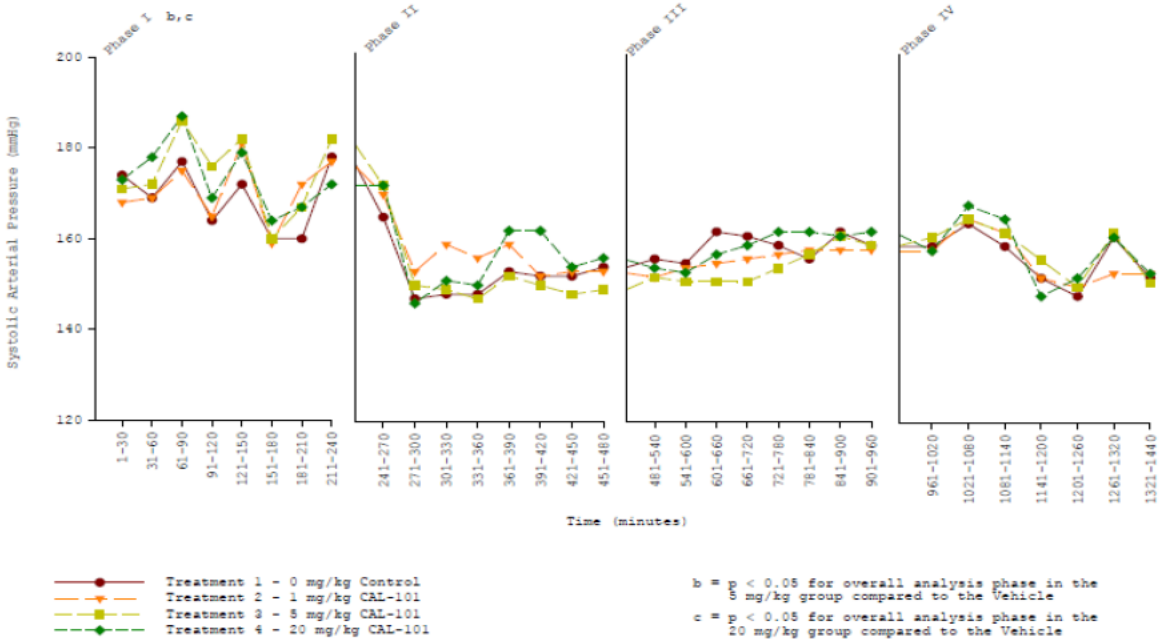


Systolic Arterial Blood Pressure (SAP): SAP was increased by 5% to 6% approximately 60 to 90 minutes after dosing with both the 5- and 20-mg/kg doses of CAL-101. This change was transient and not considered adverse.

Figure 16: SAP Changes after Oral Administration of CAL-101 to Dogs



Diastolic pressure: unremarkable

Pulse pressure: unremarkable

Electrocardiograph: unremarkable

QT/QTc intervals: unremarkable

Respiratory rate: unremarkable

Body temperature: unremarkable

Study title: CAL-101: Single-Dose Safety Pharmacology Neurofunctional Assessment in Sprague-Dawley Rats

Study no:	BHR00042
Study report location:	4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 23, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CAL-101, lot # 22033-51-38, purity 99.3%

Key study findings:

No CAL101-induced neurobehavioral effects were noted, under the conditions tested.

Methods:

Doses:	0, 50, 100 and 150 mg/kg
Frequency of dosing:	Single dose with neurobehavioral observations at 1, 2, 4, 8, and 24 hours post-dose
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) Carboxymethylcellulose (CMC) and 0.1% (v/v) TWEEN 80 in water
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	Group 1: Vehicle - 13 males and 11 females Group 2: 50 mg/kg - 11 males and 12 females Group 3: 100 mg/kg - 9 males and 8 females Group 4: 150 mg/kg – 7 males and 9 females
Age:	10-12 weeks
Weight:	375 to 440 g
Satellite groups:	3/group
Dose justification:	Based on previous studies including 28-day study in rats. -The lowest dose represented a projected no-effect dose level -The highest dose was not expected to cause severe toxicity that would interfere with collection of the Functional Observational Battery (FOB) data. -The intermediate dose was present to assess potential dose-response relationships.

Observations:

FOB observations	**Observation**
Central Nervous System (CNS) activity and excitability:	Home cage and open field, vocalization, ease of removal from the home cage, response to handling, the presence of abnormal and/or stereotypical behavior, overall activity, arousal, and the incidence of rearing in the open field.
Autonomic Nervous System (ANS) Effects	Changes in palpebral closure, pupil response and diameter, presence of salivation, piloerection, urination and defecation frequency and consistency, lacrimation, and respiration.
Sensorimotor effects	Tactile and auditory reaction to stimuli, or the reaction to a painful stimulus as a timed response via tail flick measurement.
Neuromuscular effects	Body tone, impairment or abnormalities in gait, surface and air righting reflexes, grip performance (forelimb and hindlimb), and

	landing foot splay.

Results:

CNS activity and excitability: unremarkable

Autonomic Nervous System (ANS) Effects: unremarkable

Sensorimotor Effects: unremarkable

Neuromuscular Effects: unremarkable

Body temperature: unremarkable

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Study title: Pharmacokinetics and Bioavailability of CAL-101 Following Intravenous and Oral Administration to Female Sprague-Dawley Rats

Study no.: BHR00012
Study report location: 4.2.2.2.
Conducting laboratory and location: (b) (4)
Date of study initiation: August 8, 2007
GLP compliance: No
QA statement: NA
Drug, lot #, and % purity: CAL-101, Lot No. 21905-115-20, Purity: not listed

Key Study Findings

- The oral bioavailability of CAL-101 in rats is 39%.

Study Design:

(Excerpted from the Applicant's submission)

Treatment								
Group No.	No. of Females	Test Article	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Vehicle	Dose Route	Flush
1	3	CAL 101	3	3	1	10 % Ethanol/ Citrix Acid Buffer Solution	Intravenous	0.5 mL Saline
2	3	CAL 101	3	3	1	0.5% w/v Carboxymethylcellulose/ 0.1% v/v Tween 80	Oral Gavage	NA

NA= Not Applicable

Vehicle: 10% Ethanol/Citric acid buffer solution

Methods

- Whole blood samples (0.25 mL, K2EDTA anticoagulant) were collected at pre-dose, 5, 15, 30, 60, 90 min, 2, 3, 4, 6, 8, 12, and 24 hours post-dose.
- Urine was collected on frozen cold packs from each animal, prior to dosing, 0-6, 6-12, and 12-24 hours post-dose.

Results

Table 9: Mean Pharmacokinetic Parameters for IV and Oral Dosing of CAL-101 in Rats

Test article	AUC_{inf} (hr*ng/mL)	AUC_{inf} /Dose	C_{max} (ng/mL)	C_{max} /Dose	T_{max} (hr)	$t_{1/2}$ (hr)	Vss (L/kg)	Cl (ml/min/kg)	F%
CAL-101 (IV)	1151±407	384±136	1437±220	479±73	0.08	1.89	2.49	478±19	NA
CAL-101 (oral)	422±67	141±22	129±49	43±16	3.00	1.52	NA	NA	39±13

F = Absolute bioavailability
NA = Not applicable
Dose = 3 mg/kg

Study Title: Pharmacokinetics and Bioavailability of CAL 101 Following Crossover Administration to Female Beagle dogs.

Study No.: BHR00011
Report Date: May 4, 2007
Study report location: 4.2.2.2.
Conducting Laboratory: (b) (4)
GLP: No
Drug, lot #, and % purity: CAL-101, Lot No. 21905-115-20, Purity: not listed

Key Findings

- The oral bioavailability of CAL-101 in dogs is 48%.

(Study design excerpted from Applicant's submission)

Treatment									
Session No.	Group No.	No. of Females	Test Article	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg)	Vehicle	Dose Route	Flush
I	1	3	CAL 101	1	1	1	10% Ethanol/ Citric Acid Buffer Solution	Intravenous Bolus	3 mL saline
II	1	3	CAL 101 (blend)	1	NA	NA	NA	Oral Capsule	20 mL Water
	2	3	CAL 101 (blend)	1	NA	NA	NA	Oral Capsule	20 mL Water

Methods:

- The pharmacokinetics and bioavailability of CAL-101 was investigated following crossover administration to female Beagle dogs. CAL-101 was administered to female dogs at 1 mg/kg as a bolus injection or an encapsulated powder.

- Whole blood samples (1 mL; K2EDTA anticoagulant) were collected at pre-dose, 5, 15, 30, 60, 90 min, 2, 3, 4, 6, 8, 12, and 24 hours post-dose.

- Urine was collected on frozen cold packs from each animal, prior to dosing, 0-6, 6-12, and 12-24 hours post-dose.

Table 10: Mean Plasma Pharmacokinetic Parameters for CAL-101 Following a Bolus IV Injection or Oral Administration in a Capsule at 1 mg/kg to Female Beagle Dogs (mean ± StD, n = 3)

(Excerpted from the Applicant's submission)

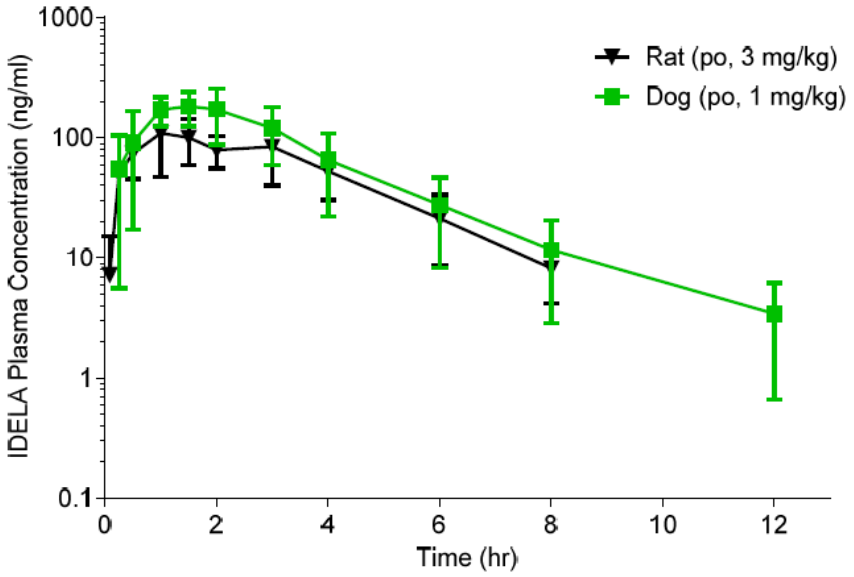
Test article	Mean Body Weight (g)	AUC_{inf}* (hr*ng/mL)	C_{max}* (ng/mL)	T_{max} (hr)	$t_{1/2}$ (hr)	Vss(L/kg)	Cl (ml/min/kg)	F%
CAL-101 (IV)	6.84±0.37	1432 ± 509	977 ± 314	0.08	2.31 ± 0.43	1.23 ± 0.30	0.76 ± 0.25	NA
CAL-101 (IV followed by oral)	7.65± 1.9	1081± 284	510± 62	0.5	2.31± 0.43	NA	NA	79± 23
CAL-101 (oral)	8.15±1.0	671 ± 277	209 ± 68	1.0	1.99 ± 0.10	NA	NA	48 ± 23

CL= systemic clearance of the drug after intravenous administration; Vss = apparent steady-state volume of distribution of the drug; NA = not applicable; F = Absolute bioavailability
*Dose = 1 mg/mL, therefore AUC_{inf} and C_{max} values are equivalent to AUC_{inf}/Dose and C_{max}/Dose, respectively

When comparing PK parameters between studies BHR00011 and BHR00012, the systemic exposure/dose of idelalisib (C_{max} and AUC) was approximately 5-fold higher in dogs after oral dosing than in rats, possibly due to the higher clearance of idelalisib in rats.

Figure 17: Mean Plasma Concentration Versus Time Profiles for Idelalisib Following Oral Administration in Suspension to Female Sprague-Dawley Rats (3 mg/kg) and in a Capsule to Female Beagle Dogs (1 mg/kg) (mean ± StD, n = 3)

(Excerpted from the Applicant's PK written summary)



Study title: Tissue Distribution of Radioactivity by Quantitative Whole-Body Autoradiography in Sprague Dawley and Long Evans Rats Following a Single Oral Dose of 14C-CAL-101

Study no.:	1503-058
Study report location:	4.2.2.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 20, 2010
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	CAL-101 [purine ring-8-14C], Lot No. 3579087 (originally from CAL-101 Lot No. 60182-09-003), Purity: 98.1% (reverse phase HPLC)

Key Study Findings

- Biliary route is primary route of excretion, with the majority of CAL-101 being detected in the feces and less than 5% in the urine.
- All tissues in Sprague-Dawley (SD) rats, except large intestine and cecum, had reached maximal concentration at 1 hour postdose (35 of 37 tissues) and decreased 24-48 hours postdose.
- In LE rats, pigmented skin and pigmented eye uvea showed higher concentrations of CAL-101 than that observed for the similar tissues in SD rats, suggesting some association of drug-derived radioactivity with melanin.

Methods

Doses:	50 mg/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% CMC, 0.1% TWEEN 80
Species/Strain:	Crl:CD(SD), Long Evans (LE)
Number/Sex/Group:	SD: 5 males + 2 spares LE: 2 males + 1 spare
Age:	6-12 weeks old
Weight:	275-300 g
Satellite groups:	None
Unique study design:	See table below for dose administration information

(Excerpted from the Applicant's submission)

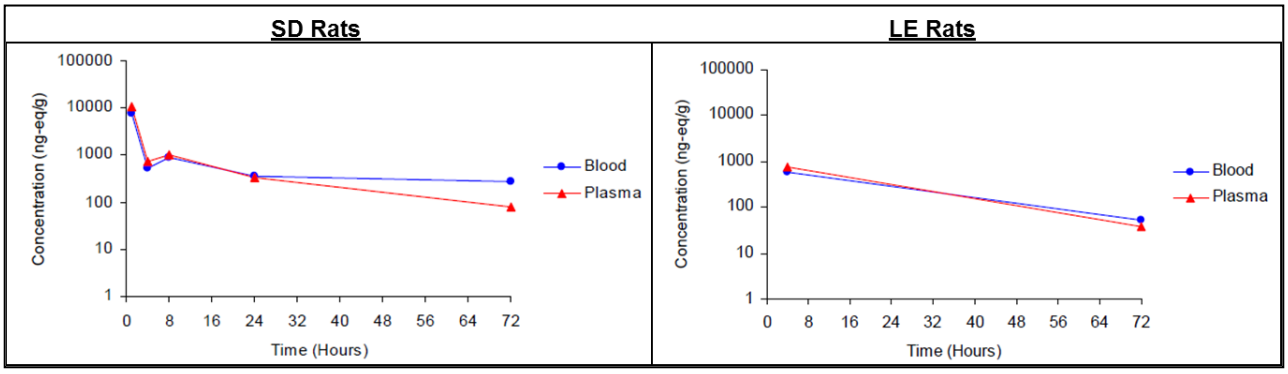
Individual Dose Administration Values					
Animal Number	Group Number[a]	Body Weight (g)	Actual Dose Level[b] (mg/kg)	(µCi/animal)	(µCi/kg)
101	1	335	53.25	69.2057	206.6
102	1	326	46.09	58.2985	178.8
103	1	328	52.25	66.4973	202.7
104	1	327	51.56	65.4108	200.0
105	1	324	51.21	64.3746	198.7
Mean		328	50.87	64.7574	197.4
SD		4	2.78	4.0344	10.8
201	2	328	49.42	62.8899	191.7
202	2	311	48.58	58.6202	188.5
Mean		320	49.00	60.7551	190.1
Overall Mean		326	50.34	63.6139	195.3
SD		7	2.46	4.0229	9.6

[a]Group 1 animals were SD and Group 2 animals were LE.
[b]The target dose level was 50 mg/kg (150 to 200 µCi/kg) and the target dose volume was 10 mL/kg.

- Blood samples were collected from one animal per time point at the designated terminal time points of 1, 4, 8, 24, and 72 hours postdose for SD rats or 4 and 72 hours postdose for LE rats.
- Urine and feces were collected from animals designated for 72 hour termination predose and at 0-24, 24-48, and 48-72 hours postdose.
- At each terminal time point of 1, 4, 8, 24, and 72 hours postdose for SD rats and 4 and 72 hours postdose for LE rats, one animal per time point was sacrificed for Quantitative Whole Body Autoradiography (QWBA) analysis.

Results

Figure 18: Concentrations of ^{14}C-CAL-101 in Blood and Plasma of Sprague-Dawley and Long Evans Rats



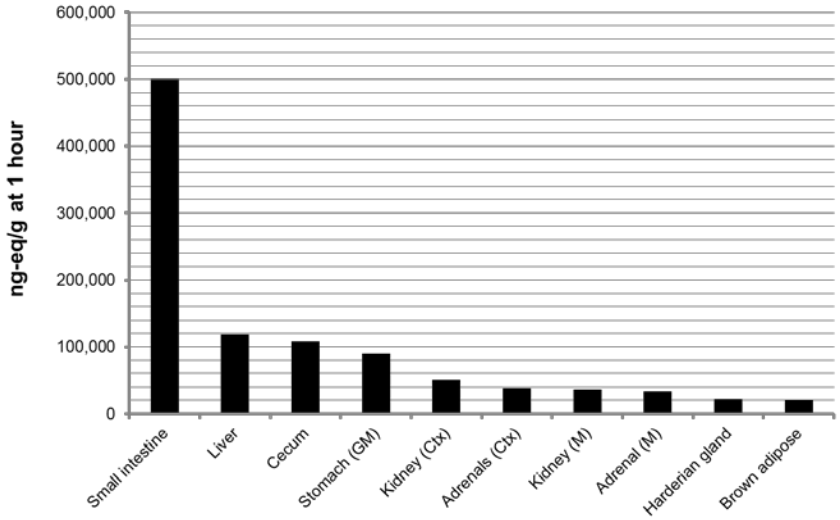
In SD rats, T_{max} in blood and plasma were observed at 1 hour postdose, then declined 93% by 4 hours and then rose 29-39% by 8 hours before declining steadily to the 72 hour time point. Similar kinetics was observed in LE rats between 8 and 72 hours.

Blood to plasma ratios were relatively constant. Of the 95% ^{14}C-CAL-101 (CAL-101 [purine ring-8-14C]) recovered at 72 hours in SD rats, 90% was in the feces, 4% in the urine, 0.9% in the cage rinses, and 0.03% in the cage wipe. Of the 90% of the dose

recovered in the feces, 89.9% was recovered within the first 48 hours, with 78.4% recovered within the first 24 hours. Similar trends were observed in LE rats.

Tissues with the highest concentrations of ^{14}C-CAL-101 in SD rats 1 hour postdose are listed in Figure 19. The lowest concentrations (<500 ng-eq/g) were observed in the bone, brain and spinal cord, and eye lens. After 72 hours postdose, most tissues were BQL except for: kidney medulla (2,178 ng-eq/g), liver (1,189 ng-eq/g), kidney cortex (ctx) (766 ng-eq/g), testis (451 ng-eq/g), thyroid (415 ng-eq/g), stomach gastric mucosa (GM) (286 ng-eq/g), lymph node (198 ng-eq/g), and bone marrow (196 ng-e/g).

Figure 19: Highest Concentrations of ^{14}C-CAL-101 in the Tissues of Sprague-Dawley Rats



The patterns and tissue concentrations observed in LE rats appeared similar to that observed in the SD rats, except that the pigmented skin of the LE rats showed a tissue concentration of 609 ng-eq/g at 72 hours postdose compared to BQL for non-pigmented skin in the SD rats at 72 hours postdose; and, the eye uvea showed a tissue concentration of 594 ng-eq/g at 72 hours postdose compared to BQL for eye uvea in the SD rats at 72 hours postdose.

Study title: Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Rats after Oral Administration of ^{14}C-GS-1101

Study no.:	AD-312-2004
Study report location:	4.2.2.4.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 20, 2010
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	GS-1101 (CAL-101) [purine ring-8-14C], Lot No. 3579087, Purity: 96.2% (reverse phase HPLC); non-radiolabeled CAL-101, Lot No. 1, no purity provided

Key Study Findings

- ^{14}C-GS-1101 was metabolized in rats via oxidative defluorination, oxidation, dealkylation, glucuronidation, and glutathione conjugation, and subsequent mercapturic acid pathway biotransformation to N-acetylcysteine.
- In rat plasma, most of the circulating radioactivity (approximately 91 to 93%) was associated with unchanged parent drug through 8 hours postdose. GS-563117 (M30A; a metabolite present at high levels in human plasma) was detected at low levels (1.4%) in plasma.
- The unchanged parent drug was 13.5% in the feces at 72 hours postdose.
- In the bile-duct cannulated rats, the unchanged parent was 1.8% at 24 hours postdose. This indicates a relatively high metabolism.
- In bile duct-intact rats, the major route of elimination was in the feces and in bile duct-cannulated, the major route of elimination was hepato-biliary.

Methods

Doses:	50 mg/kg
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% CMC, 0.1% TWEEN 80
Species/Strain:	Crl:CD(SD)
Number/Sex/Group:	See Study Design in AD-312-2003
Age:	6-8 weeks old
Weight:	205-329 g
Satellite groups:	None

- Plasma samples were obtained from rats in Study AD-312-2003.
- Plasma samples obtained from Group 2 (bile duct-intact male rats) were pooled by time point (at 0.5, 1, 2, and 8 hours postdose).

- Urine samples collected from male rats in Group 1 (bile duct-intact) or Group 3 (bile duct-cannulated) were pooled (5% or 10% from each sample by weight for Group 1 or Group 3, respectively) to generate a single 0- to 24-hour pooled sample for each group.
- Bile samples collected from rats in Group 3 were pooled by time point to generate single 0- to 4-, 4- to 8-, and 8- to 24-hour pooled samples, including 10 or 20% (equivalent percent by interval) of each sample by weight.
- Feces samples collected from rats in Group 1 or Group 3 were pooled to generate 0-24 and 24-48 hour postdose samples for each group. Acidified methanol extraction was needed to isolate parent compound and metabolites from feces.
- The radioactivity in each pooled sample was determined by liquid scintillation counting (LSC) and metabolite profiling was conducted using HPLC and TopCount solid scintillation counting.

Results

After oral administration, most of the circulating radioactivity in plasma (approximately 91 to 93%) was associated with unchanged parent drug through 8 hours postdose. GS-563117 (M30A) was also detected in plasma at 1.4%. However, the level of parent drug at 24-72 hours postdose in bile and feces indicate high metabolism.

Unchanged parent accounted for 13.5% of the administered dose through 72 hours postdose in the feces of bile duct-cannulated rats and GS-563117 (M30A)/no structure proposed (M30B) were the predominant metabolites in feces at 9.38% and 1.72% in bile duct-intact and bile duct-cannulated rats, respectively.

Table 11: Metabolite Identification in the Excreta of Sprague-Dawley Rats

Identified metabolites in excreta	Bile duct	Code	% of dose
Feces: Total Radioactivity*	intact		88.5
	cannulated		22.9
Unchanged parent	intact	GS-1101	10
	cannulated		13.5
Hydroxy-GS-1101-cysteine conjugate	intact	M10	8.21
GS-563117/ structure not proposed	intact	M30A/M30B	9.38
	cannulated		1.72
Hydroxy-GS-1101-3	intact	M21	3.93
Structure not proposed	intact	M32	3.43
desfluoro-mercapto-methoxy-GS-1101/desfluoro-mercapto-hydroxy-methoxy-GS-1101	intact	M36A/M36B[a]	6.50
	cannulated		0.356
[desfluoro-hydroxy-GS-1101-S-S-desfluoro-hydroxy-GS-1101]-dimer	intact	M40	6.12
GS-1101-$C_5H_8O_3$-adduct	intact	M31	2.68
	cannulated		1.38
Other structures	intact		<2.10
Bile: Total Radioactivity**	cannulated		63.5
Unchanged parent	cannulated		1.77
Desfluoro-oxy-GS-1101-glutathione conjugate	cannulated	M12	31.6
Desfluoro-GS-1101-glutathione conjugate	cannulated	M7	3.99
GS-563129/hydroxy-GS-1101-glucuronide-1/hydroxy-GS-1101-cysteineglycine conjugate	cannulated	M8A/M8B/M8C	6.37
GS-1101-glutathione conjugate/desfluoro-dioxy-GS-1101-glutathione conjugate	cannulated	M22A/M22B	4.43
GS-563117	cannulated	M30A	2.02
Desfluoro-hydroxy-GS-1101-cysteine conjugate	cannulated	M10	1.52
Other structures	cannulated		<1.6
Urine: Total radioactivity**	intact		3.05
	cannulated		5.54
GS-563117	intact	M30A	0.477
	cannulated		1.93
Hydroxy-purine	intact	M4	0.290
	cannulated		0.300
Unknown structure	intact	M20	0.104
	cannulated		0.193
Structure not proposed	intact	M1	1.4
	cannulated		1.3

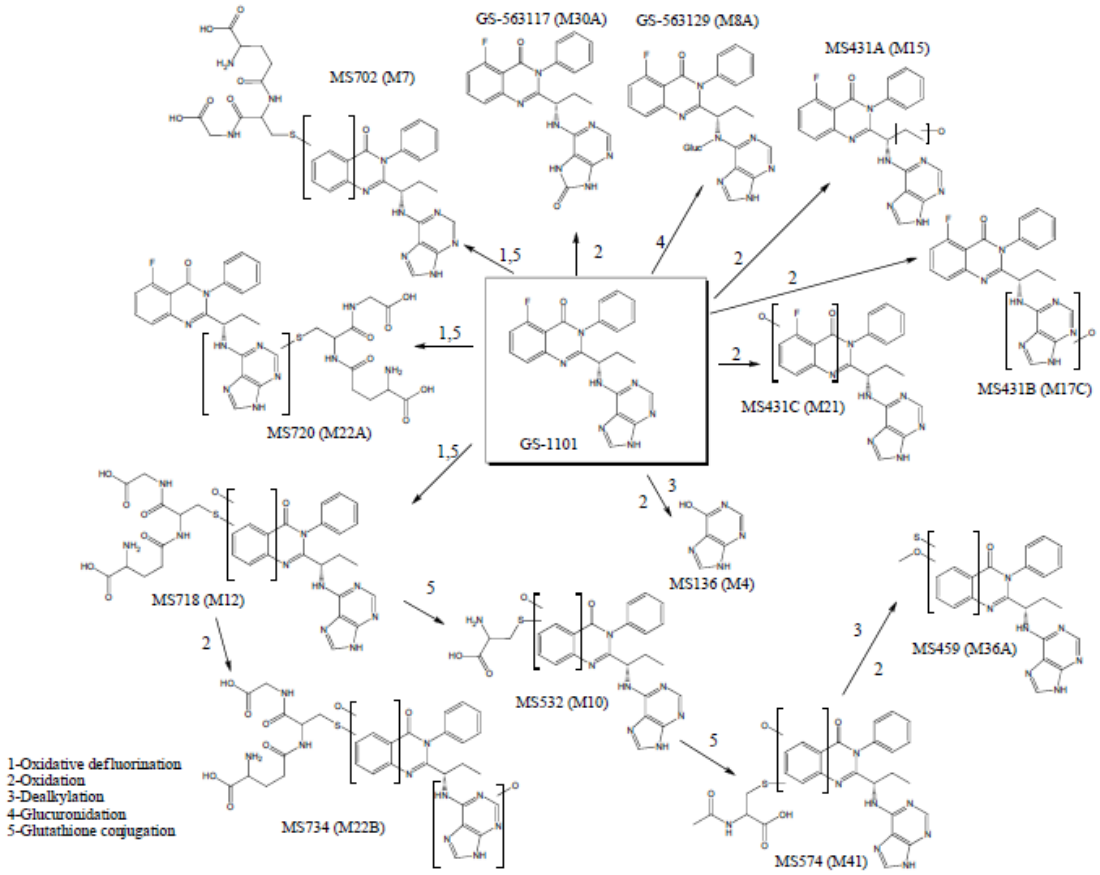
a Final metabolite designation eluted in feces only

* Bile duct-intact and bile duct-cannulated measurements from feces taken through 48- and 72-hours postdose, respectively

** All measurements from bile and urine were taken through 24 hours post dose

Figure 20: Proposed Biotransformation Pathway of GS-1101 in Rats

(Excerpted from the Applicant's submission)



Note: Pathways are proposed based on general knowledge of metabolism and do not imply definitive pathways. Direct experimentation was not performed.

Study Title: Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Dogs after Oral Administration of 14C-GS-110.

Study No.:	AD-312-2002
Report Date:	February 20, 2013
Study report location:	4.2.2.4.
Conducting Laboratory:	(b) (4)
GLP:	No
Drug, lot #, and % purity:	GS-1101 (CAL-101) [purine ring-8-14C], Lot No. 3579087, Purity: 96.2% (reverse phase HPLC); non-radiolabeled CAL-101, Lot No. 1, no purity provided

The purpose of this study was to evaluate the metabolic profiles of radioactivity derived from [^{14C}] idelalisib in pooled plasma, urine, bile, and feces from male Beagle dogs (bile duct-intact and bile duct-cannulated dogs) following oral administration at 5 mg/kg.

Key Findings

- ^{14}C-GS-1101 was metabolized in dogs via oxidative defluorination, oxidation, dealkylation, glucuronidation, and glutathione conjugation. In pooled plasma, most of the circulating radioactivity (approximately 59%) was associated with unchanged parent drug through 24 hours postdose, contributing greater than 58% of total radioactivity AUC_{0-24}. GS-563117 (M30A; a metabolite present at high levels in human plasma) contributed approximately 34% of the total radioactivity exposure through 24 hours postdose.
- The major route of elimination of radioactivity in bile duct-intact dogs was feces, and in bile duct-cannulated dogs, the major route of elimination was hepato-biliary.
- In bile duct-cannulated dogs, hepato-biliary excretion was a major route of elimination of radioactivity, with 70% of the administered dose recovered in bile through 24 hours postdose.

Methods

- Plasma samples obtained from Group 1 (bile duct-intact male dogs) were pooled by time point (at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours postdose).
- Urine samples collected from male bile duct-intact dogs in Group 1 at 0-8 and 8-24 hours postdose were pooled to generate a single 0- to 24-hour pooled sample, including 0.5% of each sample by weight.
- Urine samples collected from Group 2 (bile duct-cannulated male dogs) at 0-4, 4-8, and 8-24 hours postdose were pooled to generate a single 0- to 24-hour pooled sample, including 0.5% of each sample by weight.
- Bile samples collected from male bile duct-cannulated dogs in Group 2 at 0-1, 1-2, 2-4, 4-6, 6-8, 8-12, and 12-24 hours postdose were pooled to generate single 0- to 4-, 4- to 8-,and 8- to 24-hour pooled samples, including 5% of each sample by weight.
- Feces samples collected from male bile duct-intact dogs in Group 1 at 0-24, 24-48, and 48-72 hours postdose were pooled by collection interval, including 0.8% of each sample by weight.
- Feces samples collected from male bile duct-cannulated dogs in Group 2 at 0-24 and 24-48 hours postdose were pooled by collection interval, including 0.5% of each sample by weight.
- Radioactivity in the samples was profiled by radio-high performance liquid chromatography (HPLC) and metabolites were identified by co-chromatography with known standards and by using liquid chromatography-mass
- spectrometry (LC-MS and/or LC-MS/MS) methods.

Results

- After oral administration, most of the circulating radioactivity in pooled plasma, (approximately 59%) was associated with unchanged parent drug through 24

hours post-dose. GS-563117 (M30A) contributed approximately 34% of the total radioactivity exposure through 24 hours postdose.

- Unchanged parent drug accounted for 6.23% of the administered dose in bile duct-cannulated dogs and GS-563117 (M30A) was predominant metabolite at 5.35%.

Study title: Pharmacokinetics, Absorption, and Excretion of ^{14}C-GS-1101 Following a Single Oral Administration to Rats

Study no.:	AD-312-2003
Study report location:	4.2.2.5.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 1, 2012 (beginning of in-life portion)
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	CAL-101 [purine ring-8-14C], Lot No. 3579087, Purity: 96.2% (reverse phase HPLC); nonradiolabeled CAL-101, Lot No. 1, no purity provided

Key Study Findings

- Radioactivity derived from ^{14}C-GS-1101 (above 80%) was excreted within 24 hours after oral dosing and was below the limit of detection in plasma by 72 hours. Roughly 1% remained in the blood after 168 hours.
- A large percentage (64%) of radioactivity was eliminated in bile after oral dosing, indicating biliary excretion was the major route of elimination of ^{14}C-GS-1101-derived radioactivity.

Methods

Doses:	50 mg/kg (5.511 mg of ^{14}C-GS-1101 combined with 374.490 mg of GS-1101 and 76 mL of vehicle)
Frequency of dosing:	Once
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% CMC, 0.1% TWEEN 80
Species/Strain:	Crl:CD(SD)
Number/Sex/Group:	See table below
Age:	6-8 weeks old
Weight:	205-329 g
Satellite groups:	None

(Study design excerpted from the Applicant's submission)

Group	Number of Male Animals	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Samples Collected
1	3	Oral	50	10	Urine, Feces, and Carcasses
2	15	Oral	50	10	Blood
3	3 BDC	Oral	50	10	Urine, Feces, Bile, and Carcasses

BDC Bile duct-cannulated.
Notes: The dose was approximately 100 µCi/kg.

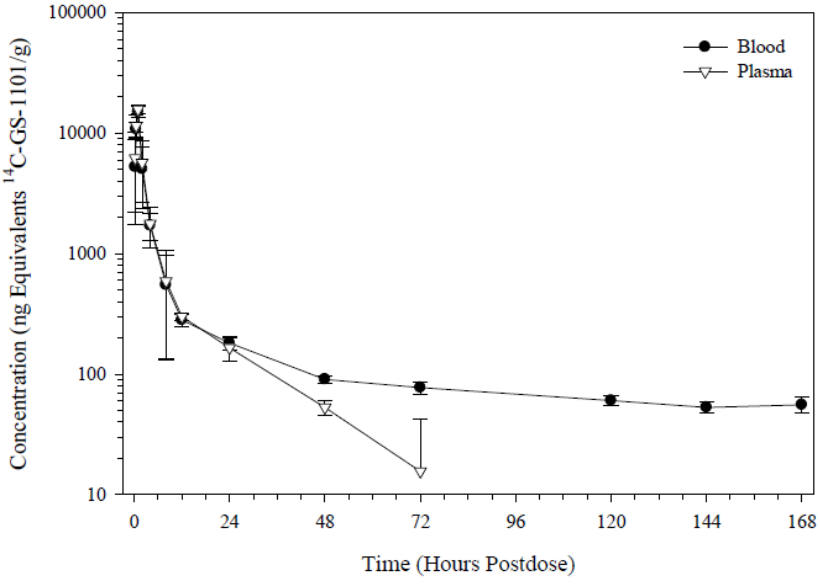
- Cages for Groups 1 and 3 were rinsed after each 24-hour excreta and wiped after the final excreta for radioanalysis.
- Group 1 and 3 - Urine was collected at 0-12 and 12-24 hours postdose, and at 24-hour intervals through 168 hours postdose. Feces were collected at 24-hour intervals through 168 hours postdose. The weight of each sample was recorded.
- Group 2 - Blood (approximately 1 mL) was collected from a jugular vein at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 120,144, and 168 hours postdose from three animals/time point.
- Group 3 - Bile was collected at 0-2, 2-4, 4-6, 6-8, 8-12, and 12-24 hours postdose, and at 24-hour intervals through 168 hours postdose. The weight of each sample was recorded.
- Radioanalysis was conducted using LSC.

Results

The mean radiopurity values from HPLC analysis of predose and postdose aliquots were 93.0 and 93.2%, respectively, confirming stability of the test article under the conditions of the study.

Figure 21: Concentrations of Radioactivity in Blood and Plasma at Specified Times after a Single Oral Administration of ^{14}C-GS-1101 to Male Sprague-Dawley Rats (Group 2, 50 mg/kg)

(Excerpted from the Applicant's submission)



Absorption was rapid (T_{max} = 1 hr), the half-life in the plasma was 14.1 hours, and the majority of excretion occurred within 24 hours postdose, although roughly 1% remained in the blood through 168 hours postdose.

Table 12: Absorption Parameters of ^{14}C-GS-1101 in Normal Rats and Route of Excretion Percentages of ^{14}C-GS-1101 in Normal and Bile Duct-Cannulated Rats

Group	Bile duct-intact	Absorption Blood (Plasma)				Excretion at 24 hours	
		T_{max} (h)	C_{max} (ng eq/g)	AUC_{0-t} (ng eq hr/g)	$t_{1/2}$	Route	%
1	Yes	ND	ND	ND	ND	Urine	3.04
						Feces	83.9
2	Yes	1.00 (1.00)	15000 (15700)	46048 (40657)	NC (14.1)	ND	ND
3	No	ND	ND	ND	ND	Urine	5.54
						Feces	19.2
						Bile	63.5

NC = Not calculated ND = Not determined
Note: The majority of urine and bile excretion occurring within 12 hours postdose.

Study title: Pharmacokinetics, Absorption, and Excretion of ^{14}C-GS-1101 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs

Study no.: AD-312-2001
Study report location: 4.2.2.5.
Conducting laboratory and location: (b) (4)
Date of study initiation: June 29, 2012 (beginning of in-life portion)
GLP compliance: No
QA statement: NA
Drug, lot #, and % purity: CAL-101 [purine ring-8-14C], Lot No. 3579087, Purity: 96.2% (reverse phase HPLC); nonradiolabeled CAL-101, Lot No. 1, no purity provided

Key Study Findings

- Radioactivity derived from ^{14}C-GS-1101 (above 88%) was excreted within 48 hours after oral dosing and was below the limit of detection in plasma by 72 hours.
- A large percentage (72%) of radioactivity was eliminated in bile after oral dosing, indicating biliary excretion was the major route of elimination of ^{14}C-GS-1101-derived radioactivity.

Methods

Doses:	5 mg/kg (6.090 mg of 14C-GS-1101 combined with 413.910 mg of GS-1101 and 420 mL of vehicle
Frequency of dosing:	Once
Route of administration:	Oral gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% CMC, 0.1% TWEEN 80
Species/Strain:	Purebred Beagle dogs (bile duct-intact and three bile duct-cannulated)
Number/Sex/Group:	See table below
Age:	8 to 11 months
Weight:	8.5 to 11.2 kg
Satellite groups:	None

(Study design excerpted from the Applicant's submission)

Group	Number of Male Animals	Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Samples Collected
1	3	Oral	5	5	Blood, Urine, and Feces
2	3 BDC	Oral	5	5	Urine, Feces, and Bile

BDC Bile duct-cannulated.
Note: The target dose was approximately 10 μCi/kg.

- Group 1 - Urine was collected at 0-8 and 8-24 hours postdose, and at 24-hour intervals through 168 hours postdose.
- Group 1 - Blood (approximately 5 mL) was collected via a jugular vein at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96, and 168 hours postdose.
- Group 1 and 2- Feces were collected at 24-hour intervals through 168 hours postdose.
- Group 2- Urine was collected at 0-4, 4-8, and 8-24 hours postdose, and at 24-hour intervals through 168 hours postdose.
- Group 2 - Bile was collected at 0-2, 2-4, 4-6, 6-8, 8-12, and 12-24 hours postdose, and at 24-hour intervals through 168 hours postdose.
- Radioanalysis was conducted using LSC.

Results

The mean radiopurity values from HPLC analysis of predose and postdose aliquots were 95.6 and 94.8%,respectively, confirming stability of the test article under the conditions of the study.

Figure 22: Concentrations of Radioactivity in Blood and Plasma at Specified Times after a Single Oral Dose of 14C-GS-1101 to Male Intact Dogs (Group 1, 5 mg/kg)

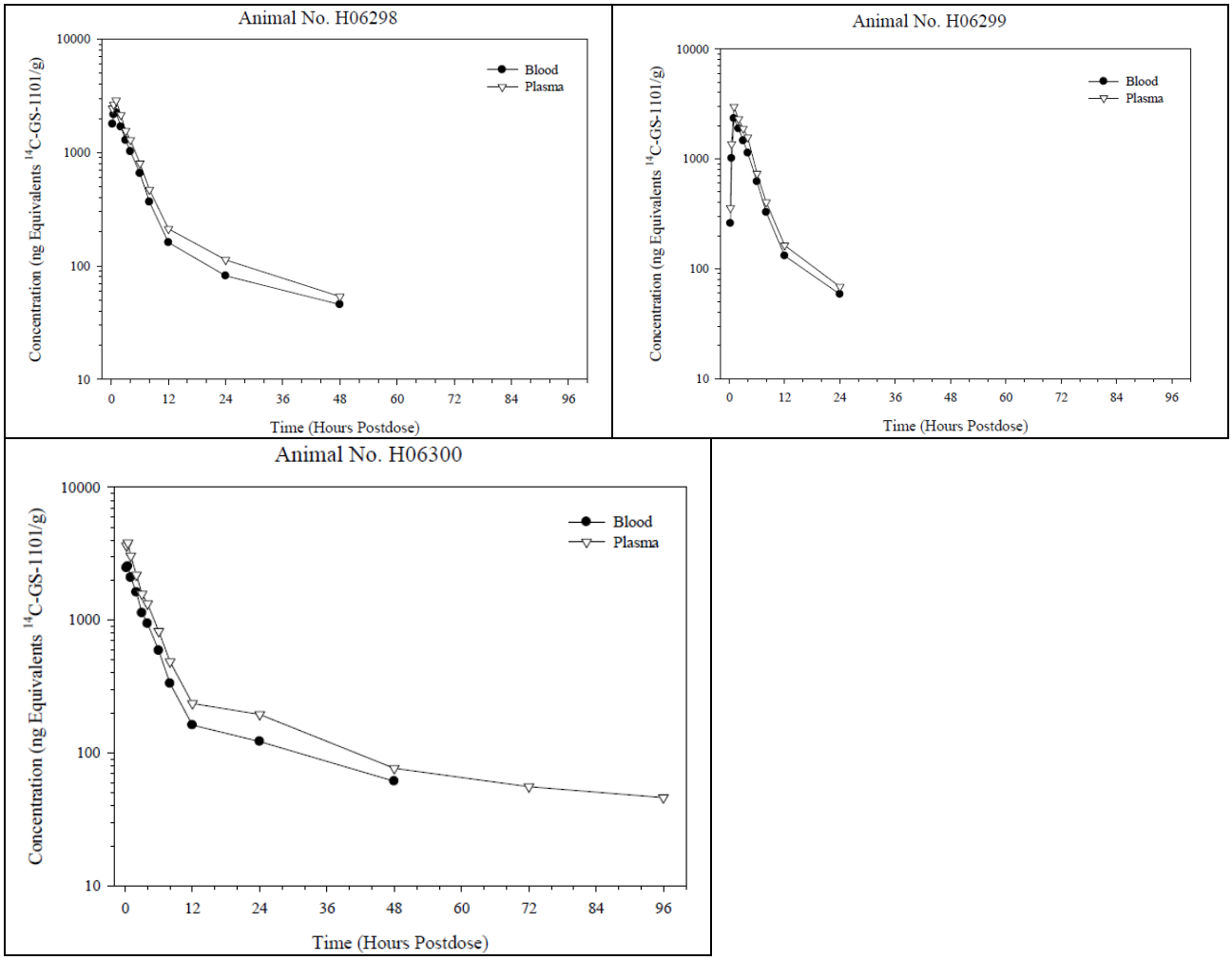


Table 13: Pharmacokinetic Parameters for Radioactivity in Blood and Plasma Collected from Male Intact Dogs after a Single Oral Administration of 14C-GS-1101 (Group 1, 5 mg/kg)

Group (Bile duct status)	Absorption Blood (Plasma)				Excretion at 24 hours	
	T_{max} (h)	C_{max} (ng eq/g)	AUC_{0-t} (ng eq hr/g)	$t_{1/2}$	Route	%
1 (intact)	0.833±0.3 (0.833±0.289)	2370±154 (3220±514)	12600±1520 (17700±4510)	17.8±9.56 (30.3±31.0)	Urine	6.04± 1
					Feces	20.2± 5
					Bile	ND
2 (cannulated)	ND	ND	ND	ND	Urine	6.04± 1
					Feces	20.2 ±5
					Bile	71.7± 4.9

ND = Not determined
Note: The majority of urine and bile excretion occurring within 12 hours postdose

Study title: Plasma Protein Binding of Idelalisib and GS-563117

Study No.: AD-312-2009
Report Date: February 08, 2013.
Study report location: 4.2.2.3.
Conducting Laboratory: (b) (4)
GLP: No

Key Findings

- Idelalisib exhibited moderately high plasma protein binding in all species with the free fraction ranging from 9 to 25%.
- The average plasma protein binding was comparable in mouse, rat, and dog, ranging from 19% free (rat) to 21% free (dog).
- GS-563117, the oxidative metabolite of idelalisib was highly protein bound in humans with a free fraction of ~12%.
- In human plasma, idelalisib had an average free fraction of 16%; GS-563117, had a free fraction of 11.6%.

Methods

Plasma protein binding of idelalisib (IDELA) was assessed by equilibrium dialysis at substrate concentrations of 0.5 and 2 µM in the mouse and rat and at 0.5 to 20 µM in the dog and human. The oxidative metabolite of idelalisib, GS-563117, was assessed in humans at a concentration of 10 µM.

Table 14: Plasma Protein Binding of IDELA and GS-563117 in Mouse, Rat, Dog, and Human
(Excerpted from the Applicant's submission)

Species	Test Compound	Average Free Fraction (%) (n = 2)						Mean ± StD (%)
		0.5 µM	1 µM	2 µM	5 µM	10 µM	20 µM	
CD-1 Mouse	IDELA	18.2	–	21.5	–	–	–	19.8 ± 2.3
Sprague Dawley Rat		19.3	–	18.1	–	–	–	18.7 ± 1.2
Dog (mixed breeds)		16.7	22.8	19.6	22.2	–	22.5	20.7 ± 4.1
Human		12.4	20.3	12.2	16.1	19.2	15.8	16.3 ± 4.2
	GS-563117	–	–	–	–	11.6	–	11.6

5.2 Toxicokinetics

Included in general toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Rat:
In a single-dose, oral gavage study (Study No. 03-0004-42, non-GLP), Sprague-Dawley rats (main study, 2 animals/sex/group; TK animals, 3 animals/sex/group) were administered vehicle (0.2% hydroxypropyl methylcellulose and 0.5% TWEEN 80), 300, 900, and 1500 mg/kg idelalisib in a dose volume of 10 mL/kg on Day 1. Necropsy for main study and TK animals were on Days 5 and 2, respectively.

Toxicological findings were overall consistent with repeat-dose studies and included histopathology findings of depletion/necrosis of lymphocytes primarily from B-cells in multiple lymphoid organs and depletion/necrosis of hematopoietic cells in the bone marrow and spleen, inflammation in the GI tract, and hepatotoxicity.

There were no sex-related differences in C_{max} or AUC.

Dog:
A single-dose dog study (04-3166-N1, non-GLP) was conducted with idelalisib to assess MTD. Male and female dogs were assigned to 2 groups (2/sex/group). Animals assigned to Group 1 received a single dose in the order of 50, 25, and 10 mg/kg in consecutive weeks. Group 2 animals received a single dose at 200 mg/kg in Week 1.

- Two females administered 200 mg/kg idelalisib died within a few hours following administration.
- Clinical observations prior to death included yellow mucus fluid, emesis, tremors and tonic-clonic convulsions, vasoconstriction (blanching of skin and gums, and cold body), dyspnea, transient cessation of breathing following a forced inspiration, lethargy, and ataxia.
- Animals administered 25 and 50 mg/kg showed clinical signs of toxicity including weight loss (>10%), loose black stool containing yellow mucus, emesis, and decreased food consumption.
- Females showed higher $AUC_{0\text{-last}}$ than males at 25 (3.4-fold) and 50 mg/kg (1.5-fold).
- MTD was considered to be < 25 mg/kg.

6.2 Repeat-Dose Toxicity

Study title: CAL-101: 28-Day Oral Toxicity Study with Toxicokinetics in Sprague-Dawley Rats with Recovery

Study no.:	BHR00009
Study report location:	4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 8, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	CAL-101, Lot. # 22033-51-38, Purity: 99.3%

Study was reviewed under IND 101254 by Dr. Timothy Kropp. The content and format of the review were slightly modified to fit this NDA review.

Key Study Findings

- There were several deaths at 100 (3 deaths) and 150 mg/kg (5 deaths).
- Target organs include bone marrow (major toxicity, but reversible), tongue, liver, and testis.
- Inflammatory changes were seen in the tongue, heart, liver parenchyma.
- Exposure was greater than dose proportional, accumulative, and higher in females than males.

Methods

Doses:	Vehicle, 50, 100, 150 mg/kg
Frequency of dosing:	Daily for 4 weeks, 4 week recovery
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% CMC, 0.1% TWEEN 80
Species/Strain:	rat/Sprague-Dawley
Number/Sex/Group:	10 animals/sex/group
Age:	7 weeks old
Weight:	242-304 g for males; 189-296 g for females
Satellite groups:	6 animals/sex/group for TK plus, 4/sex/group for recovery
Unique study design:	An FOB evaluation was performed
Deviation from study protocol:	Deviations did not negatively impact the quality or integrity of the data or the outcome of the study.

Observations and Results

Clinical signs:	Daily
Body weights:	Days -1, 7, 14, 21, 28, 35, 42, 29, 56

Food consumption:	Weekly
ECG:	Not conducted
Ophthalmoscopy:	Pre-test and within 4 days of sacrifice*
Hematology:	Before sacrifice*
Clinical chemistry:	Before sacrifice*
Coagulation:	Before sacrifice*
Urinalysis:	Pre-test and before sacrifice*
Gross pathology:	At sacrifice*
Organ weights:	At sacrifice*
Histopathology:	At sacrifice*
Toxicokinetics:	Days 1 and 28 at 0.5, 1, 2, 4, 8, 12, 16, 24 hr post-dose

* Sacrifice performed on Day 29

Mortality

- Almost all unscheduled deaths occurred during the recovery phase.
- The animal found dead on day 29 likely died due to blood collection error.

Below is a list of all animals that died early on study:

Sex	Dose (mg/kg)	Fate	Day
F	100	Found Dead	12
M	100	Found Dead	29
F	150	Moribund Euthanasia	31
F	100	Moribund Euthanasia	33
F	150	Moribund Euthanasia	34
F	150	Moribund Euthanasia	34
F	150	Found Dead	34
F	100	Found Dead	49
M	150	Moribund Euthanasia	50

Bone marrow depletion, tongue ulceration and myeloid hyperplasia may have been involved in the morbidity/mortality of these animals, but the pathology findings do not completely reveal the cause of death.

Clinical Signs

- excessive salivation starting in the 3rd week of dosing (microscopic findings in the tongue correlated with hypersalivation noted throughout the study)
- sparse hair coat, scabs, skin erythema and swelling
- soft feces was noted in all animals in the high-dose (HD) group

Body Weights

Unremarkable

Food Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Not done

Hematology

Table 15: Mean % Change in Hematology Parameters Compared to Control on Day 29 in the 4-Week Rat Study

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Hemoglobin			-9			-15
Retic.		+33			+56	+166
WBC		-50	-47		-32	-47
LYM		-26	-40		-50	-44
EOS		-45	-44		-68	-53

Abbreviations: Retic., reticulocytes; WBC, white blood cells; LYM, lymphocytes; EOS, eosinophils; LD, low-dose 50 mg/kg; MD, mid-dose 100 mg/kg; HD, high-dose 150 mg/kg

- Decreases in hemoglobin were accompanied by similar decreases in HCT and RBC.
- Mean hemoglobin, hematocrit, and red blood cell counts remained depressed on day 57 despite increased reticulocytes.

Clinical Chemistry

Table 16: Mean % Change in Clinical Chemistry Parameters Compared to Control in the 4-Week Rat Study

	MD ♂	HD ♂	MD ♀	HD ♀
Cholesterol				+27
Triglyerides				+127
ALP	-23	-31	-47	-48

- Samples taken from the animals prematurely euthanized had increases in ALT, AST, ALP, and GGT along with decreases in total protein, globulin, and albumin.

Urinalysis

Unremarkable

Gross Pathology

- In unscheduled deaths:
  - large adrenal glands
  - red, discolored mandibular and mesenteric lymph nodes
  - pale pancreas
  - discolored liver
  - small thymus
  - clear gelatinous accumulation in the thymus
  - spleen
  - dark red and gelatinous accumulation in the thoracic cavity and mediastinum
- In the scheduled sacrifice:
  - dark discoloration of left thymus in 1 low-dose male
  - enlarged mandibular lymph node and small spleen in mid-dose females

Organ Weights

Table 17: Significant Changes in Organ Weights at the End of Dosing in the 4-Week Rat Study

	LD ♂	MD ♂	HD ♂	LD ♀	MD ♀	HD ♀
Adrenal Glands						-35
Testes	-14	-22	-22			
Thymus		-26	-34	-16	-35	-39
Liver				+11	+25	+56
Pituitary				-14	-19	-19
Thyroid						+20
Salivary Glands				+13	+19	+20

- Organ weights after recovery were unremarkable

Histopathology

Adequate Battery Yes

Peer Review No signed statement, but study report stated that histopath sections would be reviewed by board certified pathologist

Histological Findings

Table 18: Incidence and Grade of Histopathology Findings at 4-Wk Sacrifice in Male Rats

		mg/kg			
	Type	**VC**	**25**	**100**	**200**
Number examined		**10**	**10**	**10**	**10**
Bone marrow – granulopoietic hyperplasia	# affected				7
	avg grade				1.3
Bone marrow – myeloid hyper plasia	# affected			6	8
	avg grade			1.2	2.1
Bone marrow – erythroid depletion	# affected			7	
	avg grade			1.1	
Heart – myocardium infiltrate	# affected		2	2	4
	avg grade		1	2	1.3
Epididymis – spermatozoa hypoplasia	# affected		1	2	7
	avg grade		1	1	1.2
Liver – parenchyma inflammation	# affected	3	4	9	7
	avg grade	2	1	1.2	1.6
Testis – seminiferous tubule degeneration	# affected		1	7	9
	avg grade		1	1	1.3
Tongue – necrosis	# affected				1
	avg grade				4
Tongue – infiltration	# affected			5	3
	avg grade			1.6	2

Grading: minimal=1, mild=2, moderate=3, severe=4. VC= Vehicle Control

Table 19: Incidence and Grade of Histopathology Findings at 4-Wk Sacrifice in Female Rats

		mg/kg			
	Type	VC	50	100	150
Number examined		10	10	10	10
Bone marrow – granulopoietic hyperplasia	# affected		1	6	7
	avg grade		1	1.3	1.2
Bone marrow – myeloid hyper plasia	# affected			4	8
	avg grade			1	1.8
Bone marrow – erythroid depletion	# affected			1	6
	avg grade			1	1
Heart – myocardium infiltrate	# affected			2	1
	avg grade			2.5	2
Heart – fibrosis	# affected				1
	avg grade				2
Liver – parenchyma inflammation	# affected		5	8	8
	avg grade		1.2	1.3	1.8
Tongue – ulceration	# affected			1	
	avg grade			3	
Tongue – infiltration	# affected		5	6	7
	avg grade		1.2	2.5	1.7

Grading: minimal=1, mild=2, moderate=3, severe=4. VC= Vehicle Control

Table 20: Incidence and Grade of Histopathology Findings at 4-Wk Recovery Sacrifice in Male Rats

		mg/kg			
	Type	VC	50	100	150
Number examined		4	4	3	3
Bone Marrow – multifocal depletion	# affected			2	1
	avg grade			3	3
Testis – seminiferous tubule degeneration	# affected		1	2	1
	avg grade		2	1	2

Grading: minimal=1, mild=2, moderate=3, severe=4. VC= Vehicle Control

- Bone marrow cytology showed shifts towards immaturity for both myeloid and erythroid precursors in all treated animals and lower erythrocyte:lymphocyte ratios in the HD female.

Toxicokinetics

Table 21: Toxicokinetic Parameters CAL-101 in Rat Plasma: Days 1 and 28

Day 1

	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{0-24} (ng*h/mL)	AUC_{0-24}/dose
50♂	2.5	6300	22000	440
100 ♂	2	9200	58000	580
150 ♂	1.5	16000	170000	1100
50 ♀	2.2	8000	47000	94
100 ♀	1.9	16000	150000	150
150 ♀	1.6	27000	340000	2300

Day 28

	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{0-24} (ng*h/mL)	AUC_{0-24}/dose
50♂	2.5	6600	36000	720
100 ♂	1.9	16000	160000	1600
150 ♂	1.3	51000	590000	3900
50 ♀	2	13000	83000	1700
100 ♀	1.8	35000	300000	3000
150 ♀	Not est.	34000	500000	3300

Study title: CAL-101: 28-Day Oral Toxicity Study With Toxicokinetics in Beagle Dogs with Recovery

Study no.: BHR00008
Study report location: 4.2.3.2.
Conducting laboratory and location: (b) (4)
Date of study initiation: August 8, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CAL-101, Lot. # 22033-51-38, Purity: 99.3%

Study was reviewed under IND 101254 by Dr. Timothy Kropp. The format and content of review were slightly modified to fit this NDA review.

Key Study Findings

- There was one death at 20 mg/kg/day on day 29. Primary toxicities were to the GI, liver, and lymphoid tissues.
- Bone marrow toxicities were also evident in hematology findings.

- There were CAL-101 findings relating to the eye (largely eye discharges); the relationship to the treatment is unclear.

Methods

Doses:	Lactose monohydrate, 2.5, 5, 20 mg/kg
Frequency of dosing:	Daily for 4 weeks, 4 week recovery
Route of administration:	Oral
Dose volume:	N/A
Formulation/Vehicle:	Capsules w/ lactose monohydrate powder
Species/Strain:	dog/Beagle
Number/Sex/Group:	4 animals/sex/group
Age:	5-8 months
Weight:	6.2-8.7 kg
Satellite groups:	3 animals/sex/group for TK and recovery
Unique study design:	None
Deviation from study protocol:	One HD male and one HD female were given Lactated Ringer's solution on multiple days due to dehydration. One HD male and two HD females were given 0.2 mg/kg meloxicam SC due to paw injury, edema, or eye pain. The female with ocular pain was also administered Vetropolycin HC topically. One MD male was given 0.2 mg/kg meloxicam due to mouth pain.

Observations and Results

Clinical signs:	Cageside twice daily and detailed weekly
Body weights:	Weekly
Food consumption:	Daily
ECG:	Pre- and postdose pretest and before sacrifice
Ophthalmoscopy/Audiology:	Pre-test and within 4 days of sacrifice
Hematology:	Pre-test and before sacrifice
Clinical chemistry:	Pre-test and before sacrifice
Coagulation:	Pre-test and before sacrifice
Urinalysis:	Pre-test and before sacrifice
Gross pathology:	At sacrifice
Organ weights:	At sacrifice
Histopathology:	At sacrifice
Toxicokinetics:	Days 1, 14 and 28 at 0.5, 1, 2, 4, 8, 12 hr post-dose. Days 2 and 29 at 16 and 24 hr post-dose

Mortality

- One HD female was euthanized in moribund condition on day 29.

Clinical Signs

- 2.5 mg/kg

  - soft feces
- 5 mg/kg
  - soft feces
  - eye squint
  - vomiting/emesis
  - skin erythema
- 20 mg/kg
  - soft feces
  - eye squint
  - vomiting/emesis
  - skin erythema
  - green discolored eyes/discharge
  - white ocular discharge
  - lacrimation
  - red discolored eyes
  - white and yellow discharge
  - swelling in left eye of one male
  - Female with unscheduled death (developing starting day 10):
    - red discolored eyes
    - fecal blood
    - marked soft feces
    - lethargy
    - salivation
    - shivering
    - mouth ulcerations
    - thin
    - mucoid eye discharge

Body Weights

HD females lost weight (-8%) while control and LD groups gained weight at 8%. MD females gained at 2% (non-significant vs controls).

Food Consumption

Observation of low food consumption was approximately 5 times higher than control animals.

Ophthalmoscopy

Unremarkable other than that noted in clinical signs.

ECG

Unremarkable

Hematology

- Lymphocyte counts were down in treated females (16-35%).
- Basophil counts were down in all treated animals (~60%).
- HCT and HGB levels were increased only in animals which had signs of dehydration.

- Monocyte count was 7.2x baseline for the early death female on day 24.
- Bone marrow toxicities were also evident in hematology findings.

Clinical Chemistry

Table 22: Change in Clinical Chemistry Parameters Compared to Control in Dog 4-Wk Study

	HD ♂	HD ♀
AST	4.7X	40.2X
ALT	2X	10.6X
ALP	2X	5.8X
GGT		2.4X

- All findings resolved by the end of recovery

Urinalysis

Unremarkable

Gross Pathology

- In the early death female:
  - red, linear discoloration of the mucosa of the GI tissues
  - red, firm discoloration of the lung lobes
  - white material in the trachea
  - red discolored thymus
- In the 20 mg/kg surviving animals:
  - small thymus in 1/4 males and 1/3 females
  - red discoloration of the GI tissues

Organ Weights

Table 23: Significant Changes in Organ Weights, Relative to Body Weight in Dogs at the End of Dosing (4-Wk)

	HD ♂	**HD ♀**
Thymus	-37%	-32%
Spleen	-38%	-22%

Table 24: Significant Changes in Organ Weights, Relative to Body Weight in Dogs at the End of Recovery (4-Wk)

	LD ♂	**MD ♂**	**HD ♂**
Testes	-31%	-33	-55%

Histopathology

Adequate Battery Yes

Peer Review Yes, signed

Histological Findings

- The histopathology of the early death female was similar to the other 20 mg/kg females with greater severity as well as congestion and hemorrhage findings in the lung and liver.

Table 25: Incidence and Grade of Histopathology Findings at 4-Wk Sacrifice in Male Dogs

		mg/kg			
	Type	**VC**	**2.5**	**5**	**20**
Number examined		**4**	**4**	**4**	**4**
Ileum (Peyer's patch) – lymphoid depletion	# affected				3/3†
	avg grade				3
Lymph node, axillary – lymphoid depletion	# affected				3
	avg grade				2.7
Lymph node, inguinal – lymphoid depletion	# affected				2
	avg grade				2.5
Thymus – lymphoid depletion	# affected				2
	avg grade				4
Liver – Chronic inflammation	# affected			2	2
	avg grade			1	1
Liver – Hepatocellular necrosis	# affected	1		1	3
	avg grade	1		1	1.3
Liver – Hepatocellular swelling	# affected				1
	avg grade				2
Colon* – multifocal hemorrhage	# affected			1	1
	avg grade			2	1
Colon* – multifocal crypt abscess	# affected				1
	avg grade				1
Colon* – mixed cell mucosal infiltration	# affected			1	
	avg grade			1	

Grading: minimal=1, mild=2, moderate=3, severe=4. VC= Vehicle Control
† No Peyer's patch was present in one of the HD males.
*Findings in the colon were replicated in the cecum, duodenum, ileum, jejunum, and rectum and are not included in the table

Table 26: Incidence and Grade of Histopathology Findings at 4-Wk Sacrifice in Female Dogs

		mg/kg			
	Type	VC	2.5	5	20
Number examined		4	4	4	3
Ileum (Peyer's patch) – lymphoid depletion	# affected				3
	avg grade				3
Lymph node, auxiliary – lymphoid depletion	# affected			1	1
	avg grade			2	3
Lymph node, inguinal – lymphoid depletion	# affected			1	1
	avg grade			2	3
Thymus – lymphoid depletion	# affected				1
	avg grade				4
Thymus – lymphoid necrosis	# affected				1
	avg grade				3
Liver – Chronic inflammation	# affected			1	2
	avg grade			1	1
Liver – Hepatocellular necrosis	# affected				2
	avg grade				1.5
Liver – Hepatocellular swelling	# affected				2
	no grade				2
Colon* – multifocal hemorrhage	# affected				1
	avg grade				1
Colon* – multifocal crypt abscess	# affected				1
	avg grade				2
Colon* – mixed cell mucosal infiltration					1
					2
Thymus – cortex necrosis	# affected				1
	avg grade				3

Grading: minimal=1, mild=2, moderate=3, severe=4. VC= Vehicle Control

Table 27: Incidence and Grade of Histopathology Findings after 4-Wk Recovery in Female Dogs

		mg/kg			
	Type	VC	2.5	5	20
Number examined		3	3	3	3
Ileum (Peyer's patch) – lymphoid depletion	# affected				2
	avg grade				2
Liver – Hepatocellular swelling	# affected				1
	avg grade				1

Grading: minimal=1, mild=2, moderate=3, severe=4. VC= Vehicle Control

Toxicokinetics

Table 28: Toxicokinetic Parameters CAL-101 in Dog Plasma: Days 1 and 28

TK parameters on day 1

Dose (mg/kg)	Sex	C_{max} (µg/mL)	$AUC_{0\text{-}lim}$ (µg*hr/mL)	AUC/dose
2.5	M	609	2130	852
5	M	1400	5390	1078
20	M	7170	56900	2845
2.5	F	700	2920	1168
5	F	1280	6420	1284
20	F	6560	47600	2380

TK parameters on day 28

Dose (mg/kg)	Sex	C_{max} (µg/mL)	$AUC_{0\text{-}lim}$ (µg*hr/mL)	AUC/dose
2.5	M	873	4760	1904
5	M	1310	8490	1698
20	M	11500	113000	5650
2.5	F	638	3850	1540
5	F	1540	9280	1856
20	F	20500	292000	14600

Study title: GS-1101: A 13-Week Oral Toxicity Study In Rats With a 4-Week Recovery Period

Study no.:	TX-312-2001
Study report location:	4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 22, 2010 (first dose)
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	GS-1101, Lot. # 60182-09-004, 99.8%

Key Findings:

- Statistically significant increase (14%-15%) in the absolute weights in the heart at 90 mg/kg/day compared to controls. There was a slight increase in the incidence and severity of cardiomyopathy in the heart in males at 90 mg/kg/day and in females at all dose levels compared to controls.
- Organs of toxicity: Heart, pancreas, tongue, and testes.

Methods

Doses:	0, 25, 50, 90 mg/kg/day
Frequency of dosing:	Daily for 13 weeks
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% high viscosity carboxymethylcellulose/0.1% TWEEN 80 in deionized water
Species/Strain:	Crl:CD(SD)
Number/Sex/Group:	15 animals/sex/group
Age:	7 weeks old
Weight:	Males, 201-259 g; Females, 141-183 g
Satellite groups:	Toxicokinetic (TK): vehicle control, 3 animals/sex/group; GS-1101-treated, 6 animals/sex/group
Unique study design:	No
Deviation from study protocol:	Deviations did not impact the quality or integrity of the data or the outcome of the study.

Mortality

Two main study females were euthanized *in extremis* and two toxicokinetic females were found dead including euthanized *in extremis*.

Table 29: Cause of Death (13-week Rat Study)

Dose of GS-1101 (mg/kg/day)	Animal #	Sex	Fate	Day	Tox/ Tk	Potential cause of morbidity/ macroscopic findings/ microscopic findings
25	1111	F	EE	61	Tox	Severe unilateral ocular hemorrhage and inflammation attributed to trauma associated with periorbital bleeding procedures
	1153	F	FD	61	Tk	No remarkable findings
	1155	F	EE	28	Tk	No remarkable findings
90	1140	F	EE	51	Tox	Inflammation of the tongue and oral cavity (present in nasal sections), which may have been associated with gavage-related trauma and/or irritation associated with the presence of the test article.

EE euthanized *in extremis,* FD found dead

Clinical Signs

Table 30: Summary of Clinical Signs in 13-Week Rat Study

Clinical signs	No. of animals affected (No. of observations)							
	Males				**Females**			
Dose (mg/kg)	0	25	50	90	0	25	50	90
Number of animals examined	15	15	15	15	15	15	15	15
During dosing phase								
Activity decreased	0	0	0	0	0	0	0	1
Hypersensitive to touch	0	0	0	0	0	0	0	1
Salivation	0	0	0	0	0	0	0	2
Material in pan/bedding, Red	0	0	0	0	0	0	0	2
Lacrimation	0	0	0	0	0	0	0	1
Material around eyes, Black	0	0	0	0	0	1	0	1
Material around mouth, Brown	0	0	0	0	0	0	0	1
Material around mouth, Red	0	0	0	0	0	0	0	1
Material around nose, Red	0	0	0	0	0	0	0	1
Swelling	0	0	0	0	0	0	0	1
Eye discolored, Dark	0	0	0	0	0	1	0	0
Deformity	0	0	0	1	0	0	0	0
Pelage/Skin Scabbed area	0	0	0	4	0	0	0	1
Hair discolored, Tan	0	0	0	0	0	0	0	1
Hair discolored, Yellow	0	0	0	0	0	0	0	1
Hair sparse	0	0	0	0	0	0	0	5
Hair wet	0	0	0	0	0	0	0	3
Scabbed area	0	0	0	0	0	0	0	1
Skin dry	0	0	0	0	0	0	0	1
Unkempt appearance	0	0	0	0	0	0	0	2
Breathing audible	0	0	0	0	0	0	0	1
During Recovery phase								
Number of animals examined	5	5	5	5	5	5	5	5
Teeth cut	0	1	1	2	0	0	1	0
Salivation	0	0	0	1	0	0	0	0
Material around nose, Red	0	0	0	1	0	0	0	0

Clinical signs	No. of animals affected (No. of observations)							
	Males				**Females**			
Dose (mg/kg)	0	25	50	90	0	25	50	90
Number of animals examined	15	15	15	15	15	15	15	15
Pelage/Skin Hair discolored, Red	0	0	0	1	0	0	0	0
Hair sparse	0	0	0	2	0	0	0	1
Hair wet	0	0	0	1	0	0	0	0
Unkempt appearance	0	0	0	1	0	0	0	0

Organ weights

- There was a 14% to 15% increase in the relative heart weights of males and females at 90 mg/kg/day compared to controls at the terminal necropsy.
- There was a 23% and 14% decrease in the relative testes and epididymides weights at 90 mg/kg.

Table 31: Summary of Organ Weight Changes in the 13-Week Rat Study

(Excerpted from the Applicant's submission)

Test Article-related Organ Weight Changes - Terminal Male and Female (Percent change relative to control)						
Dose level: mg/kg/day	25		50		90	
Sex	M	F	M	F	M	F
Number Examined	10	10	10	10	10	10
Testes (g)	↓7.3	NA	↓5.2	NA	↓23.1[b]	NA
Testes/BWt%	↓8.0	NA	↓11.2	NA	↓23.9[b]	NA
Testes/BrWt ratio	↓8.5	NA	↓7.7	NA	↓22.0[b]	NA
Epididymides (g)	↓1.6	NA	↑1.8	NA	↓13.6[a]	NA
Epididymides/BWt%	↓2.2	NA	↓4.5	NA	↓15.0[a]	NA
Epididymides/BrWt ratio	↓5.1	NA	↓0.7	NA	↓12.6[a]	NA
Heart (g)	↑7.8	↓4.6	↑5.9	↓0.8	↑13.8[a]	↑14.8
Heart/BWt%	↑7.1	↑3.4	↓0.2	↑4.2	↑12.1[a]	↑23.2
Heart/BrWt ratio	↑2.3	↓1.0	↑3.1	↓0.3	↑14.8[b]	↑15.3
Pituitary gland (g)	↓5.7	↓8.5	↑12.1	↓18.5[a]	↓4.5	↓19.9[a]
Pituitary gland/BWt%	↓6.7	0.0	↑3.3	↓13.7	↓6.7	↓15.1
Pituitary gland/BrWt ratio	↓4.1	↓4.5	↑9.5	↓17.1	↓2.7	↓18.9[a]
Thyroid/parathyroid (g)	0.0	↑17.7	0.0	↑17.7	↓8.0	↑23.5[a]
Thyroid/parathyroid /BWt%	↓2.1	↑28.8[a]	↓8.3	↑23.7	↓10.4	↑33.9[b]
Thyroid/parathyroid/BrWt ratio	↓4.1	↑24.7	↓5.8	↑16.9	↓8.3	↑28.1[a]
[a]Significantly different from control; ($p<0.05$)			↑ - Increased			
[b]Significantly different from control; ($p<0.01$)			↓ - Decreased			
BWt - Body Weight; BrWt - Brain Weight			M – Male			
NA – Not Applicable/Not Available			F - Female			

Test Article-related Organ Weight Changes - Recovery Male (Percent change relative to control)			
Dose level: mg/kg/day	25	50	90
Number Examined	5	5	5
Testes (g)	↓13.9[b]	↓8.9[a]	↓27.5[b]
Testes/BWt%	↓17.9	↑2.0	↓21.7[a]
Testes/BrWt ratio	↓12.8[b]	↓6.1	↓25.5[b]
Epididymides (g)	↑5.6	↓6.9	↓15.8[a]
Epididymides/BWt%	↑0.5	↑4.1	↓8.8
Epididymides/BrWt ratio	↑6.7	↓3.9	↓13.3
[a]Significantly different from control; ($p<0.05$)		↑ - Increased	
[b]Significantly different from control; ($p<0.01$)		↓ - Decreased	
BWt - Body Weight; BrWt - Brain Weight			

Histopathology

Adequate Battery Yes

Peer Review Yes, signed

Histological Findings

- Test article-related microscopic findings were present in the heart, pancreas, tongue, and testes.

Table 32: Microscopic Findings with Increased Incidence and/or Severity in GS-1101-Treated Rats Compared to Controls (13-Week Study)

Treatment-Related Microscopic Findings			No. of animals affected							
Terminal Necropsy			**Males**				**Females**			
Dose (mg/kg)			0	25	50	90	0	25	50	90
Number of animals examined			10	10	10	10	10	10	10	10
Organ	Finding	Severity								
Testes	Partial depletion, spermatocyte/spermatid	-minimal	0	1	1	7	NA	NA	NA	NA
		-mild	0	0	0	3	NA	NA	NA	NA
Heart	Cardiomyopathy	-minimal	4	3	3	6	0	1	2	2
		-mild	0	1	0	1	0	0	0	0
Pancreas	Inflammation, subacute/chronic	-minimal	4	2	7	2	0	1	0	0
		-mild	1	1	1	3	0	0	0	0
		-moderate	0	0	0	2	0	0	0	0
	Hemorrhage	-minimal	2	2	2	4	0	1	0	0
Tongue	Inflammation, subacute	-minimal	0	0	0	0	0	0	1	1
		-mild	0	0	0	0	0	0	0	2
		-moderate	0	0	0	0	0	0	0	1
	Erosion/ulcer	-minimal	0	0	0	0	0	0	0	2
Recovery Necropsy			**Males**				**Females**			
Testes	Partial depletion, spermatid	-minimal	0	0	0	3	NA	NA	NA	NA

NA = not present in animal

Toxicokinetics

Table 33: Toxicokinetic Parameters for GS-1101 in Rat Plasma: Days 1 and 90

(Excerpted from the Applicant's submission)

Day	Dose (mg/kg/day)	Sex	AUC_{0-24} (hr•ng/mL)	AUC_{0-last} (hr•ng/mL)	C_{max} (ng/mL)	$t_{1/2}$ (hr)	T_{max} (hr)
1	25	Male	3500	2890	1200	2.33	1.00
		Female	13300	9170	3500	2.91	0.500
		Combined	8390	6030	2350	2.62	0.750
	50	Male	12300	10900	2860	1.74	1.00
		Female	27600	27600	5110	2.32	1.00
		Combined	19900	19200	3980	2.03	1.00
	90	Male	25100	25100	4630	2.45	2.00
		Female	85400	85400	9210	2.40	1.00
		Combined	55200	55200	6920	2.42	1.50
90	25	Male	7370	7370	1980	3.46	1.00
		Female	30900	30900	6330	2.26	0.500
		Combined	19100	19100	4160	2.86	0.750
	50	Male	45000	45000	5130	2.38	1.00
		Female	73300	73300	10100	2.04	1.00
		Combined	59100	59100	7640	2.21	1.00
	90	Male	114000	114000	10200	2.49	2.00
		Female	172000	172000	15100	2.11	1.00
		Combined	143000	143000	12700	2.30	1.50

Study title: A 6-Month Oral (Gavage) Toxicity Study of GS-1101 with a 12 Week Recovery Period in Sprague Dawley Rats

Study no.:	TX-312-2005
Study report location:	4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 14, 2011 (first dose)
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	GS-1101, Lot. # 1101-AC-1P-DKS-11-1204-23, 99.8%

Key Study Findings

- There were five mortalities, four of which are likely drug-related. One mortality at the low dose was due to malignant lymphoma, which may be incidental.
- Common microscopic findings in animals found dead were decreased lymphocytes in the spleen, hemorrhage of the thymus, and dilatation of the urinary bladder. Other microscopic findings in the 90 mg/kg/day males found dead were cardiomyopathy, hypertrophy of the adrenal cortex, inflammation of the pancreas, and alveolar macrophages in the lungs.
- At all doses, there were increases in white blood cells (lymphocytes, neutrophils, monocytes, and basophils).
- Organs of toxicity: hematopoietic system, reproductive organs, kidneys, heart, liver, lungs, and pancreas. Axonal degeneration in the peripheral nerves and

hemorrhage in the brain of one male were also observed at low incidence in males at 90 mg/kg/day.

- GS-1101 was readily absorbed, accumulated with repeat-dosing, and exposures (AUC and C_{max}) were dose-dependent with greater than 2-fold higher exposures in females. The toxicokinetic profile of the metabolite GS-563117 was similar to the parent GS-1101, despite the fact that the parent compound was not readily converted to its metabolite in rats.

Methods

Doses:	0, 25, 50, 90 mg/kg/day
Frequency of dosing:	Daily for 26 weeks
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% high viscosity carboxymethylcellulose/0.1% TWEEN 80 in deionized water
Species/Strain:	Crl:CD(SD)
Number/Sex/Group:	15 animals/sex/group
Age:	7 weeks old
Weight:	Males, 201-259 g; Females, 141-183 g
Satellite groups:	Toxicokinetic (TK): vehicle control, 3 animals/sex/group; GS-1101-treated, 9 animals/sex/group
Unique study design:	No
Deviation from study protocol:	Deviations did not impact the quality or integrity of the data or the outcome of the study.

Observations and Results

Mortality:	Twice daily
Clinical signs[a]:	Twice daily (once daily during recovery); detailed examinations prior to dosing initiation, weekly during dosing and recovery, and prior to necropsy*
Body weights:	Twice prior to dosing phase initiation, weekly during the dosing and the recovery periods, at necropsy*
Food consumption[a]:	Approximately weekly throughout the study, beginning 6 days prior to randomization.
ECG:	Not conducted
Ophthalmoscopy:	Study week -1 (all animals) and on all surviving toxicology group animals near the end of the dosing period
Hematology[a,b]:	At necropsy*
Clinical chemistry[a,b]:	At necropsy*
Coagulation[a,b]:	At necropsy*
Urinalysis[a,b]:	At necropsy*
Gross pathology[a]:	At necropsy*

Organ weights[a]:	At necropsy*
Histopathology[a]:	At necropsy*
Toxicokinetics:	Days 0 and 181 Controls: • Prior to dosing and 2 hours post-dose GS1101--treated groups: • Predose and approximately 0.5, 1, 2, 4, 8, and 24 hours postdose.

a = not conducted on TK animals; b = animals were fasted overnight
*10 animals/sex/group were scheduled to be euthanized after 182 days of dosing (primary). The remaining animals, ≤ 5 animals/sex/group, were scheduled to be euthanized for necropsy following an 84-day non-dosing period (recovery). Necropsy was scheduled for TK animals on Day 182.

Mortality

Five animals were found dead prior to the dosing phase necropsy.

Table 34: Cause of Death (6-Month Rat Study)

Dose of GS-1101 (mg/kg/day)	Animal #	Sex	Day Found Dead	Potential cause of morbidity/ macroscopic findings/ microscopic findings
25	4703	Male	137	Malignant lymphoma which histologically involved the liver, spleen, bone marrow, pancreas, and axillary lymph node
	4791	Female	73	Undetermined/ unremarkable macroscopic findings/ mild acute inflammation of the renal pelvis, mild luminal dilatation of the urinary bladder, and minimal splenic marginal zone decreased lymphocytes
50	4794	Female	106	Undetermined/ unremarkable macroscopic findings/ mild luminal dilatation of the urinary bladder and mild splenic marginal zone decreased lymphocytes
90	4666	Male	55	Undetermined/ one white area on seminal vesicles/ moderate chronic inflammation in the pancreas and moderate splenic marginal zone decreased lymphocytes
	4707	Male	177	Undetermined/ multiple depressed areas of the kidneys/ minimal chronic progressive nephropathy, moderate lacrimal gland atrophy and harderian gland alteration, minimal splenic marginal zone decreased lymphocytes, and minimal thymic cortex decreased lymphocytes

Clinical Signs

Table 35: Summary of Clinical Signs in 6-Month Rat Study

Clinical signs	No. of animals affected (No. of observations)							
	Males				**Females**			
Dose (mg/kg)	0	25	50	90	0	25	50	90
Number of animals examined	15	15	15	15	15	15	15	15
During dosing phase								
Hair loss dorsal trunk				1(1)				

Clinical signs	No. of animals affected (No. of observations)							
	Males				**Females**			
Dose (mg/kg)	0	25	50	90	0	25	50	90
Number of animals examined	15	15	15	15	15	15	15	15
Swollen right hindlimb				1(2)				
Scabbing dorsal trunk			1(2)					1(1)
Upper incisor(s) broken		1(1)	2(10)					1(2)
Upper incisor(s) missing								1(1)
Reddened facial area								1(1)
Wet yellow material urogenital area		1(1)						1(2)
Dried yellow material urogenital area	1(1)							1(1)
At time of dose								
Wet red material around nose				1(1)				
Red material on cage floor				1(1)				
Swollen right forelimb				1(2)				
Swollen left hindlimb				1(1)				
Swollen right hindlimb				1(6)				
Clear discharge left eye								1(1)
Clear discharge right eye	1(1)							2(2)
Reddened facial area								1(2)
Reddened left forelimb		1(4)	1(1)					1(5)
Reddened right forelimb								1(5)
Wet yellow material urogenital area								1(2)
Dried yellow material urogenital area								2(4)
Dried yellow material anogenital area								1(1)
1-2 hours post dose								
Swollen right forelimb				1(2)				
Swollen right hindlimb				1(7)				
Wet red material around nose			1(1)	1(1)				
Soft feces				1(1)				
Clear discharge left eye						1(1)		1(1)
Clear discharge right eye								
Dried red material around mouth								1(1)
Reddened facial area								1(2)
Reddened left forelimb		1(2)						1(5)
Reddened right forelimb		1(2)						1(5)
Wet yellow material urogenital area								4(22)
Wet yellow material ventral trunk								1(1)
Wet yellow material anogenital area								1(1)
Dried yellow material urogenital area								2(3)
Dried yellow material anogenital area								1(2)
Dried red material forelimb(s)								2(4)

Body Weights

No toxicologically significant changes

Food Consumption

Unremarkable

Ophthalmoscopy

Unremarkable

ECG

Not conducted

Hematology

Males

Table 36: Summary of Hematolotgy Findings in Male Rats in the 6-Month Study

Index	Mean		Percentage deviation from Control					
	Control 0 mg/kg		25 mg/kg		50 mg/kg		90 mg/kg	
	WK 26	WK 38	WK 26	WK 38	WK 26	WK 38	WK 26	WK 38
HDW (g/dL)	2.87	2.19	3	6	11*	-1	9	2
Retic. Abs.	196.8	183.6	-2	4	6	27	11	17
Retic.%	2.1	2.0	0	0	5	30	10	15
Platelets Abs.	1165	961	2	2	11	-10	16	3
WBC Abs.	8.02	7.29	14	7	12	31	24	11
Lymphocytes Abs.	6.01	5.23	22	13	15	34	28	13
Neutrophils Abs.	1.57	1.58	-8	-7	0	28	13	6
Monocytes Abs.	0.23	0.28	13	-14	17	4	30	11
Eosinophils Abs.	0.12	0.13	-33	-23	-17	-31	-25	8
Basophils Abs.	0.2	0.2	0	0	50	100	50	50
LUC Abs.	0.07	0.05	-14	20	29	140	43	20
LUC%	0.9	0.8	-33	0	0	50	11	-13

Table 37: Summary of Hematolotgy Findings in Female Rats in the 6-Month Study

Index	Mean		Percentage deviation from Control					
	Control 0 mg/kg		25 mg/kg		50 mg/kg		90 mg/kg	
	WK 26	WK 38	WK 26	WK 38	WK 26	WK 38	WK 26	WK 38
HDW (g/dL)	2.53	2.09	8**	-6	11**	-6	12**	1
RDW%	11.6	11.8	6**	-4	9**	-2	9**	3
Retic. Abs.	152.6	153.4	12	1	21*	-5	16	21
Retic.%	1.8	1.9	11	0	22	-11	17	21
Platelets Abs.	1121	956	0	2	2	9	5	-6
WBC Abs.	4.75	5.17	4	4	30	-1	36*	9
Lymphocytes Abs.	3.86	3.55	6	19	36	16	31	24
Neutrophils Abs.	0.64	0.61[a]	2	41	3	18	81*	56
Monocytes Abs.	0.12	0.12[a]	-9	25	8	17	25	25
Eosinophils Abs.	0.07	0.08	-14	0	-14	13	-29	13
Basophils Abs.	0.01	0.01	0	0	100	0	0	100
LUC Abs.	0.04	0.02	-25	100	0	100	50	100
LUC%	0.8	0.5	-13	40	-13	40	0	20

* = $p \leq 0.05$; ** = $p \leq 0.01$; - = decrease; 0 = no change

Abbreviations: HDW, hemoglobin distribution width; RDW, red blood cell distribution width; WBC, white blood cells; LUC, large unstained cells; Abs., absolute number of cells (thousands/μL)

a – Outlier (one control recovery female, No. 4812) excluded from analysis by reviewer

Clinical Chemistry

Table 38: Summary of Clinical Chemistry Parameters in the 6-Month Rat Study

Index	Mean		Percentage deviation from Control					
	Control 0 mg/kg		25 mg/kg		50 mg/kg		90 mg/kg	
	WK 26	WK 38	WK 26	WK 38	WK 26	WK 38	WK 26	WK 38
Males								
Globulin (g/dL)	2.6	2.6	-8	0	-4	-4	-4	-4
A:G Ratio	1.55	1.62	5	-1	10**	0	10**	1
Bilirubin (mg/dL)	0.04	0.03	25	0	0	0	-25	-33
Cholesterol (mg/dL)	76	68	-12	7	11	19	24	16
Triglycerides (mg/dL)	85	86	-31	1	12	30	-9	35
Phosphorous (mg/dL)	5.9	5.7	0	0	10*	4	9	2
Females								
Globulin (g/dL)	3	2.9	-10*	0	-10	-7	-13**	-3
A:G Ratio	1.54	1.68	17**	-2	12**	4	18**	1
Bilirubin (mg/dL)	0.10	0.08	-20*	-13	-30**	-13	-40**	0
Cholesterol (mg/dL)	81	89	15	-12	24	-17	22	15

* = p ≤ 0.05; ** = p ≤ 0.01; - = decrease; 0 = no change
Abbreviations: A:G, Albumin to Globulin ration; AST, aspartate aminotransferase

- There was considerable variability in values obtained from control animals making it difficult to determine the drug-related effects of GS-1101 on clinical chemistry parameters (e.g., AST).

Urinalysis

Unremarkable

Gross Pathology

Table 39: Summary of Macrosopic Findings in the 6-Month Rat Study

Treatment-related macroscopic findings		No. of animals affected							
		Males				**Females**			
Dose (mg/kg)		0	25	50	90	0	25	50	90
Number of animals examined		0/10/**5**	1*/9/**5**	0/10/**5**	2*/8/**5**	0/10/**5**	1*/9/**5**	1*/9/**5**	0/10/**5**
Thymus	Area(s), dark red		1*/0/**0**	0/0/**1**	0/2/**1**	0/0/**1**		0/1/**1**	0/1/**1**
	Small	0/1/**0**			0/2/**0**				0/1/**1**
Uterus	Contents, clear fluid	NA				0/1/**0**	0/1/**0**	0/3/**0**	0/2/**0**
Testes	Small				0/1/**0**	NA			

Number of animals examined and affected: Early deaths*/Terminal necropsy / **Recovery necropsy**
Empty cells = no test-article related changes
NA= Not Applicable, tissue does not exist in this sex

Organ Weights

Table 40: Summary of Organ Weight Changes in the 6-Month Rat Study

Group and Dose		Percentage deviation from Control					
		25 mg/kg/day		50 mg/kg/day		90 mg/kg/day	
Necropsy		Term	Recov	Term	Recov	Term	Recov
Number of animals examined		9	5	10	5	8	5
Males							
Spleen	Absolute (g)	-12	11	-19*	62	-18*	21
	Relative BW (%)	-7	12	-13	55	-12	15
	Relative Brain (%)	-11	8	-16	59	-16	18
Epididymis	Absolute (g)	-2	0	-11*	-3	-23**	-2
	Relative BW (%)	3	0	-5	-6	-18*	-8
	Relative Brain (%)	-1	-3	-9	-5	-21**	-4
Testis	Absolute (g)	-3	-3	-13*	-6	-24**	-5
	Relative BW (%)	2	-2	-7	-9	-19*	-10
	Relative Brain (%)	-2	-5	-12*	-8	-23**	-7
Liver	Absolute (g)	-8	0	-1	4	8	10
	Relative BW (%)	-2	0	6	0	16**	4
	Relative Brain (%)	-7	-2	1	3	10	8
Number of animals examined		9	5	9	5	10	5
Females							
Spleen	Absolute (g)	-9	-10	-13	-12	-8	-4
	Relative BW (%)	-12	-5	-18	-8	-7	-1
	Relative Brain (%)	-10	-10	-13	-11	-8	-6
Uterus/ Cervix	Absolute (g)	-9	-17	-3	-16	-18	-6
	Relative BW (%)	-14	-11	-10	-12	-19	-4
	Relative Brain (%)	-10	-17	-4	-15	-21	-8

* = $p \leq 0.05$; ** = $p \leq 0.01$; - = decrease; 0 = no change; BW = body weight; Term = terminal necropsy; Recov = recovery necropsy

- There was a dose-dependent (not statistically significant) increase in lung organ weight (up to 16%) in recovery males at 90 mg/kg/day that was not observed in the dosing phase.

Histopathology

Adequate Battery Yes

Peer Review Yes, signed

Histological Findings

Table 41: Microscopic Findings with Increased Incidence and/or Severity in GS-1101-Treated Rats Compared to Controls (6-Month Study)

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				**Females**			
Dose (mg/kg)			0	25	50	90	0	25	50	90
Number of animals examined[a,b,c]			0/10/**5**	1*/t/**r**	0/t/**r**	2*/8/**5**	0/10/**5**	1*/t/**r**	1*/t/**r**	0/10/**5**
Organ	Finding	Severity								
Adrenal, Cortex[d]	Hypertrophy	Total	0/0/**2**			1*/1/**1**	0/0/**1**	0/0/**1**	0/0/**1**	0/2/**3**
		Minimal	0/0/**2**			0/1/**1**	0/0/**1**			0/0/**2**
		Mild				1*/0/**0**			0/0/**1**	0/1/**1**
		Moderate						0/0/**1**		0/1/**0**
	Angiectasis	Minimal								0/0/**1**
	Cyst	Present						0/0/**1**		
Brain	Hemorrhage	Mild				0/1/**0**				
Colon[d]	Diverticulum	Present							0/1/**0**	
Epididymides[b]	Luminal debris, cellular	Minimal				0/1/**0**	NA			
Harderian glands[d]	Degeneration	Total	0/2/**2**		0/1/**-**	0/4/**1**	0/1/**0**			0/1/**2**
		Minimal	0/1/**1**		0/1/**-**	0/2/**1**				0/0/**1**
		Mild	0/1/**1**			0/1/**0**	0/1/**0**			0/1/**1**
		Moderate				0/1/**0**				
Heart	Cardio-myopathy	Total	0/1/**2**			1*/4/**2**	0/1/**1**			
		Minimal	0/0/**1**			1*/3/**2**				
		Mild	0/1/**1**			0/1/0	0/1/**1**			
	Infiltrate, mixed cell, vascular	Mild								0/0/**1**
Kidneys[d]	Calculus	Present	0/1/**1**			0/0/**2**	0/1/**1**			0/1/**0**
	Cyst	Present								0/0/**1**
Lac. Gland Exor	Atrophy	Total	0/0/**1**			1*/0/**0**				
		Mild	0/0/**1**							
		Moderate				1*/0/**0**				
	Infiltrate, mononuclear	Minimal				2*/0/**0**				
Liver	Focus, Basophilic cell	Minimal								0/1/**0**
	Focus, Eosinophilic cell	Minimal								0/1/**0**
	Fibrosis, peribiliary	Minimal								0/0/**1**
LN, axillary	Erythrocytosis, sinus	Moderate				0/0/**1**				
LN, mandibular	Erythrocytosis, sinus	Total	0/2/**2**			0/4/**3**	0/2/**2**		1*/-/**-**	0/3/**3**
		Minimal	0/2/**2**			0/2/**2**	0/2/**1**			0/2/**1**
		Mild				0/2/**1**	0/0/**1**		1*/-/**-**	
		Moderate								0/1/**2**
LN, mediastinal[d]	Infiltrate, histiocyte	Mild	NA	NA	0/0/**1**	NA				
LN, mesenteric	Erythrocytosis, sinus	Minimal	0/0/**1**			0/2/**0**				
	Infiltrate, histiocyte	Minimal				0/0/**1**				

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				**Females**			
Dose (mg/kg)			0	25	50	90	0	25	50	90
Number of animals examined[a,b,c]			0/10/**5**	1*/t/**r**	0/t/**r**	2*/8/**5**	0/10/**5**	1*/t/**r**	1*/t/**r**	0/10/**5**
Organ	Finding	Severity								
Lungs[d]	Infiltrate, mononuclear, perivascular	Minimal				0/1/**0**				
	Infiltrate, mononuclear, subpleural	Minimal	0/1/**0**			0/2/**0**	0/1/**1**			
	Macrophages, alveolar	Total	0/1/**0**			1*/0/**1**	0/0/**1**		0/0/**1**	0/0/**1**
		Minimal	0/1/**0**			0/0/**1**	0/0/**1**			0/0/**1**
		Mild				1*/0/**0**			0/0/**1**	
Nerve, Sciatic	Degeneration, axonal	Moderate				0/1/**0**				
Ovaries[d]	Cyst, Luteal, present	Present	NA						1*/0/**-**	0/2/**0**
	Cyst, Paraovarian	Present					0/0/**1**			0/1/**0**
Pancreas	Degeneration, acinar	Minimal				0/1/**0**				
	Hyperplasia, acinar	Minimal				0/1/**0**				
	Inflammation, chronic	Total	0/0/**1**			1*/3/**0**				
		Minimal	0/0/**1**			0/2/**0**				
		Mild				0/1/**0**				
		Moderate				1*/0/**0**				
Pituitary	Cyst, Rathke's Pouch	Present								0/0/**1**
Prostate[d]	Hyperplasia	Minimal				0/0/**1**	NA			
Seminal vesicles	Decreased secretion	Mild				0/0/**1**	NA			
Skin	Infiltrate, mononuclear	Minimal				0/1/**0**				
Soft Tissue-Thor	#B Hibernoma, benign	Present	NA	NA	NA	0/0/**1**				
Spinal cord	Degeneration, axonal	Total	0/0/**1**	1*/0/**0**		0/0/**2**				
		Minimal	0/0/**1**			0/0/**2**				
		Moderate		1*/0/**0**						
Spleen[b,c]	Decreased lymphocytes, marginal zone	Total	0/1/**0**	0/6/**0**	0/8/**1**	2*/8/**3**		1*/5/**0**	1*/6/**0**	0/9/**0**
		Minimal	0/1/**0**	0/6/**0**	0/3/**1**	1*/2/**2**		1*/5/**0**	0/5/**0**	0/3/**0**
		Mild			0/4/**0**	0/4/**1**			1*/1/**0**	0/6/**0**
		Moderate			0/1/**0**	1*/2/**0**				
	Infiltrate, histiocyte	Mild			0/0/**1**					
Testes[b]	Degeneration, seminiferous tubules, bilateral	Minimal				0/1/**0**	NA			
Thymus[b]	Hemorrhage	Total	0/7/**3**	1*/5/**0**	0/5/**1**	1*/5/**2**	0/4/**1**	1*/3/**0**	1*/2/**1**	0/4/**4**
		Minimal	0/7/**3**	0/5/**0**	0/5/**0**	1*/3/**2**	0/4/**1**	0/3/**0**	0/2/**1**	0/3/**4**
		Mild		1*/0/**0**	0/0/**1**	0/2/**0**		1*/0/**0**	1*/0/**0**	0/1/**0**

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				**Females**			
Dose (mg/kg)			0	25	50	90	0	25	50	90
Number of animals examined[a,b,c]			0/10/**5**	1*/t/**r**	0/t/**r**	2*/8/**5**	0/10/**5**	1*/t/**r**	1*/t/**r**	0/10/**5**
Organ	Finding	Severity								
Thyroid glands[d]	Cyst, Ultimobranchial	Present	0/3/**2**			0/3/**4**	0/2/**2**			0/6/**3**
	Hyperplasia, follicular cell	Minimal	0/0/**0**			0/0/**1**				
Uterus[d]	#B Polyp, endometrial stromal	Present							0/0/**1**	

Number of animals examined and affected: Early deaths*/ Terminal necropsy / **Recovery necropsy**
(minus symbol (-) means animals were not evaluated). The number of animals examined for terminal (t) and recovery (r) varied for the 25 and 50 mg/kg/day groups.
empty cell = no change or not evaluated; NA = not present in animal
a - Microscopic examination was performed for all animals found dead and from all animals in the vehicle control and 90 mg/kg/day groups at the scheduled necropsies.
b - The gross lesions, epididymides, testes, thymus, Peyer's patches, and spleen (primary necropsy only) were examined from all animals in the 25 and 50 mg/kg/day groups at the primary and recovery necropsies.
c - The spleen was not examined for 25 and 50 mg/kg/day recovery females.
d - Select animals from the 25 and/or 50 mg/kg/day dosing groups were examined.

Other noteworthy findings:

- In one moribund male at 90 mg/kg/day and two moribund females, one each at 25 and 50 mg/kg/day, the ileum and jejunum were too autolyzed to examine.
- In the 25 mg/kg/day moribund female, the Peyer's patches were too autolyzed to examine.
- One moribund male at 25 mg/kg/day had lymphoma in the liver, axillary lymph nodes, bone marrow (femur and sternum), pancreas, spleen, and systemic tumors.
- The following microscopic findings were present in moribund animals, but were also present at similar incidence and severity in GS-1101 treated animals as in concurrent controls and are likely not treatment related:
  - Pigment, porphyrin (Harderian glands)
  - Infiltrates, neutrophil, pelvis (kidneys)
  - Nephropathy, chronic progressive (kidneys)
  - Mineralization (kidneys)
  - Decreased lymphocytes (cortex of thymus and Peyer's patches)
  - Alteration Harderian gland (Lac. Gland Exor)
  - Pigment, brown (pancreas)
  - Dilatation, lumen (urinary bladder and cervix and uterus (females only))
- Skeletal muscle degeneration and hemorrhage of the lungs, mesenteric lymph nodes, and Harderian glands are noteworthy microscopic findings, but were observed in control and GS-1101 treated animals at similar incidence and severity.

Special Evaluation

None

Toxicokinetics

GS-1101

- Exposures (AUC and C_{max}) to GS-1101 increased with increasing dose. The increases in C_{max} were approximately proportional between the 25 and 90 mg/kg/day dose levels while the increases in AUC_{0-t} were generally greater than dose-proportional between the 25 and 90 mg/kg/day dose levels.
- There was accumulation of GS-1101 as values for C_{max} and AUC_{0-t} were higher on study day 181 than on study day 0, with greater than 2-fold differences observed in AUC_{0-t} values.
- GS-1101 was readily absorbed, with T_{max} values ranging from 0.5 to 1.0 hours on study day 0 and from 0.5 to 4.0 hours on study day 181.
- $T_{1/2}$ values for males and females ranged from 1.2 to 3.4 hours on study day 0 and from 2.9 to 3.8 hours on study day 181.
- Females had higher C_{max} and AUC_{0-t} values than males, with greater than 2-fold differences generally observed on study day 0.

Table 42: Toxicokinetic Parameters for GS-1101 in Rat Plasma: Days 0 and 181

(Excerpted from the Applicant's submission)

Interval	Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	C_{last} (ng/mL)	T_{last} (hr)	$t_{1/2}$ (hr)	AR
Day 0	2A	25	M	1297	0.500	3394	21.7	8.00	1.32	NA
			F	3113	0.500	7855	101	8.00	1.61	NA
			MF	2205	0.500	5480	61.5	8.00	1.52	NA
	3A	50	M	2453	1.00	7090	63.4	8.00	1.24	NA
			F	5960	1.00	18738	27.4	24.0	3.37	NA
			MF	4207	1.00	13277	27.4	24.0	3.65	NA
	4A	90	M	6510	1.00	17824	285	8.00	1.80	NA
			F	10987	1.00	55727	19.0	24.0	2.55	NA
			MF	8748	1.00	37990	19.0	24.0	2.73	NA
Day 181	2A	25	M	2210	1.00	10635	10.9	24.0	3.08	3.13
			F	3930	0.500	23018	54.3	24.0	3.80	2.93
			MF	2992	1.00	16861	37.0	24.0	3.75	3.08
	3A	50	M	4837	1.00	43738	21.3	24.0	2.91	6.17
			F	8570	1.00	54954	35.9	24.0	2.87	2.93
			MF	6703	1.00	49346	28.6	24.0	2.88	3.72
	4A	90	M	8217	4.00	104217	34.6	24.0	NC	5.85
			F	15500	1.00	181567	262	24.0	3.35	3.26
			MF	10950	4.00	143074	171	24.0	NC	3.77

NA Not applicable
NC Not calculated due to the lack of a distinct elimination phase.
Note: Combined data is based on the analysis of the combined concentration data of both sexes.

AR = accumulation ratio

GS-563117

- Exposures (AUC and C_{max}) to GS-563117 increased with increasing dose in a dose-proportional manner. Gender differences were less than 2-fold.

- There was accumulation of GS-563117 after multiple dosing of the parent compound, although accumulation ratios were lower than for GS-1101.
- GS-563117 readily appeared in plasma, with T_{max} values of 1 hours on Day 0 and ranging from 1 to 8 hours on Day 181.
- The AUC_{0-t} metabolite to parent (M/P) ratios ranged from 0.00559 to 0.0328, indicating that GS-1101 is not extensively converted to GS-563117 in rats following oral gavage administration of GS-1101.

Table 43: Toxicokinetic Parameters for GS-563117 in Rat Plasma: Days 0 and 181

(Excerpted from the Applicant's submission)

Interval	Dose Group	GS-1101 Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	C_{last} (ng/mL)	T_{last} (hr)	$t_{1/2}$ (hr)	AR	M/P Ratio
Day 0	2A	25	M	33.4	1.00	82.2	11.9	4.00	NC	NA	0.0242
			F	47.4	1.00	92.4	11.2	4.00	NC	NA	0.0118
			MF	40.4	1.00	87.4	11.6	4.00	NC	NA	0.0159
	3A	50	M	97.1	1.00	218	24.7	4.00	NC	NA	0.0307
			F	92.9	1.00	218	27.8	4.00	NC	NA	0.0117
			MF	95.0	1.00	218	26.2	4.00	NC	NA	0.0164
	4A	90	M	161	1.00	585	9.10	8.00	1.68	NA	0.0328
			F	152	1.00	378	96.1	4.00	NC	NA	0.00678
			MF	157	1.00	589	10.3	8.00	1.79	NA	0.0155
Day 181	2A	25	M	52.5	1.00	133	28.5	4.00	NC	1.62	0.0125
			F	69.3	1.00	280	14.3	8.00	3.44	3.03	0.0122
			MF	60.9	1.00	247	13.1	8.00	3.52	2.83	0.0146
	3A	50	M	86.2	1.00	454	59.6	8.00	NC	2.09	0.0104
			F	179	1.00	562	29.4	8.00	3.85	2.57	0.0102
			MF	132	1.00	508	44.5	8.00	7.25	2.33	0.0103
	4A	90	M	155	8.00	980	155	8.00	NC	1.67	0.00940
			F	167	1.00	1015	157	8.00	NC	2.69	0.00559
			MF	156	8.00	997	156	8.00	NC	1.69	0.00697

NA Not applicable
NC Not calculated due to the lack of a distinct elimination phase.
Note: Combined data is based on the analysis of the combined concentration data of both sexes.

Dosing Solution Analysis

Test-article formulations were prepared weekly, divided into aliquots for daily dispensation, and stored refrigerated (2°C to 8°C). Samples for homogeneity determination were collected from the top and bottom strata. Samples for concentration analysis were collected from the middle stratum.

The analyzed dosing formulations were found to contain 97.3% to 103% of the test article which was within the [redacted] (b) (4) SOP range of target concentrations for suspensions (85% to 115%), as well as met the [redacted] (b) (4) SOP requirement for homogeneity and, after 8 days of refrigerated storage, resuspension homogeneity. The test article was not detected in the vehicle formulation that was administered to the vehicle control group (Group 1).

Study title: A 9-Month Oral (Capsule) Toxicity Study of GS-1101 with a 12-Week Recovery Period in Beagle Dogs

Study no.:	TX-312-2006
Study report location:	4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-1101, Lot # 1101-AC-1P-DKS-11-1204-23, Purity 99.8%

Key Study Findings

- Two unscheduled deaths related to GS-1101. The cause of death for these 2 animals was systemic inflammation in multiple organs and decreased lymphocytes in peripheral lymphoid tissues.
- Target organs of toxicity were the lymphoid system (decreased lymphocytes and inflammation) and gastro-intestinal tract (red areas, inflammatory changes, and crypt dilatation).
- Exposure to GS-1101 generally increased with the increase in dosage level from 2.5 to 7.5 mg/kg/day.

Methods

Doses:	0, 2.5, 5 and 7.5 mg/kg
Frequency of dosing:	Once daily for 9 months
Route of administration:	Oral
Formulation/Vehicle:	Capsule with appropriate amount of test article doses and empty capsule vehicle control
Species/Strain:	Beagle dogs
Number/Sex/Group:	4 main group 2 recovery group for control and high-dose
Age:	6-7 months
Weight:	Males –6.9 kg to 9.8 kg; Females – 6.1 kg to 8.2 kg
Dose justification:	The dose levels were selected by the Applicant based on previous 13-week repeat dose study with GS-1101 in dogs (study TX-312-2002)

Observations and Results

Mortality:	Twice daily
Clinical signs:	Twice daily
Body weights:	Weekly
Food consumption:	Daily

ECG:	Study week 2 and study week 38
Ophthalmoscopy:	Study week 1 and study week 38
Hematology:	Day -8 (study week -2) and 14 (week -2') on the report tables), study weeks 13, 38, and 51
Clinical chemistry:	Day -8 (study week -2) and 14 (week -2') on the report tables), study weeks 13, 38, and 51
Coagulation:	Day -8 (study week -2) and 14 (week -2') on the report tables), study weeks 13, 38, and 51
Urinalysis:	Day -8 (study week -2) and 14 (week -2') on the report tables), study weeks 13, 38, and 51
Gross pathology:	At necropsy
Organ weights:	At necropsy
Histopathology:	At necropsy
Toxicokinetics:	On study days 91, 182, and 272, and at 1, 2, 4, 8, 16, and 24 hours after dose administration on study days 0 and 272.

Mortality

Table 44: Cause of Death (9-Month Dog Study)

GS-1101 (mg/kg)	Animal #	Sex	Day	Potential cause of death
2.5	1376	F	231 (week 33)	GS-1101-related - systemic inflammation
7.5	1364	M	145 (week 20)	GS-1101-related - systemic inflammation

Table 45: Toxicities Observed in Dead Animals (9-Month Dog Study)

GS-1101 (mg/kg)	Toxicities	
2.5 Animal # 1376	Clinical	Injected sclera, reddened ears, reddened gums, soft feces, emesis, hunched posture, and intermittent tremors.
	Gross necropsy	Dilated lateral ventricles in the brain.
	Histopathology	Decreased lymphocytes in the spleen, thymus, mandibular lymph nodes, and Peyer's patches; moderate perivascular mixed cell infiltrate and multifocal microabscesses in the brain; mild perivascular mixed cell infiltrate in the spinal cord meninges; and mild granulocyte infiltrate in the rectum
7.5 Animal # 1364	Clinical	Clear discharge and injected sclera, excessive salivation, soft mucoid feces, thin body condition, decreased activity, impaired equilibrium, and vocalization upon handling
	Gross necropsy	1 nodule on the palate and dark red areas in the colon

	Histopathology	decreased lymphocytes in the spleen, thymus, lymph nodes (mandibular, mesenteric, and axillary), and Peyer's patches; hypercellular bone marrow; vascular inflammation in multiple tissues including the stomach, colon, cecum, heart, kidneys, liver, and skeletal muscle; granulocyte infiltrates in the duodenum, jejunum, ileum, colon, cecum, and rectum; neutrophilic inflammation in the heart and lungs; and moderate testicular seminiferous tubule degeneration.

Clinical Signs

Clear discharge and injected sclera of the eyes at 2.5, 5 and 7.5 mg/kg in males and females and reddened facial area at 7.5 mg/g in both males and females. These clinical findings generally resolved during the recovery period.

Body Weights

- At the end of 38 weeks mean body weights were 11% and 9% lower than the vehicle control group in the 7.5 mg/kg/day group males and females, respectively.
- At the end of dosing, the mean body weights in 5 mg/kg/day group males and females were 8.5% and 6.7%, respectively, lower than the vehicle control group.

Figure 23: Changes in Body Weight Gain in Male Dogs Administered GS-1101 by Oral Gavage (9-Month Study)

(Excerpted from the Applicant's submission)

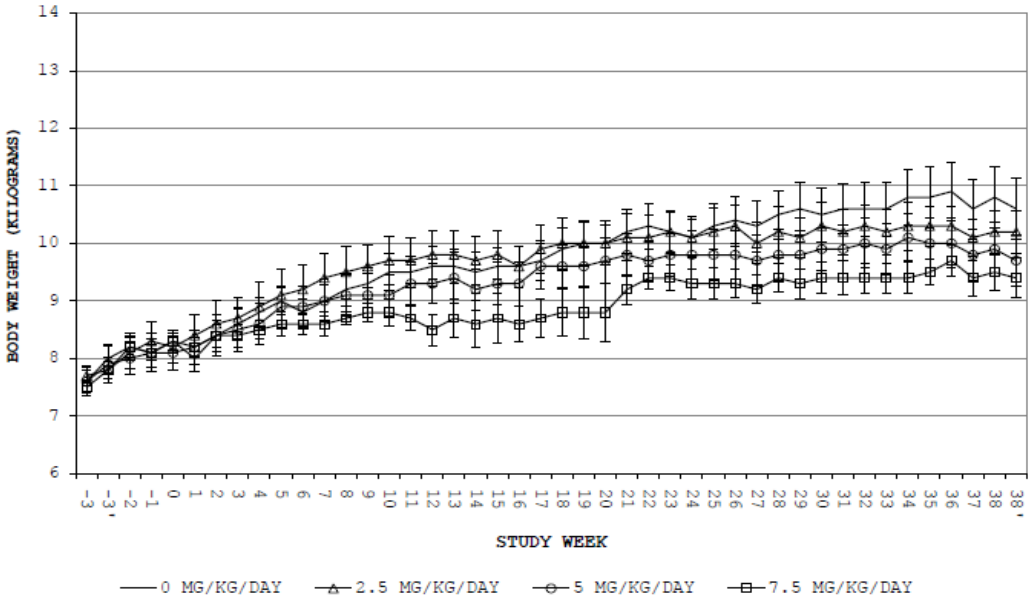


Figure 24: Changes in Body Weight Gain in Female Dogs Administered GS-1101 by Oral Gavage (9-Month Study)

(Excerpted from the Applicant's submission)

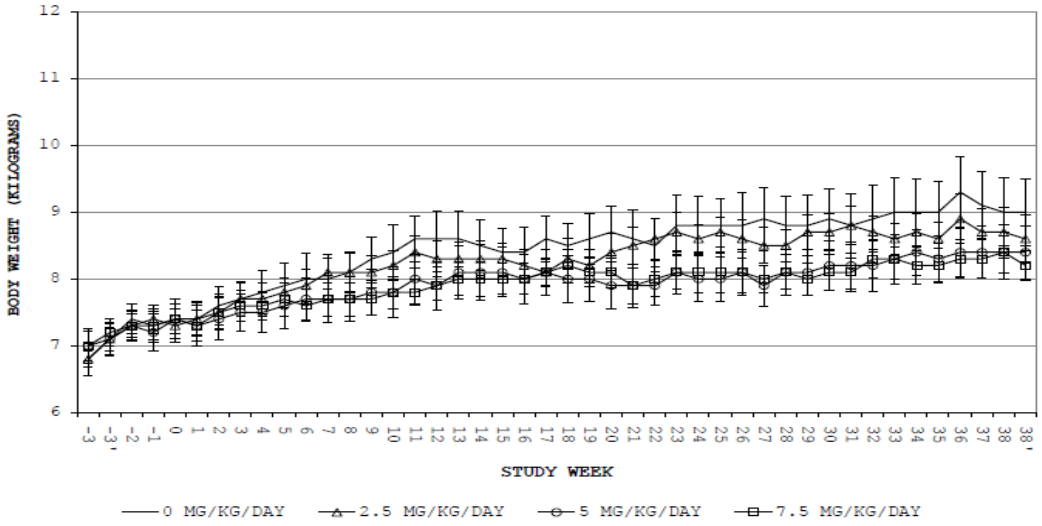


Table 46: Changes in Body Weight Gain in Male Dogs During Recovery (9-Month Study)

(Excerpted from the Applicant's submission)

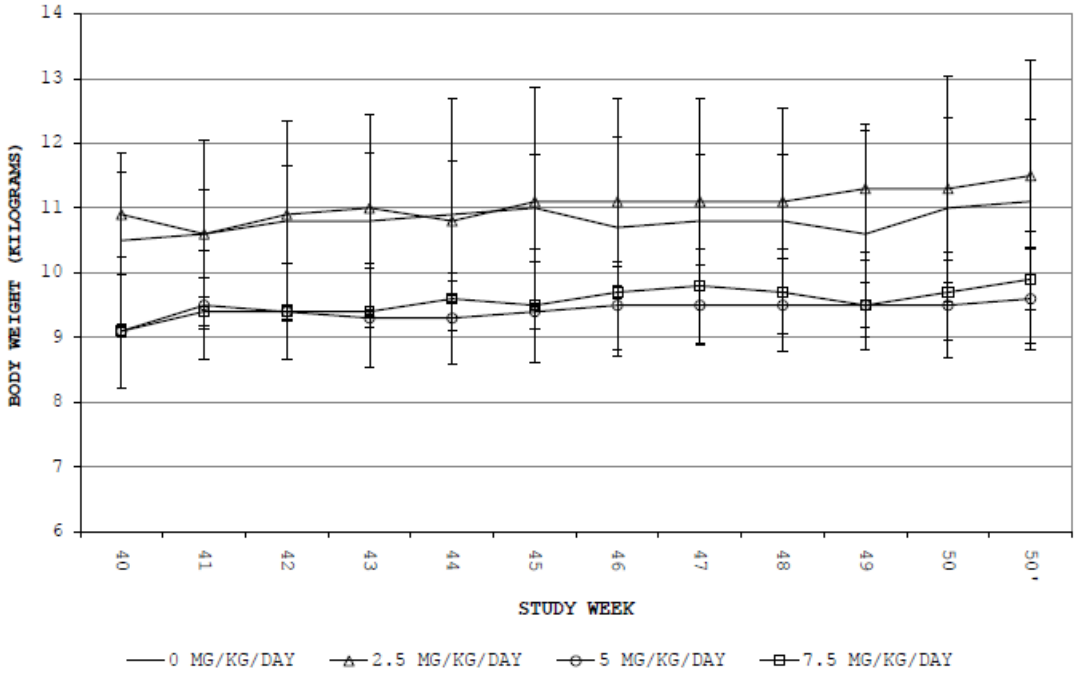
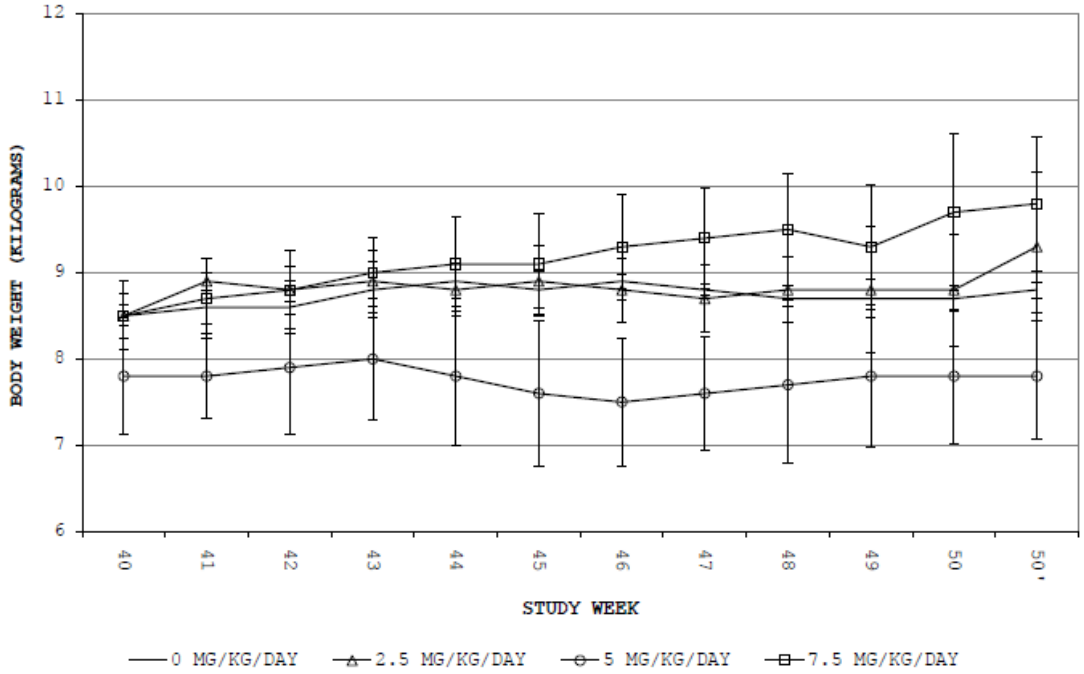


Table 47: Changes in Body Weight Gain in Female Dogs During Recovery (9-Month Study)

(Excerpted from the Applicant's submission)



Food Consumption
Unremarkable

Ophthalmoscopy
Unremarkable

ECG
Unremarkable

Hematology
Potentially GS-1101-related hematology alterations included reversible,

- Lower absolute basophil counts in the 2.5, 5, and 7.5 mg/kg/day group males and females at study weeks 13 and 38.
- Lower absolute large unstained cell (LUC) counts in the 5 and 7.5 mg/kg/day group males at study week 38, 2.5, 5, and 7.5 females at study weeks 13 and 38.

Table 48: Summary of Hematology Findings in Male Dogs in the 9-Month Study

Index	Mean			Percentage deviation from Control - Males								
	Control 0 mg/kg			2.5 mg/kg			5 mg/kg			7.5 mg/kg		
	Week			Week			Week			Week		
	13	38	51 Rec	13	38	51 Rec	13	38	51 Rec	13	38	51 Rec
Basophils Absolute	0.09	0.07	0.05	-44.4	-57.1	40.0	-66.7	-71.4	0.0	-77.8	-71.4	-20.0
LUC%	0.6	0.4	0.6	-33.3	-25.0	16.7	-50.0	-75.0	0.0	-33.3	-50.0	-33.3
LUC Absolute	0.06	0.04	0.06	-33.3	-25.0	33.3	-50.0	-75.0	-16.7	-16.7	-75.0	-50.0

Table 49: Summary of Hematology Findings in Female Dogs in the 9-Month Study

Index	Mean			Percentage deviation from Control - Females								
	Control 0 mg/kg			2.5 mg/kg			5 mg/kg			7.5 mg/kg		
	Week			Week			Week			Week		
	13	38	51 Rec.	13	38	51 Rec.	13	38	51 Rec.	13	38	51 Rec.
Neutrophils Absolute	5.39	4.70	5.11	-3.2	5.5	1.2	12.4	13.8	-26.8	62.5	52.8	13.9
Basophils	1.1	1.1	0.6	-54.5	-63.6	-50.0	-72.7	-72.7	16.7	-63.6	-81.8	0.0
Basophils Absolute	0.10	0.09	0.04	-60.0	-55.6	-25.0	-80.0	-77.8	-25.0	-60.0	-77.8	50.0
LUC%	0.7	0.7	0.7	-2.9	-42.9	-14.3	-57.1	-57.1	14.3	-57.1	-71.4	-14.3
LUC Absolute	0.07	0.06	0.06	-57.1	-50.0	-16.7	-57.1	-66.7	-16.7	-42.9	-83.3	-16.7

Clinical Chemistry

Table 50: Summary of Clinical Chemistry Parameters in Male Dogs in the 9-Month Study

Index	Mean			Percentage deviation from Control - Males								
	Control 0 mg/kg			2.5 mg/kg			5 mg/kg			7.5 mg/kg		
	Week			Week			Week			Week		
	13	38	51 Rec.	13	38	51 Rec.	13	38	51 Rec.	13	38	51 Rec.
Albumin (g/dL)	3.4	3.7	3.9	2.9	0.0	-3	-3	-8	-18	-8	0.0	0.0
Total Protein (g/dL)	5.6	5.9	6.4	-4	-5	-8	-7	-10	-20	-7	-5	-5
ALP (U/L)	60.	39.	38.	7	23	16	5	15	0.0	55	21	34
Triglyceride(mg/dL)	28.	34.	44.	7	3	-41	0.0	0.0	-34	29	21	-16
SDH (U/L)	2.	3.	4.	0	33	-25	50	100	0	50	100	25

SDH= Sorbitol Dehydrogenase

Table 51: Summary of Clinical Chemistry Parameters in Female Dogs in the 9-Month Study

Index	Mean			Percentage deviation from Control - Females								
	Control 0 mg/kg			2.5 mg/kg			5 mg/kg			7.5 mg/kg		
	Week			Week			Week			Week		
	13	38	51 Rec.	13	38	51 Rec.	13	38	51 Rec.	13	38	51 Rec.
Albumin (g/dL)	3.5	3.7	3.5	0.0	0.0	3	0.0	-3	6	3	0.0	6
Total Protein (g/dL)	5.7	6.0	5.8	-5	-7	-3	-7	-8	-3	-5	-8	-2
Globulin (g/dL)	2.2	2.4	2.3	-14	-25	-13	-14	-21	-13	-14	-25	-13
A/G Ratio	1.63	1.58	1.53	16	32	20	17	27	21	20	38	20
GGT (U/L)	1.4	1.4	1.9	7	-14	11	-50	-7	79	-29	129	63

Urinalysis
Unremarkable

Gross Pathology

Table 52: Summary of Macrosopic Findings in the 9-Month Dog Study

Treatment-Related Macroscopic Findings		No. of animals affected							
		Males				Females			
Dose (mg/kg)		0	2.5	5	7.5	0	2.5	5	7.5
Number of animals examined		4/2	4/2	4/2	*3/2	4/2	*3/2	4/2	4/2
Colon	-area dark red	2/-	1/-	-/-	-/1	1/-	-/-	-/-	-/1
Ileum	-area dark red	-/1	-/-	-/-	-/-	-/-	-/1	-/-	-/1
	-ileo-colico junction, reddened	-/-	-/-	-/-	-/-	-/-	-/1	-/-	-/-
Liver	-area yellow	-/-	-/-	-/-	-/-	-/-	-/-	-/1	-/-
Mammary gland	-swollen	-/-	-/-	-/-	-/-	1/-	1/-	-/-	-/1
Skin	-Mass	-/-	-/-	-/-	1/-	1/-	-/-	-/-	-/-
Spleen	-areas, white	-/-	-/-	1/-	-/-	-/-	-/-	1/-	-/-
Thymus	-small	-/-	-/-	1/-	-/-	-/-	-/-	-/-	-/-
Cervix	-swollen	NA	NA	NA	NA	-/-	-/-	-/-	1/-
Ovaries	-swollen	NA	NA	NA	NA	1/-	-/-	-/-	1/-
	-cyst	NA	NA	NA	NA	-/-	1/-	-/-	1/-
Pituitary	-cyst	-/-	-/-	-/-	-/-	-/-	1/-	1/-	-/-
Vagina	-swollen	NA	NA	NA	NA	-/-	-/-	-/-	1/-
Uterus	-swollen	NA	NA	NA	NA	2/-	-/-	-/-	1/-
	-nodule(s)	NA	NA	NA	NA	-/-	-/1	-/-	-/-

Number of animals examined and affected: Early deaths*/ Terminal necropsy / Recovery necropsy
- = no change; NA = not present in animal

Organ Weights
Unremarkable

Histopathology
Adequate Battery: Yes

Peer Review: Yes

Special Evaluation: Lymphoid System and Intestinal Tract

GS-1101-related microscopic finding:

Lymphoid system: Lymphoid system changes included decreased lymphocytes, primarily involving typical B-cell regions, with lesser effects in typical T-cell regions, in the spleen, lymph nodes (mandibular, mesenteric, and axillary), and Peyer's patches in the 2.5, 5, and/or 7.5 mg/kg/day group males and females at the primary necropsy.

Intestinal tract: Inflammatory changes in the intestinal tract including increased amounts of granulocyte infiltrates in the jejunum, ileum, colon, and/or rectum as compared to the vehicle control group, and crypt dilatation in the colon, cecum, and/or rectum in 5, and/or 7.5 mg/kg/day group males and females.

Recovery: Minimal to mild decreased lymphocytes in the spleen and mesenteric lymph node of 1 of 2 males in the 7.5 mg/kg/day group and increased severity of granulocyte infiltrates in the ileum in the 7.5 mg/kg/day group males as compared to the vehicle control, 2.5, or 5 mg/kg/day groups.

Histological Findings

Table 53: Incidence of Selected Lymphoid System Histopathological Findings in the Beagle Dog, Study Week 39 Primary Necropsy

(Excerpted from the Applicant's submission)

	Males				Females			
Dosage (mg/kg/day):	**0**	**2.5**	**5**	**7.5**	**0**	**2.5**	**5**	**7.5**
Spleen [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Decreased Lymphocytes	0	4	4	3	0	3	4	4
(Incidence as a %)	*(0)*	*(100)*	*(100)*	*(100)*	*(0)*	*(100)*	*(100)*	*(100)*
Minimal	-	3	0	0	-	1	0	1
Mild	-	1	2	2	-	2	1	0
Moderate	-	0	2	0	-	0	3	3
Severe	-	0	0	1	-	0	0	0
Lymph Node, Mandibular [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Decreased Lymphocytes, Cortex	0	4	4	3	0	2	4	4
(Incidence as a %)	*(0)*	*(100)*	*(100)*	*(100)*	*(0)*	*(67)*	*(100)*	*(100)*
Minimal	-	2	0	1	-	1	1	1
Mild	-	2	1	1	-	0	2	2
Moderate	-	0	3	1	-	1	1	1
Lymph Node, Mesenteric [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Decreased Lymphocytes, Cortex	0	1	4	3	0	1	4	4
(Incidence as a %)	*(0)*	*(25)*	*(100)*	*(100)*	*(0)*	*(33)*	*(100)*	*(100)*
Minimal	-	1	1	1	-	1	2	1
Mild	-	0	2	1	-	0	1	0
Moderate	-	0	1	1	-	0	1	3
Lymph Node, Axillary [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Decreased Lymphocytes, Cortex	0	2	2	3	0	2	3	4
(Incidence as a %)	*(0)*	*(50)*	*(50)*	*(100)*	*(0)*	*(67)*	*(75)*	*(100)*
Minimal	-	1	0	0	-	1	0	0
Mild	-	1	0	0	-	0	1	0
Moderate	-	0	2	2	-	1	2	3
Severe	-	0	0	1	-	0	0	1
Peyer's Patches [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Decreased Lymphocytes	0	1	3	2	0	0	2	3
(Incidence as a %)	*(0)*	*(25)*	*(75)*	*(67)*	*(0)*	*(0)*	*(50)*	*(75)*
Minimal	-	1	3	2	-	-	2	0
Mild	-	0	0	0	-	-	0	2
Moderate	-	0	0	0	-	-	0	1

[a] = Number of tissues examined from each group.

Table 54: Incidence of Selected Lymphoid System Histopathological Findings in the Beagle Dog,Study Week 51 Recovery Necropsy

(Excerpted from the Applicant's submission)

	Males				Females			
Dosage (mg/kg/day):	**0**	**2.5**	**5**	**7.5**	**0**	**2.5**	**5**	**7.5**
Spleen [a]	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Decreased Lymphocytes	0	0	0	**1**	0	0	0	0
(Incidence as a %)	*(0)*	*(0)*	*(0)*	***(50)***	*(0)*	*(0)*	*(0)*	*(0)*
Minimal	-	-	-	**1**	-	-	-	-
Lymph Node, Mesenteric [a]	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Decreased Lymphocytes, Cortex	0	0	0	**1**	0	0	0	0
(Incidence as a %)	*(0)*	*(0)*	*(0)*	***(50)***	*(0)*	*(0)*	*(0)*	*(0)*
Mild	-	-	-	**1**	-	-	-	-

[a] = Number of tissues examined from each group.

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Table 55: Incidence of Selected Intestinal Tract Histopathological Findings in Beagle Dogs, Study Week 39 Primary Necropsy

(Excerpted from the Applicant's submission)

	Males				Females			
Dosage (mg/kg/day):	**0**	**2.5**	**5**	**7.5**	**0**	**2.5**	**5**	**7.5**
Jejunum [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Infiltrate, Granulocyte	4	4	4	3	4	3	4	4
(Incidence as a %)	*(100)*	*(100)*	*(100)*	*(100)*	*(100)*	*(100)*	*(100)*	*(100)*
Minimal	4	4	3	3	3	3	4	4
Mild	0	0	1	0	1	0	0	0
Ileum [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Infiltrate, Granulocyte	4	4	**4**	3	4	3	**4**	**4**
(Incidence as a %)	*(100)*	*(100)*	***(100)***	*(100)*	*(100)*	*(100)*	***(100)***	***(100)***
Minimal	2	4	**1**	3	4	2	**1**	**2**
Mild	2	0	**3**	0	0	1	**3**	**2**
Cecum [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Dilatation, Crypts	0	0	0	0	0	0	**2**	0
(Incidence as a %)	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*	***(50)***	*(0)*
Minimal	-	-	-	-	-	-	**2**	-
Infiltrate, Granulocyte	1	2	**4**	**3**	2	2	4	2
(Incidence as a %)	*(25)*	*(50)*	***(100)***	***(100)***	*(50)*	*(67)*	*(100)*	*(50)*
Minimal	1	2	**1**	**3**	2	2	4	2
Mild	0	0	**3**	**0**	0	0	0	0
Colon [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Dilatation, Crypts	0	0	**2**	0	0	0	0	0
(Incidence as a %)	*(0)*	*(0)*	***(50)***	*(0)*	*(0)*	*(0)*	*(0)*	*(0)*
Minimal	-	-	**2**	-	-	-	-	-
Infiltrate, Granulocyte	2	2	**3**	0	0	1	**3**	**3**
(Incidence as a %)	*(50)*	*(50)*	***(75)***	*(0)*	*(0)*	*(33)*	***(75)***	***(75)***
Minimal	2	2	**2**	-	-	1	**2**	**3**
Mild	0	0	**1**	-	-	0	**1**	**0**
Rectum [a]	**4**	**4**	**4**	**3**	**4**	**3**	**4**	**4**
Dilatation, Crypts	2	2	0	2	2	0	3	**4**
(Incidence as a %)	*(50)*	*(50)*	*(0)*	*(67)*	*(50)*	*(0)*	*(75)*	***(100)***
Minimal	2	2	-	2	2	-	3	**1**
Mild	0	0	-	0	0	-	0	**3**

[a] = Number of tissues examined from each group.

Table 56: Incidence of Selected Intestinal Tract Histopathological Findings in Beagle Dogs, Study Week 51 Recovery Necropsy

(Excerpted from the Applicant's submission)

	Males				Females			
Dosage (mg/kg/day):	**0**	**2.5**	**5**	**7.5**	**0**	**2.5**	**5**	**7.5**
Jejunum [a]	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Dilatation, Crypts	0	0	1	1	0	0	1	0
(Incidence as a %)	*(0)*	*(0)*	*(50)*	*(50)*	(0)	*(0)*	*(50)*	*(0)*
Minimal	-	-	1	1	-	-	1	-
Infiltrate, Granulocyte	2	2	2	2	2	2	2	2
(Incidence as a %)	*(100)*	*(100)*	*(100)*	*(100)*	*(100)*	*(100)*	*(100)*	*(100)*
Minimal	2	2	2	2	2	1	2	1
Mild	0	0	0	0	0	1	0	1
Ileum [a]	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Infiltrate, Granulocyte	2	2	2	**2**	2	2	2	2
(Incidence as a %)	*(100)*	*(100)*	*(100)*	***(100)***	*(100)*	*(100)*	*(100)*	*(100)*
Minimal	2	2	2	**0**	2	1	2	1
Mild	0	0	0	**2**	0	1	0	1

[a] = Number of tissues examined from each group.

Table 57: Incidence of Selected Intestinal Tract Histopathological Findings in Beagle Dogs, Study Week 51 Recovery Necropsy (continued)

(Excerpted from the Applicant's submission)

	Males				Females			
Dosage (mg/kg/day):	**0**	**2.5**	**5**	**7.5**	**0**	**2.5**	**5**	**7.5**
Cecum [a]	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Infiltrate, Granulocyte	0	0	0	1	1	2	1	1
(Incidence as a %)	*(0)*	*(0)*	*(0)*	*(50)*	*(50)*	*(100)*	*(50)*	*(50)*
Minimal	-	-	-	1	0	2	1	1
Moderate	-	-	-	0	1	0	0	0
Colon [a]	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Dilatation, Crypts	0	0	0	0	1	0	0	0
(Incidence as a %)	*(0)*	*(0)*	*(0)*	*(0)*	*(50)*	*(0)*	*(0)*	*(0)*
Minimal	-	-	-	-	1	-	-	-
Infiltrate, Granulocyte	0	0	0	0	1	0	0	0
(Incidence as a %)	*(0)*	*(0)*	*(0)*	*(0)*	*(50)*	*(0)*	*(0)*	*(0)*
Mild	-	-	-	-	1	-	-	-
Rectum [a]	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Dilatation, Crypts	1	2	1	0	2	0	0	0
(Incidence as a %)	*(50)*	*(100)*	*(50)*	*(0)*	*(100)*	*(0)*	*(0)*	*(0)*
Minimal	1	2	1	-	2	-	-	-
Infiltrate, Granulocyte	1	1	1	1	2	1	1	2
(Incidence as a %)	*(50)*	*(50)*	*(50)*	*(50)*	*(100)*	*(50)*	*(50)*	*(100)*
Minimal	1	1	1	1	2	1	1	2
Intestinal Tract, Overall (Jejunum - Rectum) [a]	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Dilatation, Crypts	1	2	1	1	2	0	1	0
(Incidence as a %)	*(50)*	*(100)*	*(50)*	*(50)*	*(100)*	*(0)*	*(50)*	*(0)*
Minimal	1	2	1	1	2	-	1	-
Infiltrate, Granulocyte	2	2	2	**2**	2	2	2	2
(Incidence as a %)	*(100)*	*(100)*	*(100)*	***(100)***	*(100)*	*(100)*	*(100)*	*(100)*
Minimal	2	2	2	**0**	1	1	2	0
Mild	0	0	0	**2**	0	1	0	2
Moderate	0	0	0	**0**	1	0	0	0

[a] = Number of tissues examined from each group.

Toxicokinetics

- Exposure to GS-1101 generally increased with the increase in dosage level from 2.5 to 7.5 mg/kg/day.
- The increases in mean plasma C_{max} and AUC_{0-t} were roughly proportional between the 2.5 and 7.5 mg/kg/day dosage levels.

- Sex differences were generally less than 2-fold in GS-1101 mean C_{max} and AUC_{0-t} values.
- In general, both C_{max} and AUC_{0-t} increased on study day 272 compared to that determined on study day 0 among all treatment groups.
- Mean T_{max} values for males and females ranging from 1.5 to 5.7 hours on study day 0 and from 1.6 to 5.7 hours on study day 272.
- Mean $t_{1/2}$ values for males and females ranging from 3 to 5.2 hours on study day 0, and from 3.5 to 7.2 hours on study day 273.

Table 58: Summary of Toxicokinetic Parameters for GS-1101 in Beagle Dogs (9-Month Study)

(Excerpted from the Applicant's submission)

Dosage	AUC_{0-t} (ng•hr/mL)		C_{max} (ng/mL)	
	Day 0	Day 272	Day 0	Day 272
Males				
2.5 mg/kg/day	3053	3891	745	792
5 mg/kg/day	3579	8550	660	1372
7.5 mg/kg/day	12878	18451	2130	2401
Females				
2.5 mg/kg/day	1630	6292	490	1410
5 mg/kg/day	2707	10677	677	1690
7.5 mg/kg/day	3471	15884	770	2933
Males and Females				
2.5 mg/kg/day	2342	4982	617	1073
5 mg/kg/day	3143	9614	669	1531
7.5 mg/kg/day	8175	17051	1450	2691

Table 59: Summary of Toxicokinetic Parameters for GS-563117 in Beagle Dogs (9-Month Study)

(Excerpted from the Applicant's submission)

Dosage	AUC_{0-t} (ng•hr/mL)		C_{max} (ng/mL)	
	Day 0	Day 272	Day 0	Day 272
Males				
2.5 mg/kg/day	1145	1103	201	151
5 mg/kg/day	1511	2002	183	201
7.5 mg/kg/day	3822	3473	528	292
Females				
2.5 mg/kg/day	1024	1980	180	268
5 mg/kg/day	1395	2514	260	281
7.5 mg/kg/day	2193	4022	324	509
Males and Females				
2.5 mg/kg/day	1084	1502	191	204
5 mg/kg/day	1453	2258	222	241
7.5 mg/kg/day	3008	3772	426	410

Dosing Solution Analysis

Analysis of the test article formulations was not conducted for this study as the test article supplied by the Applicant was administered as the drug substance in capsules, without any further modification.

7 Genetic Toxicology

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

Study title: CAL-101 Bacterial Mutation Test

Study no.: 961805
Study report location: 4.2.3.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 4 Oct 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CAL-101, 22033-51-31, 99.3%

Study was reviewed under IND 101254 by Dr. Timothy Kropp. The review was slightly modified in format and content to fit this NDA review.

Key Study Findings

- CAL-101 is negative *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Methods: Plate incorporation and pre-incubation

Strains:	S. typhimurium strains TA98, TA100, TA1535, TA1537, and E. coli WP2uvrA
Concentrations in definitive study:	1.58, 5, 15.58, 50, 158, 500, 1581, 5000 µg/plate
Basis of concentration selection:	No toxicity was noted at any of the concentrations with or without activation up to 5000 µg/plate except for strain TA100 in the pre-incubation assay. No precipitation was noted at any concentration.
Negative control:	DMSO
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48-72 hr incubation @ 37±0.1°C. Pre-incubation was 30 min.

Strain	**Positive control**
All S9 activated strains	2-aminoanthracene (2-AA)
TA98	2-Nitrofluorene (2-NF)
TA100, TA1535	sodium azide (SA)
TA1537	9-aminoacridine (9-AA)
WP2*uvr*A	Methyl Meathanesulfonate (MMS)

Study Validity:

The mean revertant colony counts of the vehicle controls for each strain were close to or within the historical control range of the laboratory

Appropriate positive control compounds (with S9 mix) induced increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5x for strain TA100), confirming sensitivity of the test system and activity of the S9 mix.

Results

- No substantial increases in the revertant colony counts were obtained with any strain following exposure to the test article in either the plate incorporation or pre-incubation assay in the absence or presence of S9 mix.

The detailed results of the assays are displayed in tables below.

Table 60: Plate Incorporation Average-Revertant Colonies/Plate in the Absence of S9 Mix

Dose µg/plate	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
DMSO	28	126	19	13	42
50	29	141	22	15	39
158	30	136	27	11	37
500	26	138	20	12	36
1581	35	143	18	17	43
5000	30	120	16	10	47
Positive control	188	604	301	208	154

Table 61: Plate Incorporation Average-Revertant Colonies/Plate in the Presence of S9 Mix

Dose µg/plate	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
DMSO	38	138	21	17	58
50	43	179	23	15	61
158	43	162	25	13	51
500	48	175	23	13	61
1581	38	148	19	13	54
5000	38	130	22	12	60
Positive control	348	984	335	130	378

Table 62: Pre-incubation Average-Revertant Colonies/Plate in the Absence of S9 Mix

Dose µg/plate	TA98	TA100*	TA1535	TA1537	WP2 *uvr*A
DMSO	33	132	19	11	39
50	28	143	19	12	45
158	29	143	20	15	43
500	26	132	16	12	49
1581	24	125	23	8	48
5000	26	144	22	13	46
Positive control	141	508	334	1539	1137

*TA100 was exposed to 5.0, 15.8, 50, 158, 500, and 1581µg/plate. There was an incomplete lawn (reduced background lawn) due to toxicity at 1581 µg/plate.

Table 63: Pre-incubation Average-Revertant Colonies/Plate in the Presence of S9 Mix

Dose µg/plate	TA98	TA100*	TA1535	TA1537	WP2 *uvr*A
DMSO	45	132	22	16	57
50	39	139	20	13	51
158	42	146	20	12	59
500	37	138	27	18	64
1581	32	148	24	17	61
5000	31	145	23	11	63
Positive control	339	955	268	135	395

*TA100 was exposed to 5.0, 15.8, 50, 158, 500, and 1581µg/plate. There was an incomplete lawn (reduced background lawn) due to toxicity at 1581 µg/plate.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: CAL-101 Test Chromosome Aberration Test

Study no.: 961806
Study report location: 4.2.3.3.1
Conducting laboratory and location: (b) (4)
Date of study initiation: 4 Oct 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CAL-101, 22033-51-38, 99.3 %

Study was reviewed under IND 101254 by Dr. Timothy Kropp. The review was slightly modified in format and content to fit this NDA review.

Key Study Findings

- CAL-101 is negative in the *in vitro* chromosome aberration assay in whole blood human lymphocyte cultures in presence or absence of S9 mix up to at least 50% cytotoxic dose level.

Methods: *In vitro* chromosome aberration test in human peripheral blood lymphocytes

Cell line: Whole blood human peripheral blood lymphocytes
Concentrations in definitive study: 32, 64, 128, 256 µg/mL without activation and 64, 128, 256, 500 µg/mL with activation
Basis of concentration selection: Visible precipitate at ≥500 µg/mL and the top dose selected for analysis produced at least a 50% decrease in relative mitotic index compared to concurrent control. In

	addition, the next two lower concentrations were also subjected to examination to ensure that at least three levels were analyzed in the event of excessive cell toxicity at the selected high level.
Negative control:	DMSO
Positive control:	Mitomycin C (MMC) in the absence of S9 mix and cyclophosphamide (CP) in the presence S9 mix.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	4 hours with 17 hour recovery in presence or absence of S9 mix or 21 hours in the absence of S9 mix at approximately 37°C in a humidified atmosphere containing 5% CO_2; All cultures were treated with colcemid 2 hours prior to harvest to arrest the cells in metaphase stage.

Study Validity

The vehicle/negative control results were within the historical control range. The positive control produced a significant increase in the incidence of aberrant cells compared with the concurrent control.

Results

Table 64: Results for CAL-101 in the *In Vitro* Chromosome Aberration Assay

Conc. (µg/mL)	Treatment time	S9 Mix	RMI (%)	% aberrant cells
DMSO	4 hours	-	100	1.0
CAL-101				
64.0	4 hours	-	46	1.5
128	4 hours	-	52	1.0
256	4 hours	-	24	0.0
MMC, 0.20	4 hours	-	63	14.5**
DMSO	4 hours	+	100	2.0
CAL-101				
64.0	4 hours	+	78	1.5
128	4 hours	+	71	0.0
256	4 hours	+	51	2.5
500ppt	4 hours	+	0	NA
CP, 12.0	4 hours	+	75	12.0**
DMSO	21 hours	-	100	2.0
CAL-101				
64.0	21 hours	-	66	0.5

128	21 hours	-	94	0.5
256	21 hours	-	39	0.5
MMC, 0.20	21 hours	-	41	14.5**

RMI = Relative Mitotic Index (vehicle = 100%); ppt = visible precipitate; NA= not applicable; ** p ≤ 0.001 (highly significant)

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

Study title: CAL-101 Rat Micronucleus Test

Study no: 961807
Study report location: 4.2.3.3.2.
Conducting laboratory and location: (b) (4)
Date of study initiation: 09 October 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CAL-101, 22033-51-38, 99.3%

Study was reviewed under IND 101254 by Dr. Timothy Kropp. The review was slightly modified in format and content to fit this NDA review.

Key Study Findings

- CAL-101 was positive at high dose, 2000 mg/kg in males, but not in females.
- Statistically significant increase with dose response in the incidence of micronucleated immature erythrocytes was observed, with a group mean value for high dose males (4.8 in the initial reading and 5.6 in the additional reading) slightly outside the laboratory historical negative control range (0.0 to 4.7) at the 24 hour sampling time.
- The relevance of this positive finding of high dose in males only in humans is unknown. However, this is not an issue and acceptable for this patient population.

Methods

Doses in definitive study:	500, 1000 and 2000 mg/kg
Frequency of dosing:	Single
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% w/v carboxymethylcellulose (high viscosity)/0.1% v/v TWEEN 80
Species/Strain:	rat/Sprague-Dawley
Number/Sex/Group:	5/sex/dose/harvest time
Basis of dose selection:	Standard limit dose of 2000 mg/kg was used
Negative control:	0.5% w/v carboxymethylcellulose (high viscosity)/0.1% v/v TWEEN 80
Positive control:	Cyclophosphamide (monohydrate) 20 mg/kg
Exposure conditions	24 and 48 h (positive control, 24 h only)
Incubation/sampling:	Doses: 500, 1000, 2000 mg/kg
Analysis	Toxicity was determined out of 1000 erythrocytes per animal. Clastogenicity was determined out of 2000 polychromatic erythrocytes (PCE) per animal

Study Validity

The incidence of micronucleated immature erythrocytes for the vehicle control group was within the laboratory historical vehicle/negative control range. In addition, the positive control group showed a statistically significant increase in the incidence of micronucleated immature erythrocytes ($p \leq 0.01$).

Interpretation of Results

A positive response is normally indicated by a statistically significant dose-related (where appropriate) increase in the incidence of micronucleated immature erythrocytes ($p \leq 0.01$). In this case, individual and/or group mean values should also exceed the laboratory historical control range.

Results

Table 65: Group Mean Micronucleus Results for CAL-101

Dose (mg/kg)	Sampling time	% PCE	% MN Males	% MN Females
Vehicle control	24 hours	47.9	0.8	1.4
CAL-101				
500 mg/kg	24 hours	41.0	2.6	1.6
1000 mg/kg	24 hours	47.1	2.6	3.0
2000 mg/kg	24 hours	45.3	**4.8**	1.8
CP, 20 mg/kg	24 hours	38.7*	39.7**	32.3
Vehicle control	48 hours	54.1	1.8	2.0

Dose (mg/kg)	Sampling time	% PCE	% MN Males	% MN Females
CAL-101				
2000 mg/kg	48 hours	**37.2****	2.6	2.8
Additional Slide Reading				
Vehicle control	24 hours	40.8	2.6	2.0
CAL-101				
500 mg/kg	24 hours	37.8	2.0	2.2
1000 mg/kg	24 hours	42.9	3.8	4.4
2000 mg/kg	24 hours	41.9	**5.6**	2.6
CP, 20 mg/kg	24 hours	36.6	38.3**	33.0**

Significantly different from control group (group 1) value: * - P ≤ 0.01 ** - P ≤ 0.001

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Evaluation of Orally (by Gavage) Administered GS-1101 on Fertility in Male Rats

Study no.:	(b) (4) 604019
Study report location:	4.2.3.5.1. TX-312-2014
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June, 07 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-1101, Lot # 60182-09-004, Purity 99.8%

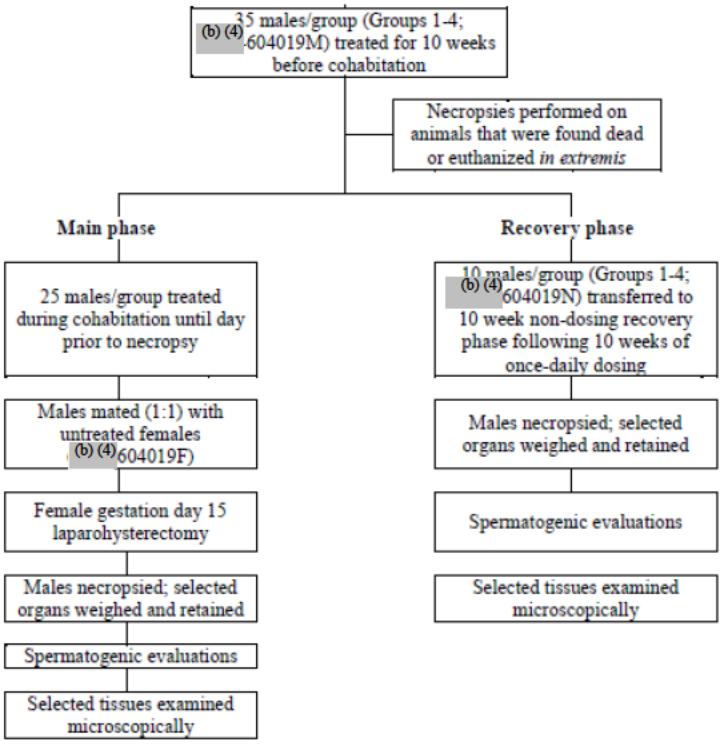
Key Study Findings:

- Dose related decreased testicular and epididymal weights (at all doses) and sperm concentration (at mid- and high-dose).
- No test article-related effects on male reproductive function: mating, fertility, or copulation indices, or pre-coital intervals (mean time to mating).
- There were no apparent adverse effects in females that were mated with treated male rats in regard to endpoints measured.

Methods

Doses:	0, 25, 50, or 100 mg/kg/day
Frequency of dosing:	Daily for ~14 weeks
Dose volume:	10 mL/kg/day
Route of administration:	Oral
Formulation/Vehicle:	0.5% high viscosity carboxymethylcellulose and 0.1% TWEEN 80 in deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Group:	35 males/group for treatment; 25* females/group untreated for breeding purposes.

Study design excerpted from Applicant's submission:



*males were paired with an additional female if the initial female did not have evidence of mating, resulting in 28 total females in Groups 1 and 4

Observations and Results

Mortality:	Males:Twice daily
Clinical signs:	Males:Twice daily; Untreated Females: on gestation days days 0, 7, and 15.
Body weights:	Males:Twice weekly; Untreated Females: on gestation days 0, 7, and 15
Food consumption:	Males: Twice weekly
Breeding Procedures:	The 25 male rats/group selected for evaluation of

	reproductive toxicity were paired with females on a 1:1 basis within each treatment group following 70 days of dosing for the males.
Spermatogenic Endpoint Evaluations	At scheduled necropsy
Gross pathology:	At necropsy
Gestation Laparohysterectomy	Day 15
Organ weights:	At necropsy
Histopathology:	At necropsy

Mortality

Treated Males

Two males at 25 mg/kg/day and one male at 50 mg/kg/day were found dead or euthanized *in extremis* during the study. See details in the table below

Table 66: Mortalities in Male Rat Fertility Study

GS-1101 (mg/kg)	Animal #	Sex	Day	Potential cause of death
25	58450	M	34	Undetermined
25	58560	M	33	Fractured hard palate
50	58491	M	91	Undetermined

Table 67: Toxicities Observed in Male Rats with Mortalities in the Fertility Study

GS-1101 (mg/kg)	Toxicities observed at necropsy
25 Animal #58450	no clinical findings were noted at the daily examinations
25 Animal #58560	41 g body weight loss occurred during study days 28-31 and gasping was noted on the day of euthanasia; red material around the eyes and nose and malaligned upper incisors on study days 30-33
50 Animal # 58491	several incidences of red material and lacrimation around the eyes and broken and malaligned upper incisors during study days 30-91 at the daily examinations.

Clinical Signs

Unremarkable

Body Weight

Unremarkable

Food Consumption

Unremarkable

Reproductive Performance

Table 68: Results of Reproductive Performance in Male Rats

(Excerpted from the Applicant's submission)

Parameter	Dosage Level (mg/kg/day) 0[a]	25	50	100[a]	(b) (4) HC[b] Mean (Range)
No. of Gravid Females	25	21	22	25	
No. of Nongravid Females with Evidence of Mating	2	1	0	0	
No. of Nongravid Females without Evidence of Mating	1	2	3	3	
Male Mating Index (%) (n)	100.0 (25)	91.7 (24)	88.0 (25)	100.0 (25)	98.9 (92.0-100.0)
Male Fertility Index (%) (n)	92.0 (25)	87.5 (24)	88.0 (25)	100.0 (25)	95.8 (84.0-100.0)
Male Copulation Index (%) (n)	92.0 (25)	95.5 (24)	100.0 (25)	100.0 (25)	97.2 (88.0-100.0)
Pre-Coital Interval (days) (n)	2.7 (25)	3.5 (22)	2.8 (22)	2.8 (25)	2.8 (1.8-4.4)

[a] = Group 1 and Group 4 males with no evidence of mating were paired with a second untreated female.
[b] = (b) (4) historical control data

Necropsy

Spermatogenic Endpoint Evaluations

Statistically significant decreases in epididymal concentrations and weights as well as testicular weights were observed compared to the concurrent vehicle control.

Table 69: Results of Spermatogenic Evaluations in Rats

(Excerpted from the Applicant's submission)

Dose (mg/kg/day):	0	25	50	100	(b) (4) HC Mean (Range)
Main Study Period:					
Motility (%)	85	83	83	81	82.6 (78.0-90.0)
Left epididymis weight (g)	0.71	0.65*	0.64**	0.60**	0.68 (0.59-0.76)
Left epididymis concentration (millions/g)	686.9	655.6	586.0*	570.9*	554.6 (307.1-786.5)
Left testis weight (g)	1.84	1.52**	1.54**	1.29**	1.75 (1.66-1.84)
Left testis concentration (millions/g)	101.9	105.6	98.6	97.5	121.9 (80.5-147.9)
Recovery Period:					
Motility (%)	87	88	88	92	NA
Left epididymis weight (g)	0.67	0.66	0.66	0.66	NA
Left epididymis concentration (millions/g)	544.7	576.9	590.2	611.1	NA
Left testis weight (g)	1.85	1.81	1.77	1.84	NA
Left testis concentration (millions/g)	109.5	84.9	99.7	94.1	NA

NA = Not Applicable

Gross pathology

Table 70: Summary of Macroscopic Findings in Male Rat Fertility Study

Dose (mg/kg/day)	0	25	50	100
# Rats examined	25	24	24	25
Left testis - small	0	1	2	3
Right testis - small	0	1	2	3
Kidneys -contents, white precipitate	0	0	0	1
Lymph node, mandibular - enlarged	0	0	0	1

Organ Weights

Table 71: Selected Mean Organ Weights at the Primary Necropsy (Grams) in the Male Rat Fertility Study

(Excerpted from the Applicant's submission)

	Males (mg/kg/day)			
Parameter	0	25	50	100
Cauda Epididymis, Left	0.3486	0.3151*	0.3056**	0.2946**
Cauda Epididymis, Right	0.3806	0.3389**	0.3323**	0.3151**
Epididymis, Left	0.71	0.65*	0.64**	0.60**
Epididymis, Right	0.75	0.69*	0.70*	0.63**
Testis, Left	1.84	1.52**	1.54**	1.29**
Testis, Right	1.83	1.53**	1.53**	1.29**

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Histopathology

Table 72: Summary of Microscopic Findings in Male Rat Fertility Study

Microscopic Cellular Debris in the Right Epididymis at the Primary Necropsy				
Dose (mg/kg/day)	**0**	**25**	**50**	**100**
# Rats examined	**25**	**NA**	**1**	**25**
Cellular debris Minimal Mild	19 16 3	- - -	0 - -	25* 2 23
* = Statistically significant compared to the control group at p<0.05 using Fisher's Exact test				

Untreated Females

Mortality

All females survived to the scheduled necropsies.

Clinical signs

Unremarkable

Gestation body weights
Unremarkable

Female Macroscopic Findings
Unremarkable

Gestation Day 15 Laparohysterectomy

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Table 73: Summary of Embryonic Data at Scheduled Necropsy in Females Mated with GS-1101 Treated Males

Dose (mg/kg)	Control	25	50	100
Number of females pregnant (gravid females)	23	21	22	25
Corpora lutea Total Average/animal (mean)	 411 17.9	 359 17.1	 388 17.6	 420 17.5
Implantation sites Total Average/animal (mean)	 379 16.5	 339 16.1	 355 16.1	 407 16.3
Preimplantation loss (%)	7.3	5.3	8.7	8.0
Postimplantation loss (%)	5.9	7.1	4.8	6.4
Resorptions (early resorptions) Total % (resorptions/implantation sites x100% Average/animal (mean)	 22 5.8 1.0	 24 7.1 1.1	 16 4.5 0.7	 24 5.9 1.0
Viable fetuses Total % (viable/total fetuses x 100%) Average/animal (mean)	 357 94 15.5	 315 92.9 15	 339 95.2 15.4	 383 94.1 15.3

Study title: Evaluation of Orally (by Gavage) Administered GS-1101 on Fertility and Early Embryonic Development to Implantation in Female Rats

Study no.:	(b) (4)-604028
Study report location:	4.2.3.5.1. TX-312-2016
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August, 17 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GS-1101, Lot # 60182-09-004, Purity 99.8%

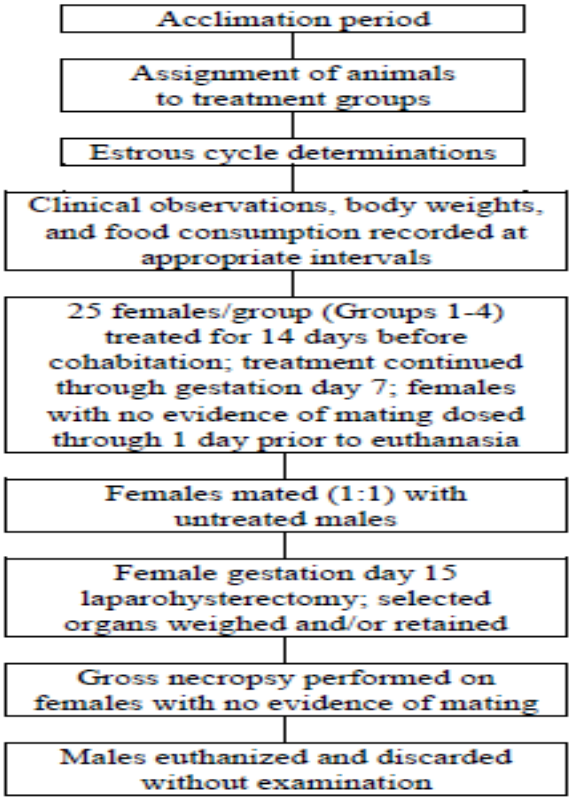
Key Study Findings:

- One female dosed at 100 mg/kg had a completely resorbed litter. This female had a normal pattern of estrous cyclicity, and normal mating and reproductive indices, including normal numbers of corpora lutea and implantation sites. This female at 100 mg/kg/day had a lower ovarian weight.
- An increase in post-implantation loss and early embryolethality at 100 mg/kg, resulting in a 20% decrease in the number of live embryos was observed.
- No test article-related effects on female reproductive function: mating, fertility, or copulation indices, or pre-coital intervals (mean time to mating).

Methods

Doses:	0, 25, 50, or 100 mg/kg/day
Frequency of dosing:	Daily for 14 days before cohabitation; treatment continued through gestation day 7
Dose volume:	10 mL/kg/day
Route of administration:	Oral
Formulation/Vehicle:	0.5% high viscosity carboxymethylcellulose and 0.1% TWEEN 80 in deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Group:	25 females/group for treatment; 25 untreated males/group

Study Design (excerpted from Applicant's NDA)



Observations and Results

Mortality:	Females: Twice daily
Clinical signs:	Females: Twice daily
Body weights:	Twice weekly; Gestation days 0, 3, 7, 10, 13, and 15
Food consumption:	Twice weekly Gestation days 0, 3, 7, 10, 13, and 15
Breeding Procedures:	The animals were cohabited on a 1:1 basis within each treatment group following 14 days of treatment for the females.
Gross pathology:	At necropsy
Gestation Laparohysterectomy	Day 15
Organ weights:	At necropsy

Mortality

All females in the vehicle control, 25, 50, and 100 mg/kg/day groups survived to the scheduled necropsies.

Clinical Signs

Unremarkable

Body Weight

Lower mean body weight gain was noted at 100 mg/kg/day (2 females; nos. 65422, the animal with complete resorption, and 65475 that lost 107 g and 41 g, respectively, of body weight during gestation days 7-15).

Food Consumption

Significantly ($p<0.01$ or $p<0.05$) lower mean food consumption was noted in the 100 mg/kg/day group (2 females; 65422, the animal with complete resorption, and 65475).

Reproductive Performance

Table 74: Results of Reproductive Performance in Female Rat Fertility Study

(Excerpted from the Applicant's submission)

Parameter	Dosage Level (mg/kg/day) 0	25	50	100	(b) (4) HC[a] Mean (Range)
No. of Gravid Females	24	24	24	23	
No. of Nongravid Females with Evidence of Mating	0	1	0	2	
No. of Nongravid Females without Evidence Mating	1	0	1	0	
Female Mating Index (%) (n)	96.0 (24)	100.0 (25)	96.0 (24)	100.0 (25)	99.3 (92.0-100.0)
Female Fertility Index (%) (n)	96.0 (24)	96.0 (24)	96.0 (24)	92.0 (23)	96.6 (84.0-100.0)
Female Conception Index (%) (n)	100.0 (24)	96.0 (24)	100.0 (24)	92.0 (23)	97.3 (85.0-100.0)
Estrous Cycle Length (days) (n)	4.1 (25)	4.1 (25)	4.0 (25)	4.0 (25)	4.2 (3.6-5.1)
Pre-Coital Interval (days) (n)	3.8(24)	2.8 (25)	2.8 (24)	3.1 (24)	2.8 (2.0-3.6)

[a] = (b) (4) historical control data

Reduction in female mating index (%) and female fertility index (%) compared to concurrent control. However, these values were within the laboratory's historical control range.

Gross Pathology

A single female (no. 65404) in the 100 mg/kg/day group did not have evidence of mating during the breeding period but delivered 1 pup 8 days following the final day of cohabitation that was found partially cannibalized; 2 live fetuses and an early resorption were present *in utero*. The breeding period for this animal was complete and separated from male.

Organ Weights

Unremarkable

GESTATION DAY 15 LAPAROHYSTERECTOMY

Table 75: Summary of Embryonic Data at Scheduled Necropsy in GS-1101 Treated Females Mated with Untreated Males

	Control	25 mg/kg	50 mg/kg	100 mg/kg
Number of females pregnant (gravid females)	24	24	24	22
Corpora lutea Total Average/animal (mean)	 389 16.2	 383 16.0	 389 16.2	 331 15.0
Implantation sites Total Average/animal (mean)	 376 15.7	 361 15	 366 15.3	 315 14.3
Preimplantation loss (%)	3.2	6.2	5.7	5.0
Postimplantation loss (%)	5.7	13.1	5.3	11.6
Resorptions (early resorptions) Total % (resorptions/implantation sites x100% Average/animal (mean)	 20 5.3 1.0	 41 11.4 1.1	 20 5.5 0.7	 32 10.2 1.0
Viable fetuses Total % (viable/total fetuses x 100%) Average/animal (mean)	356 94.3	 320 86.9	 346 94.7	 283 88.4

9.2 Embryonic Fetal Development

Study title: An Oral (Gavage) Embryo/Fetal Development Study of GS-1101 in Rats

Study no.:	TX-312-2008
Study report location:	4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 16, 2012 (first dose)
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	GS-1101, lot#1101-AC-1P-DKS-11-1204-23, Purity: 99.8% (HPLC)

Key Study Findings

- There was one test-article related maternal death at the high dose of 150 mg/kg/day.
- Clinical signs of urogenital blood loss correlated with complete resorptions, increased post-implantation loss, and decreased mean litter size in the 150 mg/kg/day group.

- At doses ≥ 75 mg/kg/day, the net reduction in the maternal body weight gain was > 10% compared to controls, and the presence of external malformations and skeletal variations in fetuses were observed.
- $AUC_{0\text{-}t}$ for GS-1101 increased greater than dose proportionally and there was accumulation with repeated dosing.

Methods

Doses:	0, 25, 75, and 150 mg/kg/day
Frequency of dosing:	Daily on Gestational Days (GD) 6-17
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% high viscosity carboxymethylcellulose (CMC) /0.1% Polysorbate 80, in deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25/females/group
Satellite groups:	TK: 4/females/group (control), 8/females/group (GS-1101 treated animals)
Study design:	See table below
Deviation from study protocol:	None that affected the integrity or overall interpretation of results

(Study design table excerpted from the Applicant's submission)

Group Number	Treatment	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females	
				EFD[a]	TK[b]
1	Vehicle Control	0	10	25	4
2	GS-1101	25	10	25	8
3	GS-1101	75	10	25	8
4	GS-1101	150	10	25	8

[a] = Embryo/fetal development phase, (b) (4) -604007
[b] = Toxicokinetic phase, (b) (4) 604007T

Observations and times:

Mortality	Twice daily
Clinical Signs	Daily from GD 0 through 20
Body Weight	GD 0, 6-18 (daily), and 20 (TK animals at GD 0, 6-17 (daily))
Food Consumption	GD 0, 6-18 (daily), and 20
Toxicokinetics	Control animals: • 0 (predose) and 2 hours post-dose GS-1101 treated animals: • GD 6 and 17 at 0 (predose), 0.5, 2, 4, 8, and 24 hours post-dose
Cesarean section data	At necropsy*
Offspring	At necropsy*

* Necropsy performed on GD 20

Observations and Results

Mortality

One female in the 150 mg/kg/day group was found dead on GD 20. Final body weight results indicated a 39 g loss from GD 6. Clinical findings for this female consisted of red material on the urogenital area (beginning on gestation day 12), red vaginal discharge (on gestation day 15), and red material on the ventral abdominal/thoracic areas and cage papers, and body and limbs that were pale and cool on day prior to death.

Clinical Signs

Table 76: Clinical Signs in Dams Treated with GS-1101 in the Embryofetal Development Study in Rats

Clinical signs	No. of animals affected (No. of observations)			
Dose (mg/kg/day)	0	25	75	150
Number of animals examined	15	15	15	15
Hair loss, right forelimb	5 (24)	4 (30)	5 (24)	7 (67)
Hair loss, left forelimb	4 (15)	3 (27)	5 (28)	6 (55)
Hair loss, urogenital area			2 (15)	1 (7)
Hair loss, right hindlimb			1 (2)	2 (16)
Hair loss, left hindlimb				3 (16)
Hair loss, ventral abdominal area			1 (4)	1 (10)
Hair loss, dorsal abdominal area			1 (2)	1 (1)
Hair loss, right lateral abdominal area				1 (8)
Hair loss, left lateral abdominal area				1 (4)
Scabbing, right forelimb				1 (4)
Wet red material, urogenital area	1 (1)			3* (10)
Dried red material found on cage papers				1* (1)
Dried red material urogenital area			1 (1)	3 (4)
Wet red material ventral abdominal area				1* (1)
Wet red material ventral thoracic area				1* (1)
Body pale				1* (1)
Body cool				1 *(1)
Limbs cool				2* (2)
Limbs pale				2* (2)

* Clinical signs in the female found dead

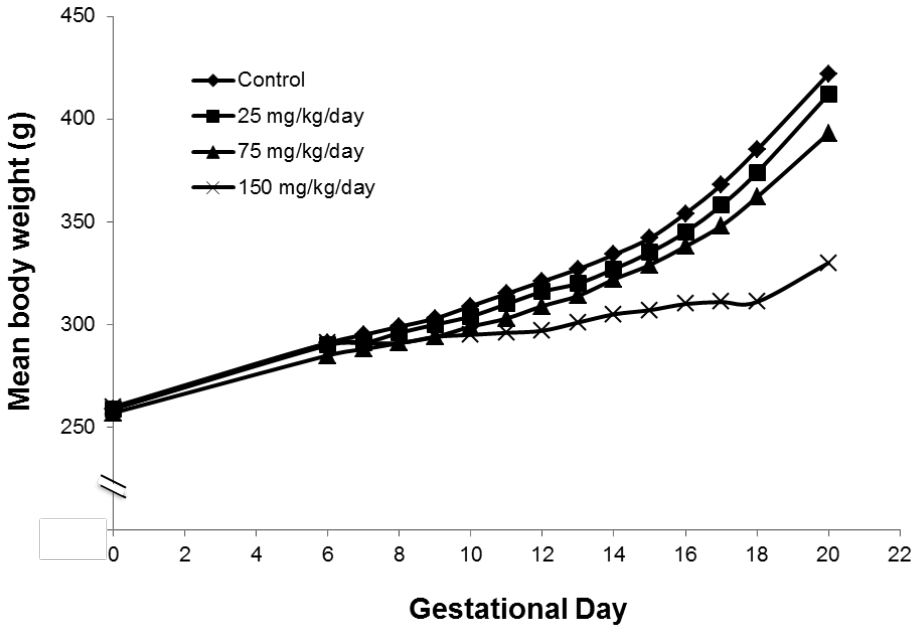
- Wet red material was observed 1-2 hours post-dose on the urogenital area in 2 females at 150 mg/kg/day and 1 female at 75 mg/kg/day beginning on GD 13. Wet red material on the cage paper (3 females) and red vaginal discharge (5 females) were also observed postdose in the 150 mg/kg/day group and corresponded to the high incidence of post implantation loss in this group.

Body Weight

Statistically significant lower mean body weight gains (including gravid uterine weight) were observed in the 150 mg/kg/day group compared to controls beginning on GD 6. At 75 mg/kg/day, there was a statistically significant decrease (~ 6 to 7%) in mean body weight gain in treated animals compared to controls on GD 18 and 20 and is likely the result of lower mean gravid uterine and fetal body weights.

Figure 25: Mean Body Weight in Female Rats During Gestation

(Including Gravid Uterine Weights)



There was a dose-dependent decrease in net body weight gain (after deducting the gravid uterine weight) between GD 6 and 20, however the decrease was only statistically significant at the high dose (25% decrease).

Table 77: Changes in Net Body Weight Gain (g) in Female Rats

(Separating out Gravid Uterine Weights)

Dose GS-1101 (mg/kg/day)	Female Rats					
	Initial BW	Terminal BW	Gravid Uterine Wt.	Net BW	Net BWΔ	Net BW %Δ
Control	259	422	88.8	333.1	74.3	
25	259	412	85.8	326.3	67.7	9%↓
75	257	393**	72.2**	320.9	63.6	**14%**↓
150	260	330**	13.9**	315.8	55.9**	**25%**↓

%Δ = percent change compared to the control mean
** = significantly different from control group; p ≤ 0.01

Food Consumption

There was a statistically significant reduction in food consumption (g/animal/day) at doses ≥ 75 mg/kg/day that correlated in reduced body weight gain.

Toxicokinetics

GS-1101:

- Exposure increased with the increase in dose level from 25 to 150 mg/kg/day.
- The increase in C_{max} was approximately dose proportional between the 25 to 150 mg/kg/day dose levels, while the increase in AUC_{0-t} was greater than dose proportional.
- Accumulation of 20 to 215 % based on AUC was observed after multiple dosing in rats.

Table 78: Toxicokinetic Parameters for GS-1101 in Plasma of Pregnant Female Rats

(Table excerpted from the Applicant's submission)

Interval	Dose Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	T_{last} (hr)	C_{last} (ng/mL)	AR
GD 6	2	25	4,418	0.500	24,391	24.0	8.19	NA
	3	75	11,777	2.00	79,606	24.0	30.6	NA
	4	150	19,275	2.00	251,445	24.0	100	NA
GD 17	2	25	5,335	2.00	29,106	8.00	1,780	1.19
	3	75	18,675	4.00	250,775	24.0	58.3	3.15
	4	150	34,975	4.00	584,117	24.0	10,605	2.32

NA = Not applicable

GS-563117 metabolite:

- Exposure increased with the increase in dose level from 25 to 150 mg/kg/day.
- The increases in C_{max} and AUC_{0-t} were approximately dose proportional between the 25 to 150 mg/kg/day dose levels.
- Accumulation of 263% based on AUC was observed at 150 mg/kg/day after multiple dosing in rats.
- GS-1101 was not readily converted to GS-563117 based on low metabolite to parent compound (M/P) ratios.

Table 79: Toxicokinetic Parameters for GS-536117 in Pregnant Female Rats

(Table excerpted from the Applicant's submission)

Interval	Dose Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng·hr/mL)	T_{last} (hr)	C_{last} (ng/mL)	AR	M/P
GD 6	2	25	62.4	0.500	279	8.00	13.5	NA	0.0114
	3	75	147	2.00	827	8.00	29.1	NA	0.0104
	4	150	308	2.00	1,651	8.00	169	NA	0.00657
GD 17	2	25	46.9	2.00	291	8.00	21.9	1.04	0.0100
	3	75	124	4.00	782	8.00	80.1	0.946	0.00312
	4	150	327	8.00	5,996	24.0	163	3.63	0.0103

NA = Not applicable

Group 2 = 25 mg/kg/day; Group 3 = 75 mg/kg/day; Group 4 = 150 mg/kg/day

Dosing Solution Analysis

Test-article formulations were prepared weekly, divided into aliquots for daily dispensation, and stored refrigerated (2°C to 8°C). Samples for homogeneity determination were collected from the top, middle, and bottom strata of the 2.5, 7.5, and 15 mg/mL dosing formulations prepared for dosing on GD 6 and from the top and bottom following 6 days for storage and mixing using a magnetic stirrer for 30 minutes. Samples for concentration determination were taken from the middle.

The analyzed dosing formulations were within [redacted] (b) (4) SOP range for suspensions (85% to 115%) and were homogeneous, with the following exception. The analyzed concentration of the 25 January 2012 Group 4 formulation was 75.2% of target. However, this formulation was not used for dose administration and a new formulation was prepared.

Necropsy

The female in the 150 mg/kg/day group that was found dead on GD 20 was noted with dark red matting on the skin (ventral abdominal area, urogenital area, and hind limbs) and dark red contents of the vagina. This female had 16 early resorptions *in utero*.

Nodules on the spleen and small thymus were noted in different female at 150 mg/kg/day.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

- Gravid uterine weights were significantly lower at doses ≥ 75 mg/kg/day (19% decrease at 75 mg/kg/day and 84% at 150 mg/kg/day when compared to controls) and it correlated with reduced maternal body weight gain.
- Fourteen of 24 surviving females in the 150 mg/kg/day group had entirely resorbed litters (primarily early resorptions), resulting in a post-implantation loss value of 89.3% per litter compared to 3.7% in concurrent controls and an 89% decrease in mean litter size (mean litter size was 1.6 in GS-1101 treated dams compared to ~15 for concurrent and historical controls).
- Decreased mean fetal weights of 37% at 150 mg/kg/day and 10.5% at 75 mg/kg/day compared to concurrent controls were observed, however the mean fetal weight of 3.4 g at 75 mg/kg/day fell within the historical range.

Table 80: Summary of Cesarean Section Data in Dams Treated with GS-1101

Dose group		1	2	3	4	Historical Control 1998-2010	
Dose (mg/kg/day)		0 (vehicle)	25	75	150	Mean	Range
Rats tested	N	25	25	25	25	4166	
Pregnant	N	24	25	25	25	4026	
	%	96	100	100	100	96.6	76.0-100

Dose group		1	2	3	4	Historical Control 1998-2010	
Dose (mg/kg/day)		0 (vehicle)	25	75	150	Mean	Range
No. died or sacrificed moribund	N	0	0	0	1	6	
	%	0	0	0	4	6	
No. aborted or with total resorptions of litter	N	0	0	0	14		
	%	0	0	0	56		
Laparohysterectomy	N	24	25	25	24*	4160	
Dams with live fetuses	N	24	25	25	24*	4015	
Corpora Lutea	N	420	405	397	408		
	MEAN	17.5	16.2	15.9	17.0	17.2	14.5-19.4
Implantation sites	N	379	375	369	368		
	MEAN	15.8	15.0	14.8	15.3	15.9	13.0-17.6
Pre-implantation loss	% per litter	9.3	7.4	6.4	8.2	7.3	1.5-15.7
Litter sizes	MEAN	15.2	14.7	13.6	**1.6****	15.1	12.2-17.1
Live fetuses	N	365	368	340	39**	60839	
	% per litter	96.3	98.2	92.7	**10.7****	95.1	90.1-98.0
	MEAN	15.2	14.7	13.6	1.6**		
Dead fetuses	N	0	0	0	0		
	MEAN	0.0	0.0	0.0	0.0		
No. Resorptions	MEAN	0.6	0.3	1.1	13.3		
Early resorptions	N	14	7	28	320		
	% per litter	3.7	1.8	7.1	**87.0****	4.8	1.5-9.9
	MEAN	0.6	0.3	1.1	13.3		
Late resorptions	N	0	0	1	9		
	% per litter	0	0	0.3	**2.3**	0.1	0.0-0.8
	MEAN	0.0	0.0	0.0	0.4		
Post-implantation loss	% per litter	3.7	1.8	**7.3**	**89.3****	4.9	2.0-9.9
Males	N	188	181	160	22		
	% per litter	51.5	49.1	45.3	53.3	49.9	43.1-56.7

Dose group		1	2	3	4	Historical Control 1998-2010	
Dose (mg/kg/day)		0 (vehicle)	25	75	150	Mean	Range
	MEAN	7.8	7.2	6.4	0.9		
Females	N	177	187	180	17		
	% per litter	48.5	50.9	54.7	46.7	50.1	43.3-56.9
	MEAN	7.4	7.5	7.2	0.7		
Fetal weights (g)	MEAN	3.8	3.8	3.4**	**2.4****	3.7	3.4-3.9
	%Δ		0	-10.5	-36.8		

* Female found dead was not evaluated
** Statistically significant; p≤0.01
Bold = value is outside of the historical control range
% per litter =sum of percent affected per litter of a given parameter / number of total litters
%Δ = percent change relative to control mean

Offspring (Malformations, Variations, etc.)

GS-1101 related external malformations were observed at doses ≥ 75 mg/kg/day that were not observed in concurrent controls and fell outside the historical range. However, evaluation of fetal morphology at 150 mg/kg/day was somewhat limited by the test article-related effects on embryo/fetal survival and the small number of viable fetuses available for examination.

Table 81: Malformations and Variations Observed in Litters from Dams Treated with GS-1101

Dose (mg/kg/day)	Control	25	75	150	Historical control data 1998-2010	
External malformations					Number affected or % per litter	Range
Number of litters	24	25	25	10	3942	
Number of fetuses examined	365	368	340	39	59744	
Short tail	L 1 F 1	L 0 F 0	L 1 F 2	L 9 F 16	L 3 F 3	
% per litter	0.0	0.0	**0.5**	**66.0***	0.0	0.0-0.3
Anury	L 0 F 0	L 0 F 0	L 0 F 0	L 3 F 10		
% per litter	0.0	0.0	0.0	13.8		
Vertebral Agenesis (with anury)	L 0 F 0	L 0 F 0	L 0 F 0	L 3 F 3	L 10[a] F 10[a]	
% per litter	0.0	0.0	0.0	**6.8**	0.0[a]	0.0-0.3[a]
Microphthalmia/anophthalmia	L 1 F 1	L 0 F 0	L 1 F 1	L 1 F 1	L 18 F 18	
% per litter	0.2	0.0	0.3	**5**	0.0	0.0-0.5

Dose (mg/kg/day)	Control	25	75	150	Historical control data 1998-2010	
% per litter with external malformations	0.2	0.0	0.8	**86.7***	0.1	0.0-1.3
Visceral malformations						
Number of litters	24	25	25	10	3942	
Number of fetuses examined	365	368	340	39	59550	
Hydrocephaly	L 0 F 0	L 0 F 0	L 0 F 0	L 1 F 1	L 9 F 9	
% per litter	0.0	0.0	0.0	**1.0**	0.0	0.0-0.8
% per litter with visceral malformations	0.0	0.0	0.0	**1.0**	0.1	0.0-0.9
Skeletal variations						
Number of litters	24	25	25	10	3941	
Number of fetuses examined	365	368	340	39	59537	
Cervical centrum no. 1 ossified	L 18 F 68	L 17 F 79	L 12 F 28	L 0 F 0	L 3004 F 11969	
% per litter	19.6	20.7	9.7	**0.0***	20.4	6.6-35.8
Sternebra(e) nos. 5 and/or 6 unossified	L 11 F 28	L 11 F 25	L 19 F 26	L 10 F 23	L 1415 F 3908	
% per litter	7.6	6.8	18.1	**73.3***	6.4	0.0-26.1
Reduced ossification of the vertebral arch(es)	L 1 F 1	L 0 F 0	L 3 F 8	L 10 F 17	L 62 F 67	
% per litter	0.2	0.0	**3.9**	**63.7***	0.1	0.0-1.1
Sternebra(e) nos. 1, 2, 3, and/or 4 unossified	L 1 F 1	L 0 F 0	L 2 F 2	L 4 F 7	L 106 F 114	
% per litter	0.2	0.0	**2.2**	**18.0**	0.2	0.0-1.3
Pubis unossified	L 1 F 1	L 0 F 0	L 0 F 0	L 3 F 6	L 36 F 42	
% per litter	0.2	0.0	0.0	**16.7**	0.1	0.0-2.3
Reduced ossification of the skull	L 0 F 0	L 0 F 0	L 2 F 2	L 1 F 1	L 45 F 52	
% per litter	0.0	0.0	**2.3**	**5.0**	0.1	0.0-1.0
% per litter with skeletal variations	35.7	36.8	39.7	**91.2***	33.7	18.0-50.4
Total Affected Fetuses with Malformations (% per litter)	0.2	0.0	0.8	**86.7***	0.3	0.0-1.6
Total Affected Fetuses with Developmental Variations (% per litter)	36.0	37.3	40.3	**91.2****	33.9	17.1-51.1

a. Historical control data was presented under skeletal malformations

* Statistically significant ($p \leq 0.01$); L = number of litters affected; F = number of fetuses affected; **bold** values = outside of the historical control range

% per litter = sum of percent malformed per litter / number of litters with live fetuses

Other noteworthy findings:

- The fetus with microphthalmia/anophthalmia at 150 mg/kg/day was one of only two live fetuses in a litter which contributed to a high 5% per litter value for this malformation. While of low incidence, this is likely a toxicologically significant finding since the percent per litter is higher than the control and higher than the historical range.
- The fetus with hydrocephaly also displayed vertebral agenesis.
- The reduced ossification sites at 75 and 150 mg/kg/day also occurred in the presence of low fetal weight.

10 Special Toxicology Studies

Study title: A 28-Day Oral Gavage Impurity Qualification Study of GS-1101 in the Sprague Dawley Rat

Study no.:	TX-312-2012
Study report location:	4.2.3.7.6.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 30, 2012
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	(b) (4) Purity 99.3%); (b) (4) Purity: 97.5) (see table below)

Key Study Findings

- Targets include organs of the lymphatic system and digestive tract, eyes, heart, organs of the exocrine system (including the kidneys), and testis.
- There were no additional toxicities related to the impurities. Toxicological findings between the lots with high (b) (4) and lower (b) (4) impurities were similar; findings were also similar to the 28-day and 6-month rat studies.

Table 82: Impurity Levels in Batches Used in Toxicology Studies

Lot #	Observed Impurities					
	(b) (4)					
Proposed Limit	NMT (b) (4)	NMT (b) (4)	NMT (b) (4)	NMT (b) (4)	NMT (b) (4)	NMT (b) (4)
(b) (4)						
(b) (4)						
(b) (4)						

Note: None of these impurities were found in any other toxicology batches except for Lot No. (b) (4) used in the 6-month rat, 9-month dog, and EFD studies, in which levels of impurities were below the ICH qualification threshold of (b) (4)

Methods

Doses:	Vehicle, 90 [redacted] lower impurity levels), 25 and 90 mg/kg [redacted] higher impurity levels)
Frequency of dosing:	Daily for 4 weeks
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% high viscosity CMC, 0.1% TWEEN 80 in deionized water
Species/Strain:	rat/Sprague-Dawley
Number/Sex/Group:	10 animals/sex/group
Age:	7 weeks old
Weight:	175 g to 224 g for males; 149 g to 187 g for females
Satellite groups:	3 animals/sex/group for TK control animals; 9/sex/group for TK for [redacted] treated animals
Unique study design:	None
Deviation from study protocol:	Deviations did not negatively impact the quality or integrity of the data or the outcome of the study.

Observations and Results

Mortality:	Twice daily
Clinical signs:	Twice daily
Body weights:	Pre-test and weekly
Food consumption:	Pre-test and weekly
ECG:	Not conducted
Ophthalmoscopy:	Pre-test and during the final week of dosing
Hematology:	At necropsy*
Clinical chemistry:	At necropsy*
Coagulation:	At necropsy*
Urinalysis:	At necropsy*
Gross pathology:	At necropsy*
Organ weights:	At necropsy*
Histopathology:	At necropsy*
Toxicokinetics:	Days 0 and 27: Control animals • 2 hours postdose [redacted] treated animals: • 0.5, 1, 2, 4, 8, and 24 hr postdose

* Scheduled necropsy was on Day 28

Mortality

- Deaths appear to be related to blood sampling and/or gavage error.

Table 83: Cause of Death in 4-Week Study Evaluating GS-1101 Impurites (Study No. TX-312-2012)

Dose of [redacted] (b) (4) (mg/kg/day)	Animal #	Sex	Day Found Dead	Potential cause of morbidity/ macroscopic findings
25	1641	Female (TK)*	27	Undetermined/none as determined by the Applicant
90	1574	Male	28	Died during blood collection at euthanasia/ Discoloration/swollen thymus; discoloration, dark red trachea
	1592	Female (TK)*	177	Gavage error/ none as determined by the Applicant

* Only macroscopic, TK, and body weight evaluations were conducted for TK animals

Clinical Signs

Table 84: Clinical Signs in Rats in the 4-Wk Impurity Study

Clinical signs	No. of animals affected (No. of observations)			
Group	Vehicle Control	[redacted] (b) (4)		
		90 mg/kg/day	25 mg/kg/day	90 mg/kg/day
Number of animals examined	10	10	10	10
Males				
Hair loss, forelimb(s)		2 (3)	1 (3)	2* (5)
Females				
Hair loss, forelimb(s)			2 (2)	
Dried red material around right eye				1 (1)

* Observed in male that died (No. 1574)

Body Weights

The level of impurities had no effect on body weight gain. Body weight gain at the 90 mg/kg [redacted] (b) (4) dose was 38% from Days 0-27 compared to 35% body weight gain for controls. Body weight gain at the 90 mg/kg [redacted] (b) (4) dose was 33% from Days 0-27.

Food Consumption

Unremarkable

Ophthalmoscopy

Corneal crystals were present in three out of ten 90 mg/kg/day [redacted] (b) (4) treated females on Day 23, which is slightly higher than the incidence in control females pre-test (2 out of 10 females) and identical to control males on Day 23. This finding is likely not treatment related.

ECG

Not conducted

Hematology

Table 85: Summary of Hematology Findings in Rats in the 4-Wk Impurity Study

Index	Mean		Percentage deviation from Control					
	Control 0 mg/kg		90 mg/kg (b) (4)		25 mg/kg (b) (4)		90 mg/kg (b) (4)	
	M	F	M	F	M	F	M	F
HDW (g/dL)	2.37	2.45	7**	13**	0	7	4	13**
RDW%	12.0	11.1	3	6**	0	3	0	4*
Retic. Abs.	141.1	123.5	7	23	6	-11	3	2
Retic.%	1.8	1.6	6	25	11	-13	0	0
Platelets Abs.	909	896	-2	6	17	16	1	5
WBC Abs.	8.46	6.20	-5	30	1	12	10	62*
Lymphocytes Abs.	6.58	5.07	1	14	12	16	22	55**
Neutrophils Abs.	1.56	0.67[a]	-28*	176	-37*	22	-38*	173
Monocytes Abs.	0.15	0.15	-7	87*	-27	-20	0	40
Eosinophils Abs.	0.09	0.08	-22	0	0	0	-44**	-13
Basophils Abs.	0.2	0.1	0	100	0	100	50	200
LUC Abs.	0.06	0.04	-33	50	-50*	0	0	125*
LUC%	0.7	0.7	-29	14	-43**	-29	-14	14

* P ≤ 0.05, ** P ≤ 0.01 compared to control
a – Outlier (one control female, No. 1649) excluded from analysis by reviewer

Clinical Chemistry

Table 86: Summary of Clinical Chemisty Findings in Rats in the 4-Wk Impurity Study

Index	Mean		Percentage deviation from Control					
	Control 0 mg/kg		90 mg/kg (b) (4)		25 mg/kg (b) (4)		90 mg/kg (b) (4)	
	M	F	M	F	M	F	M	F
Albumin (g/dL)	4.0	4.1	3	5	0	2	0	5
Globulin (g/dL)	2.5	2.5	-8	-4	-4	0	-8	0
A:G Ratio	1.61	1.67	11.2**	7.2**	3	1	10**	6*
Total bilirubin (mg/dL)	0.01	0.03	0	-67	0	-33	0	-33
Direct	0.03	0.03	-33	-67	-33	-33	-67	-33
Indirect	0.01	0.01	-100	0	-100	0	-100	0
Cholesterol (mg/dL)	63	71	-3	11	-10	-4	-6	16*
Calcium (mg/dL)	10.5	10.3	2	3	1	2	1	4*
Phosphorus (mg/dL)	9.1	7.1	-4	10	-9	1	-6	11
Potassium (mEq/L)	6.15	5.48	-9	8	-8	0	-8	4

* P ≤ 0.05, ** P ≤ 0.01 compared to control

Urinalysis

Mean urine osmolarity increased 34% and 12% compared to vehicle control in males and females at 90 mg/kg/day (b) (4) respectively, however the standard deviation was very high in all groups making the significance of this observation unclear.

Gross Pathology

Table 87: Treatment-Releated Macroscopic Findings in Rats in the 4-Wk Impurity Study

Treatment-related macroscopic findings		No. of animals affected							
		Males				**Females**			
Dose (mg/kg)		1	2	3	4	1	2	3	4
Number of animals examined		0/10	0/10	0/10	1*/9	0/10	0/10	0/10	0/10
Cavity, cranial	Contents, dark red				0/1				
Seminal vesicles	Small			0/1		NA			
Thymus	Discoloration, dark red				1*/1				
	Swollen				1*/0				
	Area(s), Dark red	0/2		0/2	0/1	0/3	0/2	0/1	0/1
Trachea	Discoloration, dark red				1*/0				
Thyroid glands	Small								0/1
Uterus	Contents, clear fluid	NA				0/2		0/1	0/1

Group 1=vehicle control; Group 2=90 mg/kg/day (b) (4) Group 3=25 mg/kg (b) (4) Group 4=90 mg/kg/day (b) (4)
Number of animals examined and affected: Early deaths*/Terminal necropsy
Empty cells = no test-article related changes
NA= Not Applicable, tissue does not exist in this sex

Organ Weights

Table 88: Treatment-Related Organ Weigt Changes in Rats in the 4-Wk Impurity Study

Group		% deviation from control					
		2		3		4	
Gender		M	F	M	F	M	F
Number of animals examined		10	10	10	10	9	10
Adrenal glands	Absolute (g)	4	15	3	8	19	14
	Relative BW (%)	6	14	0	11	25*	14
	Relative Brain (%)	4	14	1	6	20	13
Spleen	Absolute (g)	-14	-12	-7	-12	-11	-6
	Relative BW (%)	-12	-15	-9	-12	-9	-6
	Relative Brain (%)	-13	-13	-9	-13	-9	-7
Testis	Absolute (g)	-4	NA	1	NA	-11*	NA
	Relative BW (%)	-3		-2		-10**	
	Relative Brain (%)	-5		-2		-10*	
Liver	Absolute (g)	0	16**	2	5	5	13*
	Relative BW (%)	1	11**	-1	4	6	13**
	Relative Brain (%)	0	14*	0	3	6	12
Thymus	Absolute (g)	-6	-17	4	-9	-10	-20
	Relative BW (%)	-5	-20*	1	-9	-9	-20*
	Relative Brain (%)	-6	-19*	2	-10	-9	-20*
Uterus/ Cervix	Absolute (g)	NA	-31	NA	-22	NA	-25
	Relative BW (%)		-35*		-22		-27*

Group		% deviation from control					
		2		3		4	
Gender		M	F	M	F	M	F
Number of animals examined		10	10	10	10	9	10
	Relative Brain (%)		-32		-23		-25

* P ≤ 0.05, ** P ≤ 0.01 compared to control
Group 2=90 mg/kg/day (b) (4) Group 3=25 mg/kg (b) (4) Group 4=90 mg/kg/day (b) (4)

- The Applicant noted that despite differences in organ weights in test-article treated animals compared to controls, all mean values fell within the (b) (4) (b) (4) historical control reference ranges.

Histopathology

Adequate Battery Yes

Peer Review Yes (for spleen only)

Histological Findings

Table 89: Microscopic Findings with Increased Incidence or Severity in Rats in the 4-Wk Impurity Study

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				**Females**			
Dose (mg/kg)			1	2	3	4	1	2	3	4
Number of animals examined			0/10	0/10	0/10	1*/9	0/10	0/10	0/10	0/10
Organ	Finding	Severity								
Cecum	Dilatation, glandular	Minimal				0/1				
Esophagus	Hemorrhage	Minimal				1*/0				
Eyes	Dysplasia, retinal	Total		0/4	0/2†	0/4		0/2†		
		Minimal		0/4	0/2†	0/3		0/2†		
		Mild				0/1				
Heart	Cardio-myopathy	Total		0/3	0/1	0/1				
		Minimal								
		Mild								
	Cyst, valvular	Present				0/1				
Kidneys	Inflammation, Chronic	Minimal		0/2		1*/0	0/1		0/3	0/2
	Cyst	Present	0/3	0/3	0/0	0/4		0/2	0/2	0/1
	Infiltrate, mononuclear	Minimal					0/1	0/2	0/1	0/2
Larnyx	Hemorrhage	Minimal				1*/0				
LN, axillary	Erythrocytosis, sinus	Moderate								
	Hemorrhage	Minimal						0/1		
LN, mandibular	Hemorrhage	Total	0/1		0/1	0/1	0/4	0/4	0/2	
		Minimal	0/1		0/1		0/1			
		Mild				0/1	0/3	0/2	0/1	
		Moderate						0/2	0/1	
	Hyperplasia, Lymphoid	Moderate			0/1					
LN,	Erythrocytosis,	Minimal		0/1		1*/0				

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				Females			
Dose (mg/kg)			1	2	3	4	1	2	3	4
Number of animals examined			0/10	0/10	0/10	1*/9	0/10	0/10	0/10	0/10
Organ	Finding	Severity								
mesenteric	sinus									
Lungs	Infiltrate, eosinophil, perivascular	Minimal						0/1		
	Infiltrate, mononuclear	Minimal		0/1	0/1	0/1	0/1	0/1	0/2	
	Macrophages, alveolar	Minimal	0/1				0/2	0/1		
Ovaries	Cyst	Present						0/1		
Rectum	Infiltrate, mixed inflammatory cell	Minimal				0/1				
	Inflammation, subacute	Minimal						0/1		
Seminal vesicles	Decreased secretion	Severe			0/1					
Skeletal muscle	Infiltrate, mononuclear	Minimal		0/1		0/2	0/2			
Spleen	Decreased lymphocytes, marginal zone	Total	0/2	0/10	0/8	1*/9		0/8	0/8	0/10
		Minimal	0/2	0/2	0/4	0/1			0/8	0/1
		Mild		0/4	0/3	1*/1		0/3		
		Moderate		0/3	0/1	0/5	0/1	0/3		0/4
		Severe		0/1		0/2		0/2		0/5
	Fibrosis	Minimal			0/1			0/1		
Stomach, Glandular	Inflammation, subacute	Minimal						0/1		
Stomach, Non-glandular	Edema, submucosal	Mild								0/1
	Infiltrate, mononuclear	Minimal						0/1		
Thymus	Hemorrhage	Total	0/3		0/3	1*/1	0/3	0/4	0/6	
		Minimal	0/3		0/3	0/1	0/3	0/4	0/6	
		Mild				1*/0				
	Hyperplasia, epithelial	Minimal			0/2		0/2	0/4	0/3	0/3
	Decreased lymphocytes	Mild	0/1	0/2		0/2	0/1	0/8	0/2	0/6
Thyroid glands	Infiltrate, mononuclear	Minimal		0/1				0/1		
	Ectopic Thymus	Present	0/1	0/1		1*/1			0/1	
	Hemorrhage	Minimal				1*/0				
Tongue	Granuloma	Present		0/1			0/1			
	Inflammation, chronic	Minimal						0/2		
Trachea	Hemorrhage	Minimal				1*/0				
Urinary Bladder	Hyperplasia, epithelial	Moderate								0/1

Treatment-Related Microscopic Findings			No. of animals affected							
			Males				Females			
Dose (mg/kg)			1	2	3	4	1	2	3	4
Number of animals examined			0/10	0/10	0/10	1*/9	0/10	0/10	0/10	0/10
Organ	Finding	Severity								
	Inflammation, chronic active	Mild								0/1

Number of animals examined and affected: Early deaths*/ Terminal necropsy / Recovery necropsy

Group 1 = control; Group 2=90 mg/kg/day [(b) (4)] Group 3=25 mg/kg [(b) (4)] Group 4=90 mg/kg/day [(b) (4)]

empty cell = no change or not evaluated; † = similar incidence/lower severity compared to control recovery male rats in 6-month rat study and is likely a background finding.

Toxicokinetics

GS-1101:

- TK parameters including T_{max}, $T_{1/2}$, sex differences in exposure, and accumulation were generally comparable between [(b) (4)] and consistent with TK observations in general toxicology rat studies. However, in females administered 90 mg/kg/day [(b) (4)], the observed T_{max} of 8 hours on Day 27 was higher than the range of T_{max} values observed for [(b) (4)] in this study and GS-1101 in the general toxicology rat studies (between 0.5 and 4 hours).
- Exposures (C_{max} and AUC) for 90 mg/kg/day [(b) (4)] were generally higher than 90 mg/kg/day [(b) (4)] on Day 27.

GS-563117:

- Exposure to GS-563117 was similar for both [(b) (4)] and as observed in general rat toxicology studies, very little of [(b) (4)] was converted to the metabolite (M/P ratios ranged from roughly 0.003 to 0.02 at 90 mg/kg/day doses).

11 Integrated Summary and Safety Evaluation

Idelalisib is a PI3Kδ kinase inhibitor indicated for the treatment of patients with refractory indolent B-cell Non-Hodgkin Lymphoma (iNHL) and chronic lymphocytic leukemia (CLL). Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of idelalisib for this indication.

Pharmacology

Idelalisib is a kinase inhibitor that was selective for inhibition of PI3 kinases when screened against a panel of 353 kinases in an *in vitro* kinase assay. Inhibition of PI3Kδ is through binding of the drug to the ATP-binding site of P110δ (the catalytic subunit). P110δ is overexpressed in WSU-FSCCL and WSU-NHL, two cell lines derived from patients with follicular lymphoma. Idelalisib treatment inhibited cell viability in both cell lines. The concentration of idelalisib that resulted in 50% inhibition (IC_{50}) of PI3Kδ was 19 nM in *in vitro* assays using recombinant protein. The EC_{50} was 0.1-1 µM

based on Akt phosphorylation when primary tumor samples and malignant B-cell lines were treated with idelalisib. In a cell-based assay, GS-1101 inhibited PI3Kδ signaling with an EC_{50} of 8.9 nM. Idelalisib caused dose-dependent apoptosis in malignant B-cell lines and reduced the induction of chemokines in primary CLL cells stimulated by anti-IgM or by co-culture with Nurse like cells (NLCs) at concentrations ≥ 0.5 µM. Idelalisib inhibited Akt and extracellular signal-regulated kinase phosphorylation in response to anti-IgM stimulation and in response to CXCL12 or CXCL13. The secretion of chemokines CCL3 and CCL4 was significantly inhibited by idelalisib at concentrations of 1 µM or greater. Idelalisib exhibited sensitivity in cell viability in B-ALL and CLL cells compared with AML and MPN cells, suggesting a greater activity against B-cell malignancies. Idelalisib, at a concentration of 0.1 µM, inhibited P-AKT (Ser473) an average of 86% (range 55-97%) in malignant B-cells prepared from biopsy specimens of 7 patients with follicular lymphoma. In a kinase screen, idelalisib inhibited other Class I PI3K isoforms between 83 and 97.3%, however, *in vitro* assays demonstrate that GS-1101 is between 110- and 453-fold more selective for inhibition of PI3Kδ.The primary human metabolite of idelalisib, GS-563117 is an inhibitor of LOK and SLK kinases with IC_{50} values of 0.11 µM and 0.05 µM, respectively.

Both *in vivo* and *in vitro* safety pharmacology studies were conducted to assess the effects of idelalisib on neurological, cardiovascular, and respiratory function. The IC_{50} for the *in vitro* hERG potassium current was estimated to be greater than 50 µM. Idelalisib did not induce toxicologically significant cardiovascular and respiratory effects *in vivo* up to 20 mg/kg (400 mg/m^2) in telemetered dogs or neurobehavioral effects in rats up to 150 mg/kg (900 mg/m^2). However, drug-related cardiomyopathy and increased in the heart weight were observed in the rat in the repeat-dose toxicology studies (see below).

Pharmacokinetics/ADME/Toxicokinetics

Orally administered idelalisib was absorbed rapidly, with bioavailability ranging from 39 to 48% in the tested rodent (rat) and non-rodent (dog) species, respectively. Oral administration of 50 mg/kg (300 mg/m^2) radioactive idelalisib to rats reached maximal concentration at 1 hour postdose (in 35 of 37 tissues) and decreased 24-48 hours postdose, but was relatively excluded from bone, brain, spinal cord, and eye lens. In Long-Evans rats, pigmented skin and eye uvea showed higher concentrations of idelalisib than that observed for the similar tissues in Sprague-Dawley rats, suggesting some association of drug-derived radioactivity with melanin. In rats and dogs, idelalisib was the most abundant analyte in plasma (~91 to 93% in rats and ~59% dogs), with GS-563117 being the most abundant circulating metabolite. Idelalisib exhibited moderately high plasma protein binding in all species with the average free fraction values of 18.7%, 20.7%, and 16.3% for rat, dog, and human respectively; and, ~12% in humans for the oxidative metabolite GS-563117 (Table 14). Hepatobiliary route was the primary route of excretion within 24 to 48 hours in rats and dogs, with the majority of idelalisib being detected in the feces and 6% or less in the urine. Radioactivity derived from radiolabeled idelalisib (above 80% in rats and above 88% in dogs) was excreted within 24 hours in rats and 48 hours in dogs after oral dosing and was below the limit of

detection in plasma by 72 hours; roughly 1% remained in rats or was below the limit of quantitation in dogs in the blood after 168 hours. The half-life in rats following a single oral dose of 3 mg/kg (18 mg/m^2) was 1.5 hours and was 2 hours for an oral dose of 1 mg/kg (20 mg/m^2) in dogs. The pharmacokinetics of idelalisib are similar between rats and dogs and humans, however, the levels of metabolite GS-563117 differed among species and was detected at 62%, 34%, and 1.4% in human, dog, and rat plasma, respectively. Based on similarities in pharmacokinetics for idelalisib between humans and animals, the animal models for pharmacology are predictive of the time course of pharmacologic activity of the drug, and can be relied upon for predicting activity of alternative regimens in the clinical setting. The pharmacokinetics for GS-563117 was different between humans and animals, with the non-rodent (dog) species as the most comparable. Therefore, the animal models for pharmacology are not expected to be highly predictive of the pharmacologic activity of GS-563117 in the human.

General Toxicology

The primary target organ toxicities following repeated dosing of idelalisib in rats and/or dogs were the hematopoietic/lymphoid system, liver, male reproductive system, heart (in rats only), and the GI tract. The skin may also be a target based on the adverse findings in the skin in toxicology studies and a relatively high concentration of radioactive materials in the skin of pigmented rats in the ADME studies. In an in vitro assay, the human metabolite (GS-563117) caused photo-toxicity upon UVA exposure.

Repeat-dose toxicology studies with oral administration of idelalisib daily were conducted in Sprague-Dawley rats and Beagle dogs in order to fully characterize idelalisib-induced toxicities. Studies reviewed included 28-day general toxicology studies in rats and dogs, a 13-week study in rats, a 6-month study in rats, and a 9-month study in dogs. In the 28-day studies, there was mortality in rats at doses ≥ 100 mg/kg (600 mg/m^2) and in a dog at 20 mg/kg (400 mg/m^2). The probable cause of death in the 28-day rat study included liver toxicity. In the premature death in the 28-dog study, primary toxicities were to the GI tract, liver, and lymphoid tissues.

There were also mortalities at doses ≥ 25 mg/kg (150 mg/m^2) in the rat 6-month study and at doses ≥ 2.5 mg/kg (50 mg/m^2) in the 9-month dog study. The cause of deaths in the 9-month dog study was systemic inflammation. Cardiomyopathy was observed in dead and surviving rats at doses of 90 mg/kg/day (540 mg/m^2) in the 13-week and 6-month studies. There was also a 14% to 15% increase in the relative heart weights of males and females at 90 mg/kg/day compared to controls (14-15% increase in the absolute weights) in the 13-week study.

In the 6-month rat study, teeth abnormalities (broken or missing) were observed at low incidence, but are consistent with other kinase inhibitors in rats. Other clinical signs observed among studies are reddened extremities and face, alopecia, excessive salivation, yellow discharge from urogenital area, soft feces, clear discharge from eyes, and eye squint. Clinical signs are delayed and generally appear after 28-days of dosing.

There were no significant effects of idelalisib on RBC parameters in chronic toxicology studies, however, reticulocytes increased 166% in female rats dosed at 150 mg/kg/day (900 mg/m^2/day) for 28 days. The common findings in hematology parameters among the general toxicology studies were lymphoid hypocellularity in the bone marrow, decreased spleen and thymus weights, and lymphocyte infiltrates in multiple organs, confirming the lymphoid toxicities associated with the mechanism of action of idelalisib.

Genetic Toxicology
Idelalisib was negative in an *in vitro* bacterial reverse mutation (Ames) assay and *in vitro* cytogenetic assay using human lymphocytes. Idelalisib tested positive in the *in vivo* rat micronucleus assay at the highest dose, 2000 mg/kg in males with evidence of perturbation of erythropoiesis. Carcinogenicity studies have not been conducted and are not necessary for NDA approval for the proposed indication.

Reproductive and Developmental Toxicology
Reproductive toxicology studies consisted of 2 fertility studies and an embryofetal developmental (EFD) study. The studies were conducted in rats.

In the repeat-dose male fertility study, treated male rats (25, 50, or 100 mg/kg/day; 150, 300, 600 mg/m^2/day of idelalisib) were mated with untreated females. Idelalisib decreased testis and epididymis weights (all dose levels), and reduced sperm counts (at mid- and high-dose) with no effect on reproductive indices. The low dose in males resulted in an exposure (AUC) that is approximately equal to the exposure in patients at the recommended dose of 150 mg twice daily. In the repeat-dose female fertility study, treated female rats (25, 50, or 100 mg/kg/day; 150, 300, 600 mg/m^2/day of idelalisib) were mated with untreated males. There were no adverse effects on fertility parameters; however, the high dose (100 mg/kg or 600 mg/m^2) resulted in an increase in pre-implantation loss, post-implantation loss, and early embryolethality, resulting in a 20% decrease in the number of live embryos. The high dose in females resulted in an exposure (AUC) that is approximately 30-fold the exposure in patients at the recommended dose of 150 mg twice daily.

Toxicokinetic evaluations were not performed in the fertility studies, therefore, relevant comparison of exposures in animals and humans for idelalisib labeling will be based on the rat AUC at 25 mg/kg on Day 181 (10,635 ng•h/mL) in the 6-month rat study for males and the rat AUC at 100 mg/kg from the 28-day toxicology study (30,000 ng•h/mL) for females.

In the embryo-fetal developmental repeat-dose study, pregnant rats were administered oral doses of idelalisib during the period of organogenesis at doses of 25, 75, and 150 mg/kg/day (150, 450, 900 mg/m^2/day). At the high dose of 150 mg/kg/day (900 mg/m^2/day), idelalisib resulted in increased post-implantation loss, reduced mean litter size, reduced fetal body weights, reduced skeletal ossification, and increased incidences of short tail, anury, vertebral agenesis and hydrocephaly as well as skeletal developmental variations in rats at doses that also produced maternal toxicity (death of 1 female at 150 mg/kg/day; 900 mg/m^2/day). Additionally, microphthalmia/anophthalmia

was noted in 1 fetus each in the vehicle control, 75, and 150 mg/kg/day (0, 450, and 900 mg/m^2/day) groups. Skeletally, this finding consisted of orbits that were smaller than normal. Although this finding occurred similarly in these 3 groups, the mean litter proportion (5.0% per litter) in the 150 mg/kg/day (900 mg/m^2/day) group for microphthalmia and/or anophthalmia was higher than the concurrent control group (0.2% per litter) and above the maximum mean value in the (b) (4) historical control data (0.5% per litter). At doses ≥ 75 mg/kg/day (450 mg/m^2/day) short tail malformations and skeletal developmental variations such as reduced fetal skeletal ossification were observed at higher incidences than concurrent and historical controls. The dose of 75 mg/kg (450 mg/m^2/day) was also maternally toxic based on reduction in net body weight gain > 10% (after deducting the gravid uterine weight).

The reproductive and developmental toxicology studies suggest that administration of idelalisib may pose a risk for embryo-fetal toxicity. A Pregnancy category D is recommended. Relevant comparison of exposures in animals and humans for idelalisib labeling will be based on the rat AUC on GD 17 at the lowest dose with embryofetal toxicity (250,775 ng•h/mL at 75 mg/kg dose) and the human AUC of 10,081 ng•h/mL following administration of the recommended clinical dose of 150 mg BID. The dose of 75 and 150 mg/kg/day of idelalisib in rats resulted in exposures (AUC) of approximately 25 and 60 times, respectively, the human exposure at the recommended dose of 150 mg BID.

Table 90: Tabulated Summary of General Toxicology Studies

Species (Study #)	Route Duration	N/sex/dose	mg/kg (mg/m^2)	Significant findings
Rat/Sprague-Dawley (Crl) (Study No. BHR00009)	Oral gavage Once daily for 4 weeks (4 week recovery)	Main study: 14/sex/dose TK: Controls: 3/sex/dose GS-1101: 6/sex/dose	CAL-101 0[a], 50, 100, 150 (0, 300, 600, 900)	• There were several deaths at 100 (3 deaths) and 150 mg/kg (5 deaths). • Target organs include bone marrow (major toxicity, but reversible), tongue, liver, and testis. • Inflammatory changes were seen in the tongue, heart, liver parenchyma. • Exposure was greater than dose proportional, accumulative, and higher in females than males.
Dog/Beagle (Study No. BHR00008)	Oral capsule Once daily for 4 weeks (4 week recovery)	Main study: 7/sex/dose	CAL-101 0[a], 2.5, 5, 20 (0, 50, 100, 400)	• There was one death at 20 mg/kg/day on day 29. Primary toxicities were to the GI, liver, and lymphoid tissues. • Bone marrow toxicities were also evident in hematology findings. • There were CAL-101 findings relating to the eye (largely eye discharges); the relationship to the treatment is unclear.
Rat/Sprague-Dawley (Crl) (Study No. TX-312-2001)	Oral gavage Once daily for 13 weeks (4 week recovery)	Main study: 15/sex/dose TK: Controls: 3/sex/dose GS-1101: 6/sex/dose	GS-1101 0[b], 25, 50, 90 (0, 150, 300, 540)	• Statistically significant increase (14%-15%) in the absolute weights in the heart at 90 mg/kg/day compared to controls. There was a slight increase in the incidence and severity of cardiomyopathy in the heart in males at 90 mg/kg/day and in females at all dose levels compared to controls. • Organs of toxicity: Heart, pancreas, tongue, and testes.
Rat/Sprague-Dawley (Crl) (Study No. TX-312-2005)	Oral gavage Once daily for 26 weeks (12 week recovery)	Main study: 15/sex/dose TK: Controls: 3/sex/dose GS-1101: 9/sex/dose	GS-1101 0[b], 25, 50, 90 (0, 150, 300, 540)	• There were five mortalities, four of which are likely drug-related. One mortality at the low dose was due to malignant lymphoma, which may be incidental. • Common microscopic findings in animals found dead were decreased lymphocytes in the spleen, hemorrhage of the thymus, and dilatation of the urinary bladder. Other microscopic findings in the 90 mg/kg/day males found dead were cardiomyopathy, hypertrophy of the adrenal cortex, inflammation of the pancreas, and alveolar macrophages in the lungs. • At all doses, there were increases in white blood cells (lymphocytes, neutrophils, monocytes, and basophils). • Organs of toxicity: hematopoietic system, reproductive organs, kidneys, heart, liver, lungs, and pancreas. Axonal degeneration in the peripheral nerves and hemorrhage in the brain of one male were also observed at low incidence in males at 90 mg/kg/day. • GS-1101 was readily absorbed, accumulated with repeat-dosing, and exposures (AUC and C_{max}) were dose-dependent with greater than 2-fold higher

Species (Study #)	Route Duration	N/sex/dose	mg/kg (mg/m^2)	Significant findings
				exposures in females. The toxicokinetic profile of the metabolite GS-563117 was similar to the parent GS-1101, despite the fact that the parent compound was not readily converted to its metabolite in rats.
Dog/Beagle (Study No. TX-312-2005)	Oral capsule Once daily for 39 weeks (12 week recovery)	Main study: 6/sex/dose	GS-1101 0[c], 2.5, 5, 7.5 (0, 50, 100, 150)	• Two unscheduled deaths related to GS-1101. The cause of death for these 2 animals was systemic inflammation in multiple organs and decreased lymphocytes in peripheral lymphoid tissues. • Target organs of toxicity were the immune system (decreased lymphocytes and inflammation) and gastro-intestinal tract (red areas, inflammatory changes, and crypt dilatation). • Exposure to GS-1101 generally increased with the increase in dosage level from 2.5 to 7.5 mg/kg/day.
Impurities/Rat/ Sprague-Dawley (Crl) (Study No. TX-312-2012)	Oral Gavage for 28 days	Main study: 10/sex/dose	(b) (4) 0 , 90 (0, 540) (b) (4) 0 , 25 , 90 (0, 150, 540) See Table 82 for % impurities	• Targets include organs of the lymphatic system and digestive tract, eyes, heart, organs of the exocrine system (including the kidneys), and testis. • There were no additional toxicities related to levels of impurities. Toxicological findings between the lots with high (b) (4) (b) (4)) and lower (b) (4) impurities were similar; findings were also similar to the 28-day and 6-month rat studies.

a = 0.5% high viscosity carboxymethylcellulose/0.1% TWEEN 80 in water
b = lactose monohydrate in gelatin capsule
c = empty capsules
d = spiked impurity lot

Table 91: Tabulated Summary of Reproductive and Developmental Toxicology Studies

Fertility - Males	
Study #	(b) (4)-604019
Title	Evaluation of Orally (by Gavage) Administered GS-1101 on Fertility in Male Rats
Methods	Males dosed daily for 14 weeks (10 weeks before cohabitation and 4 weeks during cohabitation)
Key Study Findings	Dose related decreased epididymis, cauda epididymis, and testes weights and decreased epididymal sperm concentration in all GS-1101 dose groups with no test article-related effects on male reproductive function. There were no apparent adverse effects in females that were mated with treated male rats in regard to endpoints measured.
Species	Rat/ Sprague-Dawley (Crl)
Doses	0, 25, 50, or 100 mg/kg/day (0, 150, 300, 600 mg/m^2/day)

Mortality/ Clinical Signs	• Two males at 25 mg/kg/day and one male at 50 mg/kg/day were found dead or euthanized in extremis during the study. • One male at 25 mg/kg had significant loss of body weight (41 g) occurred during study days 28-31 and gasping was noted on the day of euthanasia; red material around the eyes and nose and malaligned upper incisors on study days 30-33. • Male at 50 mg/kg had several incidences of red material and lacrimation around the eyes and broken and malaligned upper incisors during study days 30-91 at the daily examinations.
BW/FC	Unremarkable
Fertility - Females	
Study #	(b) (4)-604028
Title	Evaluation of Orally (by Gavage) Administered GS-1101 on Fertility and Early Embryonic Development to Implantation in Female Rats.
Methods	Females were dosed daily for 14 days before cohabitation; treatment continued through gestation day 7.
Key Study Findings	• One female dosed at 100 mg/kg had a totally resorbed litter. • An increase in post-implantation loss, and early embryolethality at 100 mg/kg, resulting in a 20% decrease in the number of live embryos was observed. • No test article-related effects on female reproductive function .
Species	Rat/ Sprague-Dawley (Crl)
Doses	0, 25, 50, or 100 mg/kg/day (0, 150, 300, 600 mg/m^2/day)
Mortality/ Clinical Signs	None
BW/FC	Decreases in BW at 100 mg/kg and unremarkable FC.
Embryonic Fetal Development	
Study #	TX-312-2008
Title	An Oral (Gavage) Embryo/Fetal Development Study of GS-1101 in Rats
Methods	Females dosed daily from GD 6-17 and euthanized on GD 20
Key Study Findings	• There was one test-article related maternal death at the high dose of 150 mg/kg/day. • Clinical signs of urogenital blood loss correlated with complete resorptions, increased post-implantation loss, and decreased mean litter size in the 150 mg/kg/day group. • At doses ≥ 75 mg/kg/day, the net reduction in the maternal body weight gain was >

	10% compared to controls, and the presence of external malformations and skeletal variations in fetuses were observed. • AUC_{0-t} for GS-1101 increased greater than dose proportionally and there was accumulation with repeated dosing.
Species	Rat/ Sprague-Dawley (Crl)
Doses	0, 25, 75, and 150 mg/kg/day (0, 150, 450, 900 mg/m^2/day)
Mortality/ Clinical Signs	One female in the 150 mg/kg/day group was found dead on GD 20. Clinical findings for this female consisted of red material on the urogenital area (beginning on gestation day 12), red vaginal discharge (on gestation day 15), and red material on the ventral abdominal/thoracic areas and cage papers, and body and limbs that were pale and cool on day prior to death.
BW/FC	Decreases in net body weight gain > 10% at doses ≥ 75 mg/kg/day

Table 92: Cross-Species Comparison of Metabolite Exposure and Routes of Elimination

Human Metabolites	Matrices Where Detected		
	Human	Rat	Dog
Study Number	GS-US-313-0111	AD-312-2004	AD-312-2002
GS-563117	P 62% F 44% U 49%	P 1.4% F 9.38%* U 0.477%	P 34% F 33.4% U 0.332%

P = Plasma; F = Feces ; U = Urine
* specified in study report as GS-563117/ structure not determined (M30B)

12 Appendix/Attachments

Studies that were not reviewed:

Primary Pharmacology

Study#	Title	Module
DR-4001	The Effect of IC489666 on Activities of Cells of Hematopoietic Origin	4.2.1.1
DR-4002	PI3K P110δ Pathway is Critical for the Survival of Chronic Lymphocytic Leukemia B Cells	4.2.1.1
PC-312-2009	The Effect of GS-1101 on Anti-FcεRI and fMLP-induced Basophil Activation in Human Whole Blood	4.2.1.1
PC-312-2012	Evaluation of GS-563117 for Inhibition of Class I PI3K Isoforms in a Biochemical Assay	4.2.1.1

Secondary Pharmacology

Study#	Title	Module
1042207	Lead Profiling Screen of IDELA	4.2.1.2

Calistoga101	Evaluation of CAL-101 in Rat Derived Bone Marrow Cultures	4.2.1.2
DR-4024	Evaluation of CAL-101 in Human Hematopoietic Progenitor Cell Assays	4.2.1.2
IC0004-IC0005	Kinase Interaction Profile of IDELA	4.2.1.2

Pharmacodynamic Drug Interactions

Study #	Title	Module
	No Studies Submitted	4.2.1.4

Pharmacokinetics

Study #	Title	Module
Analytical methods and validation reports		
BA-312-2003	Abbreviated Validation of a Method for the Determination of GS-1101 and GS-563117 in Rat Plasma by HPLC with MS/MS Detection	4.2.2.1
BA-312-2004	Abbreviated Validation of a Method for the Determination of GS-1101 and GS-563117 in Dog Plasma by HPLC with MS/MS Detection	4.2.2.1
BA-312-2005	Abbreviated Validation of a Method for the Determination of GS-1101 and GS-563117 in Rabbit Plasma by HPLC with MS/MS Detection	4.2.2.1
BHR00016LX	Validation of a High Performance Liquid Chromatographic–Mass Spectrometric Method for the Analysis of CAL-101 in K2 EDTA Rat Plasma	4.2.2.1
BHR00019LX	Validation of a High Performance Liquid Chromatographic–Mass Spectrometric Method for the Analysis of CAL-101 in K2 EDTA Dog Plasma	4.2.2.1
Absorption		
400571	Determination of the Apparent Permeability Coefficients for CAL-101 and its Interaction with P-Glycoprotein in Caco-2 Monolayers	4.2.2.2
794315	*In Vivo* Rat PK - Pharmacokinetics of CAL-101 in Rats with High PO Dose (150, 500, and 2000 mg/kg)	4.2.2.2
AD-312-2007	Pharmacokinetics of GS-1101 and GS-563117 in Cynomolgus Monkeys	4.2.2.2
AD-312-2013	Pharmacokinetics of Idelalisib in Male Beagle Dogs	4.2.2.2
Distribution		
1503-059	Pharmacokinetics and Tissue Distribution of Radioactivity in Male and Female Naïve Dogs Following a Multiple Oral Dose of 14C-CAL-101	4.2.2.3
AD-312-2014	*In Vitro* Assessment of Blood Distribution of Idelalisib and GS-563117	4.2.2.3
Metabolism		
AD-312-2015	*In Vitro* Metabolism of Idelalisib in Hepatic Microsomal Fractions and Hepatocytes from Rat, Dog, and Human	4.2.2.4
AD-312-2023	Hepatic Metabolism of Idelalisib *In Vitro*	4.2.2.4
XT070003	Metabolite Characterization of 14C-CAL-101 in Human Hepatocytes	4.2.2.4
XT070008	Metabolite Characterization of 14C-CAL-101 in Dog Hepatocytes	4.2.2.4
Excretion		
AD-312-2001	Pharmacokinetics, Absorption, and Excretion of 14C-GS-	4.2.2.5

	1101 Following Oral Administration to Intact and Bile Duct-Cannulated Dogs	
Pharmacokinetic Drug Interactions		
13558	ADME-Tox: CYP Inhibition Study of CAL-101	4.2.2.6
13567	ADME-Tox: Time-Dependent CYP3A Inhibition Study of CAL-101	4.2.2.6
400571	Determination of the Apparent Permeability Coefficients for CAL-101 and its Interaction with P-Glycoprotein in Caco-2 Monolayers	4.2.2.6
794306	DME-Tox: CYP Phenotyping Study of CAL-101	4.2.2.6
AD-312-2005	*In Vitro* Assessment of GS-563117 Inhibition of Human OATP1B1, OATP1B3, Pgp and BCRP	4.2.2.6
AD-312-2006	Bi-Directional Permeability of GS-563117 Through Monolayers of P-Glycoprotein and Breast Cancer Resistance Protein Over-Expressing Cells	4.2.2.6
AD-312-2008	Evaluation of Induction Potential of GS-1101 and GS-563117 in Cultured Human Hepatocytes	4.2.2.6
AD-312-2010	*In Vitro* Assessment of GS-563117 as a Substrate for Human OATP1B1 and OATP1B3	4.2.2.6
AD-312-2011	*In Vitro* Assessment of GS-1101 Inhibition of Human OATP1B1, OATP1B3, and BCRP	4.2.2.6
AD-312-2012	*In Vitro* Inhibition Studies of GS-563117 with Renal Transporters OAT1, OAT3 and OCT2	4.2.2.6
AD-312-2016	Assessment of Human CYP3A Mechanism-Based Inhibition Potential of GS-563117 *In Vitro*	4.2.2.6
AD-312-2017	*In Vitro* Assessment of Human UGT1A1 Inhibition Potential of Idelalisib and GS-563117	4.2.2.6
AD-312-2018	*In Vitro* Assessment of Human CYP2B6 Inhibition Potential of Idelalisib	4.2.2.6
AD-312-2019	*In Vitro* Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-563117	4.2.2.6
AD-312-2022	UDP-Glucuronosyl Transferase Phenotyping of GS-1101	4.2.2.6
AD-312-2023	Hepatic Metabolism of Idelalisib *In Vitro*	4.2.2.6
AD-312-2024	*In Vitro* Assessment of Human CYP3A Mechanism-Based Inhibition Potential of Idelalisib with Midazolam 1'-Hydroxylase as the Probe Activity	4.2.2.6
CAS-2010-001	CAL-101 Studies on UDP-Glucuronosyltransferase (UGT) Reaction Phenotyping Based on Recombinant UGT1A4 and Extrahepatic UGT Pathway Investigation Using Human Kidney Microsomes	4.2.2.6
OPT-2010-087	Assessment of CAL-101 as an Inhibitor of Human BCRP, OAT1, OAT3, OCT2, OATP1B1, or OATP1B3 Mediated Transport	4.2.2.6
OPT-2010-124	Assessment of CAL-101 as a Substrate of Human P-gp, BCRP, OAT1, OAT3, OCT2, OATP1B1, and OATP1B3-Mediated Transport	4.2.2.6

Single-Dose Toxicology

Study#	Title	Module
04-3166-N1	A Pilot Dose-Range Finding Toxicity Study of IC489666 Administered Orally to the Beagle Dog	4.2.3.1
03-0004-42	Acute Toxicity Study of P110 Delta Inhibitors IC489666 and IC490098 Administered by Oral Gavage in Sprague Dawley Rats	4.2.3.1

Repeat-Dose Toxicology

Study#	Title	Module
TX-312-2002	GS-1101: A 13-Week Oral (Capsule) Toxicity Study in Dogs with a 4-Week Recovery Period	4.2.3.2
04-3166-N2	A Fourteen-Day Repeat Dose Toxicity Study of IC489666 Administered Orally to the Beagle Dog	4.2.3.2
TX-312-2009	An Oral Gavage 7-Day Tolerability Study of GS-1101 in Non-Pregnant New Zealand White Rabbits	4.2.3.2
04-R007-33	14-Day Repeat Dose Oral Toxicity Study of IC489666 in the Rat	4.2.3.2

Local Tolerance

Study#	Title	Module
9152-100254	*In Vitro* Assessment of Phototoxicity of CAL-101 and CAL-244 Using BALB/c 3T3 Cell Line	4.2.3.6

Special Toxicology

Study#	Title	Module
590089	An Assessment of the Ability of IC489666 to Interfere with the Progression of a Staphylococcus Aureus Infection in a Rat Groin Abscess Model	4.2.3.7.2
IDELA-KLH-DNP	The Effect of p110-delta Specific Inhibitor IC489666 on the Antibody Response Against Dinitrophenyl (DNP) in Rats	4.2.3.7.2
IDELA-SRBC	The Effect of p110-delta Specific Inhibitors IC489666 and IC486924 on the Antibody Response Against Sheep Red Blood Cells (SRBC) in Rats	4.2.3.7.2
TX-312-2012	A 28-Day Oral Gavage Impurity Qualification Study of GS-1101 in the Sprague Dawley Rat	4.2.3.7.6
TX-312-2013	GS-567201, GS-606710, and GS-606709 Salmonella-E.Coli/Mammalian Microsome Reverse Mutation Assay	4.2.3.7.6
09N-U12-RR	Evaluation of Bone Marrow Recovery After Cyclophosphamide Administration and Impact of Oral Administration of CAL-101 in Male Sprague Dawley Rats	4.2.3.7.7
PC-312-2001	CAL-101: A Mechanistic Evaluation in Female Beagle Dogs on Inflammatory Effect in the Liver	4.2.3.7.7
PC-312-2005	GS-1101: A Mechanistic Evaluation in Female Beagle Dogs on Transaminase Elevation in the Intestine, Lymphoid Tissues, and Liver	4.2.3.7.7

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/s/

NATALIE E SIMPSON
04/03/2014

RAMADEVI GUDI
04/03/2014

HALEH SABER
04/03/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA Number: 205858 **Applicant: Gilead Sciences Inc.** **Stamp Date: Sept. 11, 2013**

Drug Name: Idelalisib (GS-1101) **NDA Type: 505(b)(1); new molecular entity**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	✓		NDA is submitted in the eCTD format.
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	✓		Electronic submission
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	✓		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	✓		All studies needed for this indication have been conducted. In addition, other studies (for example: fertility studies) have been conducted.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	✓		Oral formulations were used in clinical and nonclinical studies.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	✓		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	✓		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	✓		

File name: 9_Pharmacology_Toxicology Filing Checklist for NDA 205858

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	✓		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	✓		The Applicant has provided a justification (e.g. based on nonclinical studies) for the proposed specifications and the acceptability of the levels will be a review issue.
11	Has the applicant addressed any abuse potential issues in the submission?			Not Applicable
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not Applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? ____Yes____

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

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

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

File name: 9_Pharmacology_Toxicology Filing Checklist for NDA 205858

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/s/

NATALIE E SIMPSON
09/27/2013

HALEH SABER
09/27/2013