CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

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

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Applicant:	Pfizer, Inc
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
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TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Glasdegib (DAURISMO) is a new molecular entity antagonist of smoothened (SMO), a transmembrane protein that activates the Hedgehog (Hh) signaling transduction pathway. (b) (4) There are other two approved drugs in this class. Glasdegib has not been approved outside the U.S.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical pharmacology, pharmacokinetic and toxicology studies have been submitted to support the approval of glasdegib for the proposed indication. Pharmacology studies provided evidence that glasdegib inhibits the transmembrane protein SMO and attenuates Hh signal transduction. In an in vitro competition binding assay with a radiolabeled human SMO chemical ligand, glasdegib showed an IC_{50} of 7.7 nM. Gli1 is one of three transcription factors in the Gli family that mediate Sonic Hedgehog (Shh)-induced pathway activation. Glasdegib showed an IC_{50} of 5.2 nM in a cell-based reporter gene assay used to measure the potency to inhibit SMO-induced Hh signaling via the Gli1 transcription factor. The upregulation of Gli1 mRNA levels were stimulated in normal human fibroblasts with a recombinant human Shh. Glasdegib inhibited the Shh-induced Gli1 levels at 40 nM assessed through quantitative PCR. In vivo, the combination of glasdegib and low dose cytarabine (LDAC) significantly improved the anti-tumor effect over LDAC alone in AML (AML-183) patient-derived xenotransplanted (PDX) models. The established pharmacological class for glasdegib is a hedgehog pathway inhibitor.

Glasdegib was evaluated for its off-target activity across a panel of more than 50 receptors, enzymes, ion channels, and transporters. Glasdegib only demonstrated activity at potentially clinically relevant concentrations for human adenosine A1, histamine H1 and sodium channel site 2 (Nav 1.5).

In safety pharmacology studies in rats, glasdegib did not adversely affect Irwin screen or pulmonary parameters. Glasdegib had effects on grip strength and locomotor activity that occurred at toxic (≥50 mg/kg/day) doses in rats, which may be secondary to animal health issues that occurred in the 26-week rat study. In in vitro studies, glasdegib produced a concentration-dependent inhibition of the potassium current in a hERG channel stably expressed in human embryonic kidney (HEK-293) cells with an IC_{50} of 2.8 µM (1049 ng/mL), which is approximately 9-fold over the human clinical exposure at the recommended dose. Glasdegib also produced a concentration-dependent inhibition of the sodium channel (Nav1.5) current in a functional patch clamp study with an IC_{50} of 32.2 nM. Glasdegib increased heart rate, and QT, QTc and QRS intervals in the dog cardiovascular (CV) study. These findings were consistent with results from the in vitro studies with hERG and Nav1.5 channels. Glasdegib systemic exposures in the dog CV study were approximately 0.9 to 3.5-fold the clinically relevant exposure at the

recommended dose of 100 mg daily. A QTc prolongation effect of glasdegib (single dose of 150 mg and 300 mg) was also detected in trial B1371023 and the effect was dose/concentration-dependent.

In general, oral bioavailability was higher in fasted rats (35%) than in fed rats (19%), and glasdegib was 68% bioavailable in dogs. Glasdegib was bound to plasma proteins (86% to 98%) in rat, dog and human samples, and binding was independent of drug concentration. Glasdegib was distributed throughout the body with the highest concentration in the uveal tract, liver, kidney and renal structures, adrenal gland, and pancreas but had minimal distribution across the blood brain barrier.

Oxidative metabolite profiles were qualitatively similar in all species. One primary and two secondary N-glucuronide metabolites were identified in human, but not rat or dog, plasma and urine. Overall, the major metabolic pathways of glasdegib were oxidation (N-demethylation, hydroxylation, and dehydrogenation), primary glucuronidation, and secondary N-glucuronidation of oxidative metabolites. The average elimination half-life was 1.3 hours in rats and 2.3 hours in dogs. There were no apparent gender-related differences in exposure in both species. Accumulation of glasdegib after repeated exposure was evident in rats (≥2x) but it did not occur in dogs.

Repeat-dose toxicology studies were conducted of 1-month (M), 13-week (W) and 26-W in rats with doses up to 100 mg/kg/day, and 1-M, 13-W and 39-W in dogs with doses up to 30 mg/kg/day. The 1-M toxicology studies in rats and dogs were used to support the FIH glasdegib dose of 5 mg/day. Doses for the toxicity studies of 26-W in rats and 39-W in dogs were selected based on the results of the 13-W toxicology studies in each species. Body weights and food consumption were decreased in rats and dogs treated with glasdegib. In both species, the major target organs of toxicity were the kidney, liver, skin, and bone. Species specific target organs included testis and peripheral nerve in the rat, and the adrenal, gastrointestinal tract, lymph nodes, and lung in dogs. Clinical pathology effects corresponded to the target organ toxicities. The primary toxicity was azotemia [abnormally high levels of nitrogen-containing compounds (such as urea, creatinine, and nitrogenous waste) in the blood] with subsequent damage to the kidneys (e.g., necrosis, mineralization, and evidence of inflammation in the renal tubules). Azotemia lead to the moribund sacrifice of rats and dogs.

In rats, glasdegib caused bone and teeth effects that are characteristic of SMO inhibitors, such as epiphysis alterations (decrease in number of chondrocytes and disorganization of chondrocytes) and decreased medullary trabeculae of femur and sternum bone sections with marrow hypercellularity. Teeth abnormalities consisted of missing or broken teeth (incisors) with histopathological atrophy leading to decreased food consumption and body weight loss. The adverse effect of glasdegib on teeth was severe enough to warrant the early sacrifice of some rats in the 13-W and 26-W toxicity studies. The effects on bones and teeth may have little significance for the intended adult patient population but they are of potential relevance for pediatric use of DAURISMO. Additional findings included alopecia and muscle tremors/twitching. These

effects occurred in both rats and dogs at high doses with overt toxicity and/or moribund sacrificed animals.

Glasdegib-related gastrointestinal tract toxicities in dogs included hemorrhage in the gut-associated lymphoid tissue (GALT)/Peyer's patch, ectasia in the ileum, and congestion in the jejunum. Liver toxicity in both rats and dogs consisted of elevated cholesterol, increased alanine aminotransferase (ALT), and decreased alkaline phosphatase (ALP) with microscopic findings of hypertrophy, inflammation, single cell necrosis and pigment in macrophage and Kupffer cells (iron). In dogs, administration of glasdegib also resulted in adrenal vacuolation with corresponding increased organ weight ratio. Alterations of serum chemistry parameters occurred in early decedents in the 39-W study in dogs.

Glasdegib was not mutagenic in vitro in the bacterial reverse mutation assay (Ames test) and was not clastogenic or aneugenic in an in vitro human chromosome aberration assay or in vivo rat bone marrow micronucleus test.

The potential for glasdegib or its metabolites to be excreted in the breast milk of nonclinical species has not been evaluated. Women will be advised to not breastfeed during treatment with DAURISMO and for at least 30 days after the last dose. Toxicity in the testis in rats at ≥50 mg/kg/day included degeneration of seminiferous tubules and hypospermatogenesis which showed lack of recovery after cessation of dosing. This finding was included in the DAURISMO label. Preliminary embryofetal development studies were conducted in rats and rabbits at doses up to 100 mg/kg/day. Glasdegib induced embryofetal lethality in rats and rabbits at approximately 0.6-times and 2.5-times, respectively, the human exposure at the recommended dose based on total C_{max}. Glasdegib was clearly teratogenic in rats at ≥10 mg/kg/day and in rabbits at ≥5 mg/kg/day. A warning for embryo-fetal risk was recommended for DAURISMO and a black box warning for embryo-fetal toxicity is included in the label. Females and males of reproductive potential will be advised to avoid pregnancy and to use effective contraception during treatment with DAURISMO and for at least 30 days after the last dose.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, approval for glasdegib (DAURISMO) is recommended.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

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

2 Drug Information

2.1 Drug

CAS Registry Number
2030410-25-2

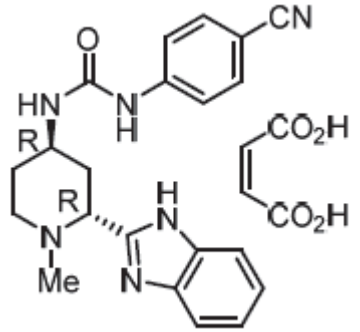
Generic Name (INN; USAN)
Glasdegib
Code Name
PF-04449913-11

Chemical Name
CAS: N-[(2R,4R)-2-(1H-benzimidazol-2-yl)-1-methyl-4-piperidinyl]-N'-(4-cyanophenyl)-urea, (2Z)-2-butenedioic acid (1:1)
IUPAC: 1-((2R,4R)-2-(1H-benzo[d]imidazol-2-yl)-1-methylpiperidin-4-yl)-3-(4-cyanophenyl)urea maleate

Molecular Formula
$C_{25}H_{26}N_6O_5$
$C_{21}H_{22}N_6O$ (drug) $C_4H_4O_4$ (maleate)

Molecular Weight
374.5 Daltons (free drug)
490.51 Daltons (as the maleate)

Structure or Biochemical Description



Chiral centers are indicated in the structure. Glasdegib has two asymmetric centers, giving four possible stereoisomers. The absolute configuration is 2R, 4R.

Pharmacologic Class
Hedgehog pathway inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 105453

2.3 Drug Formulation

Glasdegib maleate (DAURISMO) is formulated as film-coated tablets provided at 25 and 100 mg strengths. The 25 mg tablets are presented as round, yellow film-coated tablets debossed with "Pfizer" on one side and "GLS" over "25" on the other side. The 100 mg tablets are presented as round, pale orange film-coated tablets debossed with "Pfizer" on one side and "GLS" over "100" on the other side. The drug product is packaged in (b) (4) high density polyethylene bottles. The detail composition of glasdegib maleate 25 and 100 mg film-coated tablets are provided in the following tables.

Table 1 Composition of DAURISMO 25 mg Film-Coated Tablet

(Excerpted from Submission)

Name of Ingredients	Reference to Standards	Function	Unit Formula (mg/tablet)
Glasdegib maleate	Pfizer	Active Ingredient	(b) (4)
Microcrystalline cellulose	NF/Ph.Eur./JP		
Dibasic calcium phosphate anhydrous	USP/Ph.Eur./JP		
Sodium starch glycolate (b) (4)	NF/Ph.Eur./JP		
Magnesium stearate (b) (4) (b) (4)	NF/Ph.Eur./JP		
(b) (4)			
Opadry® II yellow (33G120011)	Pfizer		
(b) (4)			
			(b) (4)

Note: NF = National Formulary; USP = United States Pharmacopeia; Ph.Eur. = European Pharmacopoeia, JP = Japanese Pharmacopoeia

Table 2 Composition of DAURISMO 100 mg Film-Coated Tablet

(Excerpted from Submission)

Name of Ingredients	Reference to Standards	Function	Unit Formula (mg/tablet)
Glasdegib maleate	Pfizer	Active Ingredient	(b) (4)
Microcrystalline cellulose	NF/Ph.Eur./JP		(b) (4)
Dibasic calcium phosphate anhydrous	USP/Ph.Eur./JP		
Sodium starch glycolate (b) (4)	NF/Ph.Eur./JP		
Magnesium stearate (b) (4) (b) (4)	NF/Ph.Eur./JP		
(b) (4)			
Opadry® II beige (33G170003)	Pfizer		
(b) (4)			

Note: NF = National Formulary; USP = United States Pharmacopeia; Ph.Eur. = European Pharmacopoeia, JP = Japanese Pharmacopoeia

Table 3 Composition of Opadry® II Yellow (33G120011)

(Excerpted from Submission)

Name of Ingredients	Reference to Standards	Unit Formula (% w/w)
		(b) (4)

Table 4 Composition of Opadry® II Beige (33G170003)

(Excerpted from Submission)

Name of Ingredients	Reference to Standards	Unit Formula (% w/w)
		(b) (4)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

All impurities were either within acceptable limits according to ICHQ3A and ICHQ3B or qualified in nonclinical studies.

2.6 Proposed Clinical Population and Dosing Regimen

(b) (4)

The recommended dose and schedule of glasdegib (DAURISMO) is 100 mg, taken orally once per day. The summary of PK evaluations for 100 mg QD alone (Cycle 1 Day 21) and in combination with LDAC (Cycle 1 Day 10) tested in patients with previously untreated AML or high-risk MDS is provided in the following table as reference for exposure margins with pharmacology and animal TK data.

Table 5 Summary of Glasdegib Plasma Pharmacokinetic Parameters (Dose Compliant) - Phase 1b Arm A (Study B1371003)

(Excerpted from Submission)

Parameters (Units)	Cycle 1 Day 10 (N = 13)[a] Glasdegib 100 mg +LDAC	Cycle 1 Day 21 (N = 8)[b] Glasdegib 100 mg
C_{max} (ng/mL)	1074 (63)	1242 (56)
T_{max} (hr)	1.8 (0.75-24)	1.3 (0.53-2.0)
AUC_{tau} (ng•hr/mL)[c]	15020 (49)	16660 (43)
AUC_{last} (ng•hr/mL)	13320 (71)	16670 (43)
C_{avg} (ng/mL)	554 (71)	695 (43)
C_{trough} (ng/mL)	270 (73)	375 (55)

Source: Study B1371003 CSR Table 32.
Pharmacokinetic parameters are defined in Table 3.
Geometric mean (geometric %CV) for all except: median (range) for T_{max}.
%CV = percent coefficient of variation; LDAC = low-dose cytarabine; N = number of patients in each group; n = number of patients evaluable for the indicated PK parameters.
a. On Cycle 1/Day 10, n = 10 for AUC_{tau}. n = N otherwise.
b. On Cycle 1/Day 21, n = 7 for C_{trough}. n = N otherwise.
c. For AUC_{tau}, tau = 24 hours.

2.7 Regulatory Background

Glasdegib (code name PF-04449913-11) was investigated under IND 105453 for the treatment of (b) (4) AML. (b) (4)

3 Studies Submitted

3.1 Studies Reviewed

Study No.	Title
Primary and Secondary Pharmacology	

Study No.	Title
191616	Pharmacology Summary for PF-04449913 in Nonclinical Cancer Models In Vitro and In Vivo
075357	Anti-Tumor Efficacy of Glasdegib (PF-04449913) in Combination with Chemotherapy in AML PDX Models
7570637	In Vitro Pharmacology: Pfizer Tier 0 Profile
7571398	In Vitro Pharmacology and ADME-Tox: Pfizer Tier 1 Profile
Safety Pharmacology	
PF04449913NA15	Effect of PF-04449913 on Nav1.5 Sodium Current Stably Expressed in HEK293 Cells
PF4449913/ESD/ 1108/HERG	Effects of PF-04449913-01 on HERG Potassium Channels Stably Expressed in HEK.293 cells
08SN228	Neurofunctional Assessment of PF-04449913 in Male Rats
08SN229	Pulmonary assessment of PF-04449913 in male rats
08SN238	Cardiovascular assessment of PF-04449913 in Beagle dogs
Pharmacokinetics	
PF-04449913/ 14Nov08/143627	Single Dose Pharmacokinetics and Oral Bioavailability of PF-04449913 (b) (4) in Sprague-Dawley Rats Following Intravenous and Oral Administration
PF-04449913/ 14Nov08/143413	Single Dose Pharmacokinetics and Oral Bioavailability of PF-04449913 (b) (4) in Beagle Dogs Following Intravenous and Oral Administration
PF-04449913/ 17Nov08/142017	Protein Binding of PF-04449913 in Rat, Dog, Mouse, and Human Plasma
PF-04449913 08Dec15-022737	Protein Binding of PF-04449913 in Rabbit Plasma
PF-04449913 23Feb16-104614	Protein Binding of PF-04449913 in Human Serum Albumin (HSA), and α1- Acid Glycoprotein (AAG)
PF-04449913/ 12Nov08/104333	Red Blood Cell to Plasma Partitioning of PF-04449913 in Human, Rat, and Dog Whole Blood
PF-04449913 08Dec15-031858	Red Blood Cell to Plasma Partitioning of PF-04449913 in Rabbit Whole Blood
Toxicology	
6348-650 (08LJ094)	1-Month Oral Toxicity Study of PF-04449913 in Rats with 1-Month Recovery with Micronucleus Assessment
8299121 (14LJ052)	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04449913 in Rats
8303065 (14LJ108)	26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with PF-04449913 in Rats with a 13-Week Recovery Phase
6348-651 (08LJ093)	1-Month Oral Toxicity and Toxicokinetic Study of PF-04449913 in Dogs with 1-Month Recovery
8299265 (14LJ055)	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04449913 in Dogs
8303066 (14LJ109)	39-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with PF-04449913 in Dogs with a 16-Week Recovery Phase
6348-654 (08GR352)	Bacterial Reverse Mutation Assay with a Confirmatory Assay
09GR016	Genetic Toxicology Human Lymphocyte Assay of PF-04449913-01 (Clastogenicity Assay)
20069225 (15GR056)	A Preliminary Embryo-Fetal Development Study of PF-04449913 by Oral Gavage in Rats
20069230 (15GR057)	A Preliminary Embryo-Fetal Development Study of PF-04449913 by Oral Gavage in Rabbits

3.2 Studies Not Reviewed

Study No.	Title
Pharmacology	
121934	Glasdegib (PF-04449913) Expanded Human Kinome Biochemical Selectivity
8850718	In Vitro Pharmacology - Study of PF-04449913-01
SP1008	Cell-based investigation into the functional activity of PF-04449913 at the μ opioid receptor using the DiscoveRx technology
17GR212	Assessment of the Activity of PF-04449913 on the Peak Nav1.5 Sodium Current
17GR318	Summary of the Isolated Tissue Studies with the Smo inhibitor PF-04449913
Safety Pharmacology	
16GR392	Effect of PF-04449913 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
Pharmacokinetics	
6348-659	Method Validation for PF-04449913 Rat Plasma using LC/MS/MS
8361925	Validation of a Method for the Determination of PF-04449913 in Long Evans Rat Plasma by HPLC with MS/MS Detection
8325312	Validation of a Method for the Determination of PF-04449913 in New Zealand White Rabbit Plasma by HPLC with MS/MS Detection
6348-660	Method Validation for PF-04449913 Dog Plasma using LC/MS/MS
161811	Single Dose Pharmacokinetics and Excretion Evaluations of PF-04449913 in Sprague-Dawley Rats Following Intravenous and Oral Administration
8283508-144549	Quantitative Whole-Body Autoradiography of Male Rats Following Oral Administration of [^{14C}]PF-04449913
090256	Biotransformation of [^{14C}]PF-04449913 in Plasma and Excreta following Single Oral Administration to Rats
122431	Identification of Drug-Related Material Excreted in the Urine and Feces Following a Single 10 mg/kg Oral Dose of [^{14C}]PF-04449913 Administered to Male Long Evans Rats
090417	Biotransformation of [^{14C}]PF-04449913 in Plasma and Excreta following Single Oral Administration to Dogs
102952	Biotransformation of [^{14C}]PF-04449913 in Plasma and Excreta following Single Oral Administration to Healthy Male Human Volunteers
184200	Assessment of Circulating Metabolites of PF-04449913 in Human Plasma from Clinical Study Protocol B1371010
104634	Chiral Inversion Analysis of PF-04449913 in Human Plasma (Study Protocol B1371010)
153629	Investigation of the Glucuronide Conjugates of Glasdegib (PF-04449913) following Incubation with Human Liver Microsomes and Recombinant Human UDP-Glucuronosyltransferase (UGT)1A9 in Human Urine following Single Oral Administration
130612	Preliminary Assessment of the Biotransformation of PF-04449913
081555	Exploratory Reaction Phenotyping of PF-04449913 using Human Hepatocyte Relay Method
172724	In Vitro Cytochrome P450 Reaction phenotyping of PF-04449913
112959	Enzyme Kinetics and Identification of Cytochrome P450 Isoforms Involved in the In Vitro Metabolism of PF-04449913
050630	Enzyme Kinetics and Identification of UDP-Glucuronosyltransferase (UGT) Isoforms Involved in the In Vitro Metabolism of PF-04449913
163216	Preliminary In Vitro Recombinant UDP-Glucuronosyltransferase (UGT) Phenotyping of Glasdegib (PF-04449913)
8309182-084046	Determination of Routes of Excretion of [^{14C}]PF-04449913 in Sprague Dawley Rats After a Single Oral Dose
8309181-083752	Determination of Routes of Excretion of [^{14C}]PF-04449913 in Dogs After a Single Oral Dose

Study No.	Title
150827	In Vitro Assessment of Uptake and Biliary Excretion of Glasdegib using Sandwich Culture Human Hepatocytes (SCHH)
XT135086-115159	In Vitro Evaluation of PF-04449913 as an Inhibitor of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes
135232	An Investigation of the Potential for PF-04449913 to Induce CYP3A4 and CYP1A2 in Human Hepatocytes
XT133102-120525	In Vitro Investigation of the Potential for PF-04449913 and Rifampin to Induce Cytochrome P450 (CYP2B6 and CYP3A4) in Cultured Human Hepatocytes
152325	In Vitro Evaluation of PF-04449913 as an Inhibitor of UDP-Glucuronosyltransferase (UGT) Enzyme Activities in Human Liver Microsomes
131858	PF-04449913 Exploratory in Vitro PgP Substrate Profiling Determination in MDR-MDCK Cell Monolayers
101906	In Vitro Evaluation of PF-04449913 as a Substrate of BCRP (ABCG2) in Transfected MDCK Cells
1153561	In Vitro Renal Transporter Substrate of PF-04449913
154108	In Vitro Evaluation of Glasdegib (PF-04449913) as an Inhibitor of Major Drug Transporters
Toxicology	
09LJ058	7-Day Oral In Vivo Toleration Study of PF-05171171 and PF-04449913 in Rats
07GR162	In Vivo Toleration Study of PF-04449913 in Rats
08LJ052	7-Day Repeat Oral Dose In Vivo Toleration Study of PF-04449913 in Dogs
16GR159	Single Dose Intravenous and Perivascular Study of PF-04449913 in Female New Zealand White Rabbits
17GR041	1-Month Oral Toxicity Study of PF-04449913 in Sprague-Dawley Rats
16LJ129	A Multiple Dose Phototoxicity Study to Determine the Effects of Oral Gavage Administration of PF-04449913 on Eyes and Skin in Pigmented Rats
17GR182	1-Day In Vitro Hemolysis Compatibility Study of PF-04449913 with Human Blood

3.3 Previous Reviews Referenced

The Pharmacology/Toxicology and other discipline reviews for IND 105453.

4 Pharmacology

4.1 Primary Pharmacology

Study No. 191616: Pharmacology Summary for PF-04449913 in Nonclinical Cancer Models In Vitro and In Vivo.

A series of in vitro and in vivo assays were conducted to characterize glasdegib potency against SMO/Hh pathway signaling, including inhibition of Gli-1-associated endpoints. Table 6 shows a summary of data from the different assays described below.

Table 6 Summary of Key Pharmacological Properties of PF-04449913

(Excerpted from Submission)

In Vitro	
Inhibition of:	**Potency**
^{3}H-PF-03451358 interaction with SMO (181-787) (IC_{50})	7.7 nM
Shh Induced Gli-luciferase reporter in C3H10T1/2 (IC_{50})	5.2 nM
75% Inhibition of Shh-induced Gli-1 mRNA in Human Fibroblasts	40 nM
In Vivo	
Ptc+\-p53+\- medulloblastoma tumor growth inhibition (IC50)	73.2 nM[a] (6.8 nM free)
Gli-1 expression in tumor (IC50)	101.8 nM[a] (9.4 nM free)
Gli-1 expression in skin (IC50)	675.7 nM[a] (62.9 nM free)

IC_{50} = 50% inhibitive concentration; PF-03451358 = A cyclopamine-competitive SMO antagonist; Ptc = Patched; SMO = Smoothened; TGI = Tumor growth inhibition.
a. PF-04449913 molecular weight is = 374.4 Daltons; (X ng/mL/(374.4))*1000 = nM.

Competition Binding Assay

The ability of glasdegib to bind to the human SMO target was evaluated in a competition binding assay using a characterized cyclopamine-competitive SMO antagonist designated PF-03451358. This assay used membranes with surface expression of SMO 181-781 protein prepared from a stable cell line created in HEK293FlpIn-TetR cells (Invitrogen). The IC_{50} of glasdegib competing with the radiolabeled chemical ligand for binding to human SMO (amino acids 181-787) was 7.7 ± 7.2 nM (n = 3).

Gli Reporter Assay and the Shh-induced Gli-Luciferase Reporter Assay

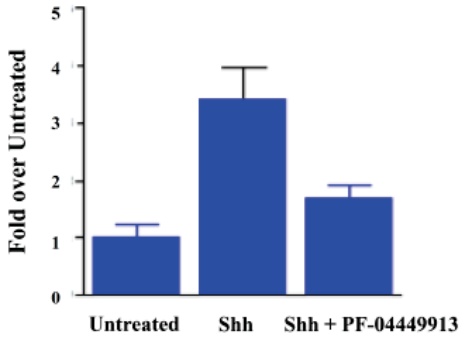
The Gli Reporter – cell line contains the firefly luciferase gene under the control of Gli responsive elements stably integrated into the cells. Luciferase expression correlates with activation of Hh signaling. To evaluate the functional effects of glasdegib on a ligand-stimulated Hh pathway, a mouse primary fibroblast cell line, C3H10T1/2, was used in transient transfection-based assays with a Gli responsive luciferase reporter. The IC_{50} of glasdegib inhibition of Shh-induced Gli reporter activity was 5.22 ± 1.65 nM (n = 12) under low serum (0.5% calf serum) conditions.

Inhibition of Shh Signaling in Normal Human Fibroblasts

Normal human fibroblasts were used to demonstrate the effects of glasdegib on an endogenous (non-engineered) Hh pathway signaling. Prepared Shh\PF-04449913 mix containing medium was added to cultured cells and incubated for 24 h prior to extraction and purification of Gli1 mRNA using a Qiagen RNA purification kit and detection with quantitative PCR. Shh stimulated the upregulation of Gli1 mRNA levels approximately three-fold in a 24-hour stimulation. Glasdegib at 40 nM inhibited the Shh-induced Gli1 levels assessed through quantitative PCR (See Figure 1).

Figure 1 Glasdegib Functional Effects in Normal Human Fibroblasts

(Excerpted from Submission)



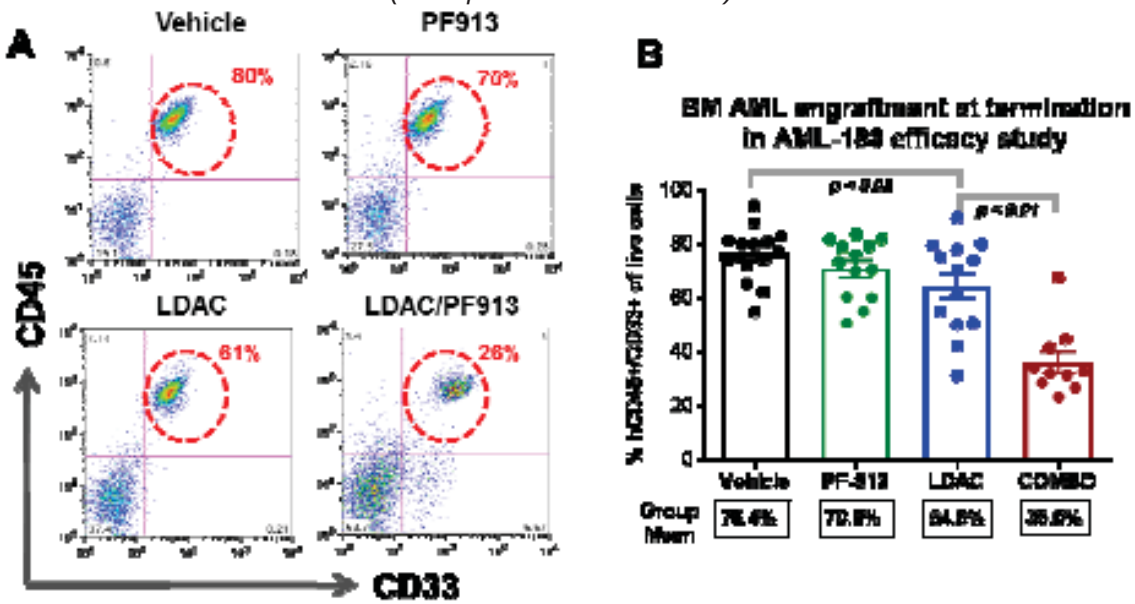
Inhibition of the endogenous Hedgehog signaling pathway in normal human lung fibroblasts (4 replicates per group). PF-04449913 inhibits Shh-induced Gli1 mRNA levels assessed through quantitative PCR. mRNA = Messenger ribonucleic acid; PCR = Polymerase chain reaction; Shh = Sonic Hedgehog.

Study No. 075357: Anti-Tumor Efficacy of Glasdegib (PF-04449913) in Combination with Chemotherapy in AML PDX Models.

The studies were performed with two of the commonly used standard of care chemotherapies in AML patients: LDAC or high dose induction chemotherapy (daunorubicin and cytarabine or DA). Glasdegib showed enhanced activity when combined with LDAC in one (AML-183) of the two tested AML PDX models. In another AML PDX model (BM2407), a combinatorial effect of glasdegib plus high dose induction chemotherapy was also observed.

Figure 2 The Combination of Glasdegib with LDAC in the AML-183 PDX Model

(Excerpted from Submission)



A. Representative image of CD45+/ CD33+ disease burden in BM following 56 days of treatment with vehicle, 100 mg/kg PO PF-913, 4 mg/kg SC LDAC, or the combination. **B.** Quantitative assessment and the mean values of the BM AML burden at study termination on day 56 post treatment. (N = 10 to 14 mice/group). AML = Acute myeloid leukemia; BM = Bone marrow; LDAC = Low dose cytarabine; PF913 = PF-04449913 = glasdegib; PO = oral; SC = Subcutaneous.

Glasdegib was tested in combination with LDAC in AML-183 xenotransplanted mice. The 4 mg/kg SC dose for cytarabine was selected based on exposure (AUC) in in non-obese SCID gamma mice. Mice that received oral glasdegib at 100 mg/kg exhibited minimal antileukemic activity (70.8% CD45+/CD33+ cells) compared to vehicle (76.4% CD45+/CD33+ cells), while LDAC treated mice had a significant decrease in CD45+/CD33+ cells compared to vehicle ($p < 0.05$). The combination of glasdegib and LDAC significantly (36.0% CD45+/CD33+; $p < 0.01$) improved the effect over LDAC alone (64.5% CD45+/CD33+ cells) (See Figure 2).

4.2 Secondary Pharmacology

Study No. 7570637: In Vitro Pharmacology: Pfizer Tier 0 Profile
Study No. 7571398: In Vitro Pharmacology and ADME-Tox: Pfizer Tier 1 Profile
These studies were conducted to investigate the effects of glasdegib in various in vitro receptor binding and enzyme assays screens at an initial concentration of 10 µM. Glasdegib demonstrated activity, defined by a response greater that 50% of a maximal response, against several targets (See Table 7).

Table 7 Summary of Glasdegib Off-Target Binding

Receptor	Assay type	Ki (nM)
human adenosine A1	Binding	370
human alpha 2a adrenoceptor	Binding	1900
human alpha 2c adrenoceptor	Binding	1600
human mu opioid	Binding	3500
human histamine H1	Binding	470
sodium channel site 2 (Nav 1.5)	Binding	470
Cell based functional assays	Result	Kb (nM)
human adenosine A1 receptor	Antagonist	550
mu opioid receptor	Antagonist	31600
histamine H1 receptor	Antagonist	40

The Applicant noted that all observed functional activities were at multiples greater than approximately 2-fold above the observed unbound C_{max} (300 nM or 113 ng/mL) at the 100 mg QD recommended clinical dose except for histamine H1 antagonism which was 7-fold lower. Centrally active histamine H1 antagonists are known to cause sedation in the clinic, and in studies in rats, have been shown to induce changes in locomotor activity. In the rat neurofunctional studies (Study 08SN228) no effect was observed on locomotion (up to 30 mg/kg), however, hypoactivity was observed in the 26-week repeat dose toxicity study in rats at doses with adverse effects (≥50 mg/kg/day). In addition, glasdegib was shown to be an antagonist of the adenosine A1 receptor in both a functional cell-based assay (Kb 550 nM) and in a radioligand binding assay (Ki 370 nM). Given a potential role for adenosine A1 antagonism in promoting natriuresis and diuresis, inhibition of this receptor at higher glasdegib exposures in animals may be a contributing factor to the dehydration, electrolyte changes and weight loss observed in the rat and dog toxicity studies.

4.3 Safety Pharmacology

(Parts were excerpted from Dr. T. Kropp's review of IND 105453)

The Applicant conducted the required safety pharmacology studies with glasdegib. Glasdegib did not cause any adverse effects in an Irwin screen nor in a pulmonary study in rats but did increase HR, QT, QTc and QRS in ECG measurements in dog CV study, small decreases in QTc at the low dose (LD) in the 39-W dog study, and concentration-dependent inhibition of the Nav1.5 and hERG currents. Glasdegib systemic exposures in the dog CV study ranged 0.9 to 3.5-fold the clinically relevant exposure at the recommended clinical dose of 100 mg QD.

Study title: Effect of PF-04449913 on Nav1.5 Sodium Current Stably Expressed in HEK293 Cells

Study no.:	PF04449913NA15
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer Global Research & Development Drug Safety Research & Development Eastern Point Road Groton, CT USA
Date of study initiation:	Not indicated
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	PF-04449913, Lot # PF-04449913-01-0001, purity not indicated

Key Study Findings

- Glasdegib produced a concentration-dependent inhibition of the Nav1.5 current with an IC_{50} = 32.2 µM.

Table 8 Inhibition of Nav1.5 Sodium Currents by Glasdegib

(Excerpted from Submission)

Cell Number	% Inhibition 10 µM	% Inhibition 30 µM	% Inhibition 100 µM	% Inhibition 300 µM
1	19.2	52.1	87.2	100.6
2	14.4	54.5	87.7	99.9
3	5.9	40.3	73.1	94.3
4	30.5	56.5	82.2	Not tested
5	8.2	42.1	76.6	93.3
Mean ± SEM	15.6 ± 4.4 P <0.05	49.1 ± 3.3 P <0.05	81.3 ± 2.9 P <0.05	97.0 ± 1.9 P <0.05

SEM = Standard error of the mean.

Study title: Effects of PF-04449913-01 on HERG Potassium Channels Stably Expressed in HEK.293 cells.

Study no.:	PF4449913/ESD/1108/HERG
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer Global Research & Development Drug Safety Research & Development Eastern Point Road Groton, CT USA
Date of study initiation:	Not indicated
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	PF-04449913, Lot # PF-04449913-01-0001, impurity not indicated

Key Study Findings

- Glasdegib inhibited hERG currents in a concentration-dependent manner. The magnitude of inhibition was statistically significant at 1 µM (20.4 ± 6.4%), 3 µM (49.7 ±4.6%) and 10 µM (78.5 ± 3.7%), respectively. The IC_{50} value for hERG current inhibition by PF-04449913-01 is 3.1 µM.

Table 9 Effect of Glasdegib on hERG Current Amplitude

(Excerpted from Submission)

Observation	% Inhibition		
	1 µM	3 µM	10 µM
1	35.4	56.1	77.1
2	2.5	33.9	67.9
3	34.2	59.8	78.9
4	11.0	45.1	77.5
5	19.1	53.8	90.9
Mean ± SEM	20.4 ± 6.4*	49.7 ± 4.6**	78.5 ± 3.7*

Study title: Neurofunctional Assessment of PF-04449913 in Male Rats

Study no.:	08SN228
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer, Sandwich, Kent, UK
Date of study initiation:	September 23, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913-01, 121657-119-8, >99%

Key Study Findings

- No glasdegib-related effects up to high dose tested of 50 mg/kg.

Methods

Doses:	vehicle, 1, 5, or 50 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (aqueous)
Species/Strain:	Rat/Crl:CD(SD)IGS BR
Number/Sex/Group:	6 males/group
Age:	56-70 days
Weight:	271-320 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that had an impact on study data or interpretation of results.

Study title: Pulmonary assessment of PF-04449913 in male rats

Study no.:	08SN229
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer, Sandwich, Kent, UK
Date of study initiation:	September 17, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913-01, 121657-119-8, >99%

Key Study Findings

- No glasdegib-related effects on tidal volume, respiratory rate or minute volume in the conscious male rat at doses of 1, 5, and 50 mg/kg P.O.

Methods

Doses:	vehicle, 1, 5, or 50 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (aqueous)
Species/Strain:	Rat/Crl:CD(SD)IGS BR
Number/Sex/Group:	6 males/group
Age:	56-70 days
Weight:	271-320 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that had an impact on study data or interpretation of results.

Study title: Cardiovascular assessment of PF-04449913 in Beagle dogs

Study no.:	08SN238
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer Global Safety Pharmacology Kent, UK
Date of study initiation:	September 12, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913-01, 121657-119-8, >99%

Key Study Findings

- Glasdegib-dose dependent increase in QT and QTc that did not resolve by the end of study (22-hour post-dose) in the high dose (30 mg/kg).
- Significant increases in HR and QRS in HD were also seen but resolved by 22-hour post dose.

Methods

Doses:	vehicle, 1, 5, or 30 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (aqueous)
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4 males
Age:	Not given
Weight:	14.6-16.9 kg
Satellite groups:	None
Unique study design:	Latin square design with 3-5-day washout. Hemodynamics and ECG from 0.5-6 hour, 7-14 hour, 14-22 hour.
Deviation from study protocol:	None that had an impact on study data or interpretation of results.

Results

- 1 mg/kg unremarkable
- 5 mg/kg
  - 7-14 hour
    - QTc ↑ 5 msec*
- 30 mg/kg
  - 0.5-6 hr
    - QRS ↑3 msec*; QT ↑12 msec*; QTc ↑18 msec*; HR ↑10 bpm*
  - 7-14 hour
    - QRS ↑2 msec*; QT ↑22 msec*; QTc ↑24 msec*;
  - 14-22 hour
    - QT ↑18 msec*; QTc ↑ 18 msec*

- Emesis occurred between 0.6 to 1.2 h post-dose

- Plasma C_{max} 1972.5 ng/mL (range 680.0 to 2780.0 ng/mL; unbound 96 to 392 ng/mL) after dosing at 5 mg/kg occurred between 0.25-1-hour post-dose
- Glasdegib C_{max} at 5 mg/kg in this study ranged 0.9 to 3.5-fold the clinical relevant exposure at the 100 mg QD

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Study PF-04449913/14Nov08/143627. Single Dose Pharmacokinetics and Oral Bioavailability of PF-04449913 (b) (4) in Sprague-Dawley Rats Following Intravenous and Oral Administration.

The Applicant evaluated the PK and oral bioavailability of glasdegib in male Sprague Dawley rats after administration of a 1 mg/kg single IV bolus or oral dose of glasdegib (b) (4) salt. Following oral administration, glasdegib had an oral bioavailability of 33% (See Table 10).

Table 10 Summary of Pharmacokinetics of PF-04449913 in Rats

(Excerpted from Submission)

PK Parameters:	IV	PO
Clearance (mL/min/kg)	50.9	n/a
Volume of Distribution (L/kg)	4.78	n/a
Half-life (h)	1.4	n/a
Cmax (ng/mL)	n/a	38.6
tmax (h)	n/a	0.33
$AUC_{(0-\infty)}$ (ng·h/mL)	329	109
Bioavailability (%)	n/a	33
Estimated Blood Clearance[a] (mL/min/kg)	30.3	n/a

[a]Blood clearance = Plasma clearance/blood to plasma ratio (PF-04449913/12Nov08/104333)
PK = Pharmacokinetics; IV = Intravenous; PO = Oral; n/a = Not applicable; C_{max} = Maximum observed plasma concentration; t_{max} = Time to reach Cmax; $AUC_{(0-\infty)}$ = Area under plasma concentration-time curve from time zero to infinity

Study PF-04449913/14Nov08/143627. Single Dose Pharmacokinetics and Oral Bioavailability of PF-04449913 (b) (4) in Beagle Dogs Following Intravenous and Oral Administration.

The Applicant evaluated the PK and oral bioavailability of glasdegib in male Beagle dogs after administration of a 0.5 mg/kg single IV bolus or 3 mg/kg oral dose of glasdegib (b) (4) salt. Following oral administration, glasdegib had an oral bioavailability of 68% (See Table 11).

Table 11 Summary of Pharmacokinetics of PF-04449913 in Dogs

(Excerpted from Submission)

PK Parameters	IV	PO
Clearance (mL/min/kg)	22.9	n/a
Volume of Distribution (L/kg)	4.21	n/a
Half-life (h)	2.3	n/a
C_{max} (ng/mL)	n/a	1070
t_{max} (h)	n/a	0.50
$AUC_{(0-\infty)}$ (ng·h/mL)	368	3860
Estimated Bioavailability (%)[a]	n/a	68
Estimated Blood Clearance (mL/min/kg)	1.99	n/a

[a]Bioavailability was estimated by converting plasma concentrations to blood concentrations to obtain blood AUCs
PK = Pharmacokinetics; IV = Intravenous; PO = Oral; n/a = Not applicable; C_{max} = Maximum observed plasma concentration; t_{max} = Time to reach C_{max}; $AUC_{(0-\infty)}$ = Area under plasma concentration-time curve from time zero to infinity

Distribution

Study PF-04449913/17Nov08/142017. Protein Binding of PF-04449913 in Rat, Dog, Mouse, and Human Plasma.
Study PF-04449913_08Dec15_022737. PF-04449913 Protein Binding in Rabbit Plasma.
Study PF-04449913_23Feb16_104614. PF-04449913 Protein Binding in Human Serum Albumin (HSA), and α1- Acid Glycoprotein (AAG).

The Applicant determined the extent of in vitro binding of glasdegib to CD-1 mice, SCID mice, Sprague Dawley rats, NZW rabbits, Beagle dogs, and human plasma proteins using an equilibrium dialysis method at concentrations ranging from 1 to 50 µM, depending on the species, and samples analyzed using LC/MS/MS. There was no concentration dependent change in free fraction of binding to plasma proteins in any of the species tested. The Applicant calculated the mean unbound fraction of drug (f_u) and used to determine unbound concentrations and exposure margins in the toxicity studies (See Table 12). Exposure margins in this review were calculated based on total exposure. The Applicant determined the in vitro binding of glasdegib at 2 µM to human serum albumin (HSA) and alpha-1 glycoprotein (AAG) at physiological concentrations for these proteins using the same methodology as indicated above and the f_u calculated (See Table 12). Results suggests that glasdegib likely binds to both HSA and AAG in human plasma.

Table 12 Calculated Unbound Fraction of Glasdegib in Mouse, Rat, Rabbit, Dog and Human Plasma

Species / Matrix	F_u
CD-1 Mice	0.047
SCID mice	0.0932
Sprague Dawley rats	0.110
NZW rabbit	0.0203
Beagle dog	0.141
Human	0.0899
Human Serum Albumin	0.204
Alpha-1-Acid Glycoprotein	0.478

Study PF-04449913/12Nov08/104333. PF-04449913 Human, Rat, and Dog RBC Distribution.
Study PF-04449913_08Dec15_031858. PF-04449913 Rabbit RBC Distribution.
The Applicant determined the extent of glasdegib partitioning into blood cells in rat, dog, rabbit, and human whole blood over a concentration range of 0.2 to 50 µM (See Table 13). The in vitro results with human blood/plasma (Cb/Cp range 1.59 to 2.32) were consistent with those observed in vivo after oral administration of [^{14}C]glasdegib, with Cb/Cp values for glasdegib radioactivity ranging from 1.36 to 1.49 (Phase 1 open-label ADME of [^{14}C]glasdegib in healthy male volunteers Protocol B1371009). The in vitro and in vivo data suggest that glasdegib demonstrated species dependent blood partitioning with a modest preferential distribution into the blood cells of rats and humans, a limited distribution into the blood cells in rabbits, and a marked, concentration-dependent and saturable preferential distribution into the blood cells of dogs.

Table 13 Partition Coefficients and Blood to Plasma Concentration Ratios of Glasdegib in Human, Rat, Dog and Rabbit

(Excerpted from Submission)

	Human		Rat		Dog	
Substrate Conc. (µM)	Whole Blood (Cb)/Plasma Conc. (Cp)Ratio (Cb/CP)	RBC/Plasma Ratio or Partition Coefficient (Kp)	Whole Blood (Cb)/Plasma Conc. (Cp)Ratio (Cb/CP)	RBC/Plasma Ratio or Partition Coefficient (Kp)	Whole blood (Cb)/Plasma Conc. (Cp)Ratio (Cb/CP)	RBC/Plasma Ratio or Partition Coefficient (Kp)
0.2	1.25 ± 0.04	1.59 ± 0.10	1.28 ± 0.04	1.59 ± 0.08	6.90 (n=2)	11.6 (n=2)
0.5	1.36 ± 0.04	1.84 ± 0.08	1.33 ± 0.30	1.69 ± 0.62	3.68 ± 0.80	5.82 ± 1.43
2	1.27 ± 0.07	1.62 ± 0.16	1.79 ± 0.23	2.65 ± 0.48	2.24 ± 0.49	3.23 ± 0.87
5	1.36 ± 0.09	1.84 ± 0.21	1.80 ± 0.16	2.66 ± 0.34	2.33 ± 0.24	3.39 ± 0.43
20	1.46 ± 0.04	2.08 ± 0.08	1.86 ± 0.26	2.80 ± 0.53	1.09 ± 0.04	1.16 ± 0.07
50	1.56 ± 0.06	2.32 ± 0.15	2.01 ± 0.15	3.11 ± 0.31	1.27 ± 0.09	1.48 ± 0.16

Values are mean ± standard deviation (n =3 except where noted).

Species	Substrate Concentration (µM)	N	Time (hours)	BPR (Cb/Cp) (Mean ± SD)	RBC/Plasma Ratio or Partition coefficient (Kp) (Mean ± SD)
Rabbit	0.2	4	1	0.672±0.066	0.284±0.144
Rabbit	0.5	4	1	0.648±0.139	0.231±0.304
Rabbit	2	4	1	0.745±0.094	0.442±0.206
Rabbit	5	4	1	0.745±0.114	0.443±0.249
Rabbit	20	4	1	0.703±0.080	0.352±0.175
Rabbit	50	4	1	0.809±0.064	0.582±0.140
Mean				0.720±0.101	0.389±0.221

BPR = blood to plasma ratio; Cb = Concentration in blood; Cp = Concentration in plasma; n = Number of replicates; RBC = Red blood cell; SD = Standard Deviation.

Metabolism

(Adapted from Submission)
The Applicant evaluated the metabolism of glasdegib in vivo after oral administration of a single dose of [^{14}C]glasdegib to rats, dogs, and humans, and in vitro in liver microsomes from nonclinical species and humans and in human hepatocytes. In humans, oxidative metabolism was the major route of elimination, with minor contributions from glucuronidation, and approximately 20% of glasdegib was excreted

unchanged in urine. After oral administration, the primary metabolic pathways of [^{14}C]glasdegib in humans were N-demethylation of the N-methylpiperidine group, glucuronidation, oxidation of the benzimidazole and N-methylpiperidine rings, and dehydrogenation of the piperidine ring. Oxidative metabolite profiles were qualitatively similar across all species. One primary and two secondary N-glucuronide metabolites (each <10%) were identified in the plasma and urine of humans only; however, additional safety testing of these metabolites was not considered necessary. Glasdegib was the most abundant circulating component in plasma, constituting 69% of plasma radioactivity, with minor circulating metabolites each being <8% of radioactivity. Chiral inversion to glasdegib epimers was considered minimal. (See Figure 3).

Excretion

(Adapted from Submission)

Following oral administration of [^{14}C]glasdegib to rats, dogs, and humans, recovery of radioactivity was essentially complete. The major route of excretion of drug-derived radioactivity was via feces and/or bile in rats and dogs; whereas, in humans there was relatively equal excretion of drug-derived radioactivity in urine and feces with unchanged glasdegib being the major drug-related components in each matrix.

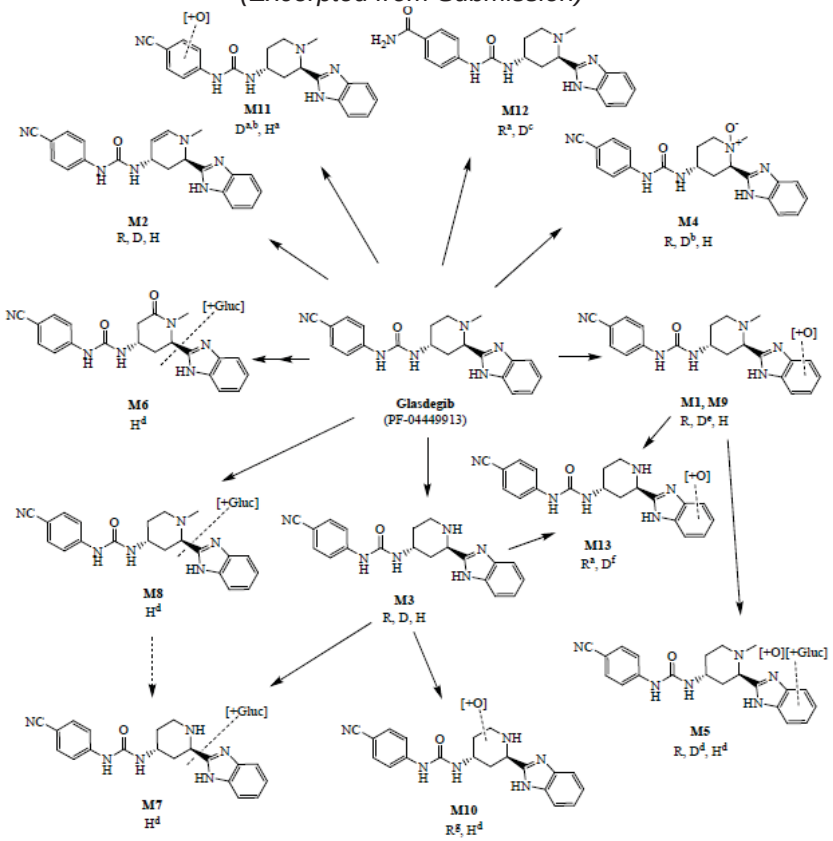
Table 14 Excretion of Radiolabeled Glasdegib

Species	Dose [^{14}C]glasdegib	Fecal (%)	Renal (%)	Total Radioactivity Recovery over 168h (%)
Rat	10 mg/kg PO	M: 93.7	<6	100
		F: 89.2	<6	96.7
Dogs	5 mg/kg PO	M: 82.0	7.2	~93
		F: 83.0	7.5	
Human	100 mg (100 µCi)	42	49	~91

The potential for glasdegib or its metabolites to be excreted in the breast milk of nonclinical species has not been evaluated.

Figure 3 Proposed Glasdegib In Vivo Metabolites Present in Rat, Dog, and Human Plasma and/or Excreta

(Excerpted from Submission)



Note: Excretory metabolites detected in urine and feces from rat, dog, or human. Metabolites M1, M2, M3, M4, M5, and M13 were detected in rat bile. Metabolite to metabolite pathways were not confirmed experimentally.
Abbreviations: D = Dog; H = Human; M = Metabolite; R = Rat.
a. Detected in feces only.
b. M4 and M11 co-elute in feces.
c. Detected in plasma and feces only.
d. Detected in plasma and urine only.
e. M9 detected in plasma and feces only.
f. Detected in urine and feces only.
g. Detected in plasma only.

5.2 Toxicokinetics

The Applicant evaluated the TK of glasdegib after once daily oral administration as part of oral repeat-dose toxicity studies in rats and dogs, embryo-fetal development (EFD) toxicity studies in rats and rabbits, and in support of a phototoxicity study in pigmented rats. The summary of all TK evaluations in animals is presented in the following tables as reference.

Table 15 Mean Toxicokinetics of Glasdegib in Sprague Dawley Rats (Male and Female Combined) Following Oral Administration in Repeat-Dose Toxicity Studies

(Excerpted from Submission)

Dose (mg/kg/day)	N/Sex	Study Day	C_{max} (ng/mL)	Unbound[a] C_{max} (ng/mL)	T_{max} (hour)	AUC_{24} (ng·hour/mL)	Unbound[a] AUC_{24} (ng·hour/mL)
1-Month Study (6348-650; 08LJ094)[b]							
1	4/M; 4/F	1	31.5	3.47	2.9	306	33.7
10	4/M; 4/F	1	743	81.7	3.4	7030	773
50	4/M; 4/F	1	4280	471	3.6	53400	5870
1	4/M; 4/F	29	36.0	3.96	2.4	288	31.7
10	4/M; 4/F	29	884	97.2	1.5	8700	957
50	4/M; 4/F	29	5610	617	2.3	66900	7360
1-Month Impurity Study (17GR041)							
10	4/M	1	286	31.5	0.5	1830	201
10	4/F	1	1060	117	1.4	5440	598
10	4/M	29	333	36.6	1.4	2500	275
10	4/F	29	2810	309	0.5	10200	1120
10[c]	4/M	1	380	41.8	0.5	2680	295
10[c]	4/F	1	847	93.2	1.5	5170	569
10[c]	4/M	29	614	67.5	0.6	3510	386
10[c]	4/F	29	1570	173	0.5	6970	767
13-Week Study (8299121; 14LJ052)[b]							
10	4/M; 4/F	87	1060	117	2.0	10000	1100
50	4/M; 4/F	87	5790	637	2.0	84200	9260
100	4/M; 4/F	87	14400	1580	6.0	215000	23700
26-Week Study (8303065; 14LJ108)							
10	4/M; 4/F	1	170	18.7	3.3	1740	191
50	4/M; 4/F	1	3560	392	4.8	46600	5130
100	4/M; 4/F	1	6320	695	4.8	81900	9010
10	4/M; 4/F	Week 13	813	89.4	2.1	7080	779
50	4/M; 4/F	Week 13	7460	821	3.5	92400	10200
100	4/M; 4/F	Week 13	13600	1500	4.4	178000	19600
10	4/M; 4/F	Week 26[d]	1050	116	1.4	7800	858
50	4/M; 4/F	Week 26[d]	8530	938	4.1	110000	12100
Embryo-Fetal Development Study (20069225; 15GR056)							
10	5/F	17[e]	726[f]	79.9[f]	4.0[f]	ND	ND
50	5/F	17[e]	4500[f]	495[f]	4.0[f]	ND	ND
100	5/F	17[e]	7140[f]	785[f]	4.0[f]	ND	ND
Phototoxicity Study (20111045; 16LJ129)							
10	3/F	3	976	107	0.50	5110	562
30	3/F	3	4460	491	0.50	22900	2520
100	3/F	3	14200	1560	0.67	137000	15100

AUC_{24} = Area under the concentration-time curve from zero to 24 hours postdose; C_{max} = Maximal plasma concentration; F = Female; f_u = Unbound fraction of drug; GD = Gestation day; M = Male; N = Number; ND = Not determined; T_{max} = Time to reach C_{max}.

a. Rat f_u = 0.110

b. Mean pharmacokinetic parameters were calculated from N=4/sex/timepoint.

c. Glasdegib blended with 0.65% PF-06658120 and 0.70% PF-06772998 impurities.

d. No data for animals in the 100 mg/kg/day dose group for Week 26, dosing suspended on Day 126.

e. Repeat oral dosages were administered to gravid rats from GD6 to GD17.

f. Results are from a single time point at 4 hours post dosing on GD17.

Plasma concentrations of glasdegib in these toxicology species are reported as total drug and unbound drug, and were used to calculate exposure ratios for various safety pharmacology and toxicity studies to assist in the assessment of safety margins relative to human exposure.

Table 16 Mean Toxicokinetics of Glasdegib in Beagle Dogs (Male and Female Combined) Following Oral Administration in Repeat-Dose Toxicity Studies

(Excerpted from Submission)

Dose (mg/kg/day)	N/Sex	Study Day	C_{max} (ng/mL)	Unbound[a] C_{max} (ng/mL)	T_{max} (hour)	AUC_{24} (ng•hour/mL)	Unbound[a] AUC_{24} (ng•hour/mL)
1-Month Study (6348-651; 08LJ093)							
1	3/M; 3/F	1	111[b]	15.7[c]	1.0	510[b]	71.9[c]
5	3/M; 3/F	1	2860	403	0.50	8300	1170
30	5/M; 5/F	1	14400	2030	0.75	115000	16200
1	3/M; 3/F	29	151	21.3	0.92	723	102
5	3/M; 3/F	29	3350	472	0.75	13500	1900
13-Week Study (8299265; 14LJ055)							
1	3/M; 3/F	89	65.5	9.24	2.3	487	68.7
5	3/M; 3/F	89	1100	155	2.0	8010	1130
10	3/M; 3/F	89	3060	431	2.2	25800	3640
39-Week Study (8303066; 14LJ109)							
1	4/M; 4/F	1	137	19.3	0.94	692	97.6
5	6/M; 6/F	1	1830	258	0.92	9060	1280
10	6/M; 6/F	1	5050	712	1.0	35200	4960
1	4/M; 4/F	Week 13	139	19.6	1.1	834	118
5	6/M; 6/F	Week 13	2340	330	1.2	13000	1830
10	4/M; 6/F	Week 13	4560	643	1.2	20100	2830
1	3/M; 3/F	160	112	15.8	0.92	381	53.7
5	2/M; 4/F	160	1400	197	1.3	6300	888
10	3/M; 6/F	160	4070	574	1.2	20000	2820
1	3/M; 3/F	Week 39[d]	95.9	13.5	0.67	372	52.5

AUC_{24} = Area under the concentration-time curve from zero to 24 hours postdose; C_{max} = Maximal plasma concentration; F = Female; f_u = Unbound fraction; h = Hour; M = Male; N = Number; NC = Not calculated due to insufficient quantifiable concentration data; ND = Not determined; T_{max} = Time to reach C_{max}.

a. Dog f_u = 0.141

b. C_{max} in males and females was 145 and 77.3 ng/mL on Day 1 and 201 and 100 ng/mL on Day 29, respectively while the AUC_{24} in males and females was 701 and 318 ng*hr/mL on Day 1 and 1020 and 427 ng*hr/mL on Day 29, respectively.

c. The unbound C_{max} in males and females was 20.5 and 10.9 ng/mL on Day 1 and 28.3 and 14.1 ng/mL on Day 29, respectively while the unbound AUC_{24} in males and females was 98.8 and 44.8 ng*hr/mL on Day 1 and 144 and 60.2 ng*hr/mL on Day 29, respectively.

d. No data for animals in the 5 or 10 mg/kg/day dose groups due to mortality prior to Week 39.

Table 17 Mean Toxicokinetics in Pregnant Female Rabbits Following Repeat Oral Doses of Glasdegib

(Excerpted from Submission)

Study	GD Day[a]	Dosage (mg/kg/day)	C_{max} (ng/mL)	Unbound[b] C_{max} (ng/mL)	T_{max} (hour)	AUC_{24} (ng•hour/mL)	Unbound[b] AUC_{24} (ng•hour/mL)
20069230 (15GR057)	19	5	3120	63.3	2.0	43200	877
		10	22700	461	1.0	262000	5320
		50	79800	1620	5.0	1790000	36300

AUC_{24} = Area under the concentration-versus-time curve from time 0 to 24 hours after dosing; C_{max} = Maximal plasma concentration; GD = Gestation day; f_u = Unbound fraction.

a. Once daily oral doses were administered to gravid rabbits from GD 7 to 19; results are from GD19.

b. Rabbit f_u = 0.0203

6 General Toxicology

In both rats and dogs, body weight was negatively affected by glasdegib, and the major target organs were the kidney, liver, skin, bone, and general body weight. Rat specific target organs of toxicity included testis and peripheral nerve; dog specific target organs of toxicity included the adrenal, gastrointestinal track, lymph nodes, and lung. Corresponding clinical pathology effects occurred in both species. The primary toxicity was azotemia with subsequent damage to the kidneys, leading to the moribund sacrifice of dogs.

6.1 Single-Dose Toxicity

No studies were conducted.

6.2 Repeat-Dose Toxicity

Study title: 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with PF-04449913 in Rats with a 13-Week Recovery Phase

Study no.:	8303065 (14LJ108)
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 18, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; GR08041; 99.4%

Key Study Findings

- Glasdegib-related mortality and early termination of the 100 mg/kg/day dose group during Week 19 and subsequent mortality in 50 mg/kg/day females during Week 26.
- Glasdegib-related and adverse clinical signs at ≥50 mg/kg/day included tremors; malocclusions; missing teeth; thin appearance; clear oral discharge; labored respiration; hypoactivity, and hunched posture.
- Glasdegib-related and adverse effects at ≥50 mg/kg/day included lower grip strength, reductions in locomotor activity, lower mean body weights, body weight gains, food consumption, and microscopic findings in the kidney with corresponding clinical pathology findings, physis of the femur and sternum, loss of one or more incisor teeth.
- Target organs of toxicity included kidney, bone/cartilage, peripheral nerve, general body weight and the testis.

Methods

Doses:	0, 10, 50, or 100 mg/kg/day Doses selected based on the 13-W rat study (8299121 (14LJ052))
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) methylcellulose (Methocel A4M, 4000 cps) in reverse osmosis water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	20/sex/group; 5-6 rats/sex in control, mid dose (MD) and high dose (HD) were allocated for the 13-w recovery phase
Age:	7 weeks
Weight:	M: 212 to 285 g; F: 163 to 208 g
Satellite groups:	4/sex/group for TK blood collection
Unique study design:	FOB elicited behavior and open field observations were evaluated
Deviation from study protocol:	None that affected the overall interpretation of study findings or compromised the integrity of the study

Observations

Table 18 Experimental Design for the 26-W Rat Study

(Excerpted from Submission)

Group	Subgroup	No. of Animals[a,d] Male	No. of Animals[a,d] Female	Dose (mg/kg/day)	Dose Concentration[b] (mg/mL)
1 (Control)[c]	1 (Toxicity)	20	20	0	0
	2 (Toxicokinetic)	4	4	0	0
2 (Low)	1 (Toxicity)	16	16	10	1
	2 (Toxicokinetic)	4	4	10	1
3 (Mid)	1 (Toxicity)	20	20	50	5
	2 (Toxicokinetic)	4	4	50	5
4 (High)	1 (Toxicity)	20	20	100	10
	2 (Toxicokinetic)	4[d]	4[d]	100	10

a All surviving toxicity animals in Group 4 (except the last 5 animals/sex in Group 4) were euthanized at Terminal Euthanasia 1 (Day 127 of the dosing phase). On Day 127 of the dosing phase, the last 5 toxicity animal/sex in Group 4 began the recovery phase (considered Day 1 of the recovery phase for these animals). The first 14 toxicity animals in Groups 1 and 3 (dependent on survival), and all surviving toxicity animals in Group 2 were euthanized at the Terminal Euthanasia 2 (Day 183 of the dosing phase). All surviving toxicity animals (Groups 1, 3, and 4) were euthanized at the Recovery Euthanasia on Study Day 277. All surviving toxicokinetic animals were euthanized after respective final blood collections.

b Concentrations were corrected for salt content and lot-specific purity. A correction factor of 1.243 was used.

c Group 1 received vehicle control article only.

d The final blood collection interval for the Group 4 toxicokinetic animals was considered Week 13 of the dosing phase and animals were euthanized and discarded at Terminal Euthanasia 1 (Day 127 of the dosing phase).

Results

Mortality

Glasdegib-related mortality occurred at ≥50 mg/kg/day. The extent of mortality at 100 mg/kg/day by Week 19 prompted euthanasia of all surviving toxicity rats at 100 mg/kg/day (except the last 5 rats/sex) on Day 127 (Week 19) of the dosing phase. The last 5 rats/sex administered 100 mg/kg/day went on an extended recovery phase of 21 weeks (+ 4 days). A total of nineteen (19) unscheduled deaths of toxicity animals and 2 toxicokinetic animals occurred between Days 22 and 176 of the dosing phase or Day 68 of the recovery phase, and 11 of the toxicity animal deaths were considered glasdegib-related due to dental abnormalities and/or degeneration/necrosis of the renal tubule cells noted histologically (See Table 19).

Table 19 Incidence of Glasdegib-related Mortality in the 26-W Rat Study

Excerpted from Submission)

	Group 1	Group 2	Group 3	Group 4
Dose (mg/kg/day)	0	10	50	100
Males	1/20	0/16	1/20[a]	7/20 Tox 2/4 TK
Females	1/20[b]	2/16[c]	4/20[d]	3/20[e]

a Attributed to blood collection procedure (Animal B13913 on Day 127)
b Attributed to blood collection procedure (Animal B14078 on Day 27)
c Both deaths attributed to blood collection procedure (Animal B14012 on Day 27; Animal B14045 on Day 90)
d One death attributed to blood collection procedure (Animal B14079 on Day 27). One moribund euthanasia occurred during the recovery phase (Animal B14091 on Day 68) and was not TA-related.
e One death attributed to the blood collection procedure (Animal B14015 on Day 54)

Clinical Signs

Clinical signs of early decedents included continuous or intermittent body tremors, labored respiration, piloerection, hypoactivity, hunched posture, clear oral discharge, thinning hair coat, malocclusion, missing teeth, thin and/or body weight loss.

Clinical signs in surviving rats at ≥50 mg/kg/day included continuous or intermittent tremors (various body regions or whole body); malocclusions; missing teeth; thin appearance; clear oral discharge; squinting eyes; rough haircoat and piloerection; rough, thinning, or discolored (red, yellow, black, or brown) haircoat in various body regions; and alopecia. Onset of observations was typically noted earlier in males than in females at the same respective dose levels and with an earlier onset with increasing dose level.

Reviewer Note: Exposure to glasdegib tended to be higher in rat females in all toxicology studies, and males were more sensitive to glasdegib toxicity.

Body Weights

Glasdegib-related and dose-dependent lower mean body weights and body weight gains relative to controls were noted at ≥50 mg/kg/day (See Table 20).

Table 20 Glasdegib-related Effects on Body Weight in the 26-W Rat Study

Glasdegib (mg/kg/day)	Percent Difference vs Control in Mean Body Weight (Day 182)	Percent Difference vs Control in Mean BW Gain (Day 1 to 182)
MALES		
10	↑4	↑5
50	↓27*	↓45*
100	↓38[A]*	Early termination
FEMALES		
10	↑4	↑3
50	↓22*	↓48*
100	↓25[A]*	Early termination

[A] Differences compared to control on Day 120 before early termination
* Significant different compared to control P≥0.05

Feed Consumption

Table 21 Glasdegib-related Effects on Food Consumption in the 26-W Rat Study

Glasdegib (mg/kg/day)	Percent Difference vs Control in Mean Food Consumption
MALES	
10	↑4
50	↓6*
100	↓11[A]*
FEMALES	
10	↑5
50	↓7*
100	↓10[A]*

[A] Differences compared to control on Day 120 before early termination
* Significant different compared to control P≥0.05

Ophthalmoscopy

No Glasdegib-related findings.

ECG

Not conducted

Hematology

Table 22 Percent Change in Hematology Parameters on Day 183 in the 26-W Rat Toxicology Study

Parameter	Glasdegib (mg/kg/day)			
	50		100[A]	
	Males	Females	Males	Females
RBC	↓7[B]*	↓4*	↓14[B]*	↓11[B]*
Hemoglobin	↓7[B]*	↓3	↓12[B]*	↓9[B]*
HCT	↓7[B]*	↓4*	↓13[B]*	↓9[B]*
RDW	↑11[B]*		↑24[B]*	↑9[B]*
Reticulocytes	↑50[B]*	↑32*	↑73[B]*	↑74[B]*
WBC		↑3*	↑55[B]*	↑64[B]*
Neutrophils			↑85[B]*	↑32[B]*
Lymphocytes		↑31[B]*	↑48[B]*	↑48[B]*
Monocytes			↑34[B]*	↑95[B]*
LUC			↑86[B]*	↑100[B]*

Platelet	↑4	↑3	↑26[B*]	↑11[B*]
MPV	↑6*	↑5	↑8*	↓5
Fibrinogen			↑27[B*]	↑24

Empty spaces denote no changes occurred compared to control
[A] Differences compared to control on Day 127 before early termination
[B] Differences also occurred on other assessment days
* Significant different compared to control P≥0.05

Clinical Chemistry

Table 23 Percent Change in Clinical Chemistry Parameters on Day 183 in the 26-W Rat Toxicology Study

Parameter	Glasdegib (mg/kg/day)			
	50		100[A]	
	Males	Females	Males	Females
Glucose	↓8*			
Urea nitrogen	↑25[B*]	↑39[B*]	↑31[B*]	↑27[B*]
Creatinine	↑29[B*]	↑25[B*]	↑67[B*]	↑29[B*]
Albumin			↓11[B*]	↓9[B]
Globulin			↑14[B*]	↑19[B*]
A:GR		↓9[B]	↓20[B*]	↓23[B*]
ALT		↑26	↑31[B*]	↓14[B*]
Alkaline Phosphatase	↓18[B*]	↓33[B*]	↓25[B*]	↓31[B*]
Phosphorus	↑16[B*]	↑20[B*]	↑32[B*]	↑53[B*]

Empty spaces denote no changes occurred compared to control
[A] Differences compared to control on Day 127 before early termination
[B] Differences also occurred on other assessment days
* Significant different compared to control P≥0.05

Urinalysis

Glasdegib-related effects in urinalysis parameters were consistent with microscopic evidence of adverse changes in the kidney.

Gross Pathology

Glasdegib-related macroscopic observations in ≥50 mg/kg/day consisted of 1 or more missing incisors, which corresponded histologically to fibrosis/new bone formation, and bilaterally discolored kidneys, which were associated with degeneration/necrosis of the tubular epithelial cells.

Organ Weights

No Glasdegib-related organ weight differences occurred.

Histopathology

Adequate Battery: Yes
Peer Review: No
Histological Findings
Glasdegib-related microscopic observations for were present in the kidney, bone and marrow of femur and sternum, 1 or more incisor teeth, oral mucosa, testis (males), and peripheral nerve adjacent to the mesenteric lymph node (nerve-(other)). In the testis, Glasdegib-related minimal to severe hypospermatogenesis occurred in

50 mg/kg/day males and was characterized by partial to complete loss of spermatogonia, spermatocytes, and spermatids; some animals also exhibited degeneration of seminiferous tubules (See Table 24).

Table 24 Incidence and Severity of Glasdegib-related Microscopic Findings at Terminal Sacrifice of the 26-W Study in Rats

(Excerpted from Submission)

	PF-04449913							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	10	50	100	0	10	50	100
Kidney								
Number Examined	14	16	14	8	14	14	12	12
Cast, proteinaceous								
Not Present	14	14	14	4	14	14	10	8
Minimal	0	2	0	4	0	0	2	4
Degeneration/necrosis, tubule								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	14	0	0	0	12	0
Moderate	0	0	0	8	0	0	0	12
Karyomegaly, renal tubule epithelium								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	14	8	0	0	12	12
Regeneration								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	14	0	0	0	12	0
Moderate	0	0	0	8	0	0	0	12
Tooth, Left Lower Incisor								
Number Examined	14	16	14	8	14	14	12	12
Dental hard substance, altered								
Not Present	14	16	3	6	14	14	4	4
Present	0	0	11	2	0	0	8	8
Fibrosis/New bone formation								
Not Present	14	16	0	0	14	14	0	0
Mild	0	0	1	0	0	0	0	0
Moderate	0	0	0	0	0	0	2	0
Marked	0	0	13	8	0	0	10	12
Infiltrate, mixed cell								
Not Present	14	16	12	5	14	13	12	12
Minimal	0	0	1	1	0	1	0	0
Mild	0	0	1	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Tooth, Left Upper Incisor								
Number Examined	14	16	14	8	14	14	12	12
Dental hard substance, altered								
Not Present	13	16	4	2	14	14	4	6
Present	1	0	10	6	0	0	8	6
Fibrosis/New bone formation								
Not Present	13	16	0	1	14	14	0	0
Minimal	1	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	4	0
Marked	0	0	14	7	0	0	8	12
Infiltrate, mixed cell								
Not Present	12	15	9	8	14	14	11	11
Minimal	2	1	5	0	0	0	1	0
Mild	0	0	0	0	0	0	0	1
Tooth, Right Lower Incisor								
Number Examined	14	16	14	8	14	14	12	12
Dental hard substance, altered								
Not Present	14	16	7	4	14	14	6	5
Present	0	0	7	4	0	0	6	7
Fibrosis/New bone formation								
Not Present	14	16	0	0	14	14	0	0
Moderate	0	0	1	0	0	0	3	0
Marked	0	0	13	8	0	0	9	12
Infiltrate, mixed cell								
Not Present	14	16	11	5	14	13	12	12
Minimal	0	0	2	1	0	1	0	0
Mild	0	0	1	2	0	0	0	0

Continued…

	PF-04449913							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	10	50	100	0	10	50	100
Tooth, Right Upper Incisor								
Number Examined	14	16	14	7	14	14	12	12
Degeneration/necrosis/absence, apical portion								
Not Present	13	16	14	7	14	14	11	12
Mild	1	0	0	0	0	0	1	0
Dental hard substance, altered								
Not Present	12	16	3	1	14	14	4	3
Present	2	0	11	6	0	0	8	9
Fibrosis/New bone formation								
Not Present	12	16	0	0	14	14	1	0
Mild	2	0	0	0	0	0	1	0
Moderate	0	0	2	0	0	0	3	0
Marked	0	0	12	7	0	0	7	12
Infiltrate, mixed cell								
Not Present	13	16	12	7	14	14	11	10
Minimal	1	0	2	0	0	0	1	2
Oral Mucosa								
Number Examined	14	16	14	8	14	14	12	12
Infiltrate, mixed cell								
Not Present	14	14	14	6	14	14	10	12
Minimal	0	2	0	2	0	0	0	0
Mild	0	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	1	0
Ulcer								
Not Present	14	16	14	5	14	14	12	12
Mild	0	0	0	2	0	0	0	0
Marked	0	0	0	1	0	0	0	0
Femur								
Number Examined	14	16	14	8	14	14	12	12
Disorganization, chondrocytes, physis								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	4	0	0	0	8	0
Mild	0	0	5	0	0	0	2	0
Moderate	0	0	5	8	0	0	2	12
Sternum								
Number Examined	14	16	14	8	14	14	12	12
Disorganization, chondrocytes, physis								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	3	0	0	0	9	0
Mild	0	0	7	8	0	0	2	12
Moderate	0	0	4	0	0	0	1	0
Marrow, Femur								
Number Examined	14	16	14	8	14	14	12	12
Hypercellular								
Not Present	14	16	10	0	14	14	12	0
Minimal	0	0	3	0	0	0	0	0
Mild	0	0	1	8	0	0	0	12
Marrow, Sternum								
Number Examined	14	16	14	8	14	14	12	12
Hypercellular								
Not Present	14	16	10	0	14	14	12	0
Minimal	0	0	3	0	0	0	0	0
Mild	0	0	1	8	0	0	0	12

Continued…

	PF-04449913							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	10	50	100	0	10	50	100
Testis								
Number Examined	14	16	14	8	NA	NA	NA	NA
Degeneration, seminiferous tubule								
Not Present	14	16	11	8	NA	NA	NA	NA
Minimal	0	0	3	0	NA	NA	NA	NA
Hypospermatogenesis								
Not Present	14	16	10	8	NA	NA	NA	NA
Minimal	0	0	2	0	NA	NA	NA	NA
Marked	0	0	1	0	NA	NA	NA	NA
Severe	0	0	1	0	NA	NA	NA	NA
Nerve-*(other)*								
Number Examined	14	15	14	8	14	14	12	12
Degeneration, axon								
Not Present	14	15	12	1	14	14	4	4
Minimal	0	0	2	3	0	0	7	6
Mild	0	0	0	0	0	0	1	2
Moderate	0	0	0	2	0	0	0	0
Marked	0	0	0	2	0	0	0	0

NA = Not applicable.

Table 25 Incidence and Severity of Glasdegib-related Microscopic Findings at Recovery Sacrifice of the 26-W Study in Rats

(Excerpted from Submission)

	PF-04449913					
Sex	Males			Females		
Dose Level (mg/kg/day)	0	50	100	0	50	100
Kidney						
Number Examined	5	5	5	5	4	5
Cast, proteinaceous						
Not Present	5	5	5	5	4	3
Minimal	0	0	0	0	0	2
Karyomegaly, renal tubule epithelium						
Not Present	5	0	0	5	1	5
Minimal	0	5	5	0	3	0
Regeneration						
Not Present	5	2	2	5	2	5
Minimal	0	3	3	0	2	0
Tooth, Left Lower Incisor						
Number Examined	5	5	5	5	4	5
Degeneration/necrosis/absence, apical portion						
Not Present	5	5	4	5	4	5
Minimal	0	0	1	0	0	0
Dental hard substance, altered						
Not Present	5	1	2	5	2	4
Present	0	4	3	0	2	1
Fibrosis/New bone formation						
Not Present	5	0	0	5	0	0
Mild	0	2	1	0	1	0
Moderate	0	0	0	0	1	0
Marked	0	3	4	0	2	5
Infiltrate, mixed cell						
Not Present	5	4	5	5	4	5
Mild	0	1	0	0	0	0
Tooth, Left Upper Incisor						
Number Examined	5	5	5	5	3	5
Dental hard substance, altered						
Not Present	5	2	3	5	2	4
Present	0	3	2	0	1	1
Fibrosis/New bone formation						
Not Present	5	0	0	5	0	0
Mild	0	1	1	0	0	0
Moderate	0	0	0	0	2	0
Marked	0	4	4	0	1	5
Infiltrate, mixed cell						
Not Present	5	4	4	5	2	4
Minimal	0	1	1	0	1	1

At recovery necropsies, glasdegib-related microscopic observations persisted in one or more incisor teeth, femur, and sternum of ≥50 mg/kg/day rats; karyomegaly, proteinaceous casts, and regeneration of renal tubule epithelium in the kidney, and hypospermatogenesis in the testis persisted indicating a lack of recovery (See Table 25). The kidney degeneration/necrosis, bone marrow hypercellularity, testicular degeneration of seminiferous tubules, and axonal degeneration nerve-(other) observed at the terminal euthanasia were not observed at the recovery euthanasia.

Special Evaluation

(Adapted from Submission)
FOB
Glasdegib-related effects on quantitative FOB parameters included reduced forelimb grip strength at ≥50 mg/kg/day beginning at Week 9; and hindlimb grip strength at ≥50 mg/kg/day in males and at 100 mg/kg/day in females. Some of the differences were statistically significant. The maximum magnitude of the reductions relative to controls in 100 mg/kg/day rats was 0.62x during Week 9, and in 50 mg/kg/day rats was 0.71x during Week 26. There was a lack of recovery in grip strength.

Locomotor Activity
Glasdegib-related effects on locomotor activity were noted at 100 mg/kg/day beginning at the Week 9 evaluations, and was related to poor animal health.

Toxicokinetics

Due to early termination, 100 mg/kg rats were unavailable for blood sample collections during Week 26. Toxicokinetic parameters were calculated for LD, MD and HD on Day 1 and during Week 13 and for LD and MD during Week 26. No marked (≤2-fold) sex-related differences were noted in exposure.

Systemic exposure, as assessed by C_{max} and AUC_{24} increased with increasing dose from 10 to 100 mg/kg/day on Day 1 and during Week 13, and from 10 to 50 mg/kg/day during Week 26 (See Table 26). The increases in exposure were generally greater than dose-proportional from 10 to 50 mg/kg/day on Day 1 and during Weeks 13 and 26. The increases in exposure were approximately dose-proportional from 50 to 100 mg/kg/day on Day 1 and during Week 13. Exposure was higher during Weeks 13 and 26 when compared with Day 1. During Week 13, mean C_{max} values were 4.8x, 2.1x, and 2.2x and mean AUC_{24} values were 4.1x, 2.0x, and 2.2x when compared with Day 1 for the 10, 50, and 100 mg/kg/day dose groups, respectively. During Week 26, mean C_{max} values were 6.2x and 2.4x and mean AUC_{24} values were 4.5x and 2.4x when compared with Day 1 for the 10 and 50 mg/kg/day dose groups, respectively.

Table 26 Mean Toxicokinetic Parameters for Glasdegib in Combined Sex on Day 1 and during Weeks 13 and 26 in the 26-W Rat Study

(Excerpted from Submission)

Interval	Dose Group	Dose Level (mg/kg/day)		C_{max} (ng/mL)	T_{max} (hr)	AUC_{24} (ng·hr/mL)	AUC_{24}/Dose [(ng·hr/mL)/(mg/kg/day)]
Day 1	2	10	Mean	170	3.3	1740	174
			SD	76.6	1.4	771	77.1
			N	8	8	8	8
	3	50	Mean	3560	4.8	46600	933
			SD	1280	1.0	18000	360
			N	8	8	8	8
	4	100	Mean	6320	4.8	81900	819
			SD	1700	1.0	19400	194
			N	8	8	8	8
Week 13	2	10	Mean	813	2.1	7080	708
			SD	448	1.6	3600	360
			N	8	8	8	8
	3	50	Mean	7460	3.5	92400	1850
			SD	2900	1.7	24500	490
			N	8	8	8	8
	4	100	Mean	13600	4.4	178000	1780
			SD	2500	1.7	32200	322
			N	8	8	8	8
Week 26	2	10	Mean	1050	1.4	7800	780
			SD	596	1.1	3860	386
			N	8	8	8	8
	3	50	Mean	8530	4.1	110000	2200
			SD	3450	1.6	53500	1070
			N	8	8	8	8

AUC_{24}	Area under the concentration-time curve from hour 0 to hour 24.
AUC_{24}/Dose	Area under the concentration-time curve from hour 0 to hour 24 divided by dose level.
C_{max}	Maximum concentration in plasma.
N	Number of values in calculation.
SD	Standard deviation.
T_{max}	Time to maximum plasma concentration.
Note:	Group 4 (100 mg/kg/day) animals were sacrificed prior to their Week 26 TK sample collection.

Dosing Solution Analysis

Stability of samples prepared at concentrations of 0.1 and 10 mg/mL was established under (b) (4) Study 8299121 (13-W rat study). Dose formulations from the Day 1 preparations (1 and 10 mg/mL) and Week 21 preparations (1 and 5 mg/mL) met specifications and were considered homogeneous. The percent relative standard deviation (RSD) ranged from 1.1 to 3.2. All dose formulations from the Days 1, 57, 120, and 176 preparations were within ±10% of the target concentrations (mean values ranging from 95.5% to 101.5% of theoretical). No glasdegib was detected in the control vehicle.

Study title: 39-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with PF-04449913 in Dogs with a 16-Week Recovery Phase

Study no.:	8303066 (14LJ109)
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 17, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; GR08041; 99.4%

Key Study Findings

- Glasdegib-related mortality at all dose levels occurred due to body weight loss, inappetence and adverse clinical observations of thin appearance, hypoactivity, periodic vomiting, evidence of dehydration, and being cold to the touch.
- Glasdegib adverse microscopic findings in the kidney (tubular degeneration/ necrosis, tubular regeneration, granular/cellular casts, and tubular dilatation) and associated clinical pathology findings of increased relative to baseline urea nitrogen and creatinine concentrations, positive urine glucose, and granular casts and adverse microscopic findings in the liver (centrilobular/midzonal mixed cell inflammation, individual hepatocyte necrosis, Kupffer cell/macrophage pigment, and centrilobular hepatocyte vacuolation).
- Findings in the kidney were not present at the end of the 16-week recovery period. Partial recovery observed for liver findings.
- Target organs for toxicity included the kidney, liver, skin, gastrointestinal trach, lung and general body weight.

Methods

Doses:	0, 1, 5, or 10 mg/kg/day Doses selected based on the 13-W dog study (8299265 (14LJ055))
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% (w/v) methylcellulose [Methocel A4M (4000 cps)] in reverse osmosis water
Species/Strain:	Beagle dogs
Number/Sex/Group:	6/sex/group except at LD 4/sex.
Age:	10 months
Weight:	M: 7.1-9.7 kg; F: 5.5-8.6 kg
Satellite groups:	None
Unique study design:	None

Deviation from study protocol: None that affected the overall interpretation of study findings or compromised the integrity of the study

Observations

Table 27 Experimental Design for the 39-W Dog Study

(Excerpted from Submission)

Group	No. of Animals[a] Male	Female	Dose Level (mg/kg/day)	Dose Concentration[b] (mg/mL)
1 (Control)[c]	6	6	0	0
2 (Low)	4	4	1	0.2
3 (Mid)	6	6	5	1.0
4 (High)	6	6	10	2.0

a All surviving animals, except 2 animals/sex in Group 1 and all surviving animals in Group 2 were euthanized on Day 274 of the dosing phase (Terminal Euthanasia 2). All surviving animals in Group 3 and all surviving animals, except 3 animals/sex in Group 4 were euthanized on Day 162 of the dosing phase (Terminal Euthanasia 1). The last 2 animals/sex in Group 1 and last 3 animals/sex in Group 4 underwent recovery beginning on Day 162 of the dosing phase (also considered Day 1 of recovery phase); these animals were assigned to the recovery euthanasia (Study Day 274; Day 113 of the recovery phase).

b Concentrations were corrected for salt content and lot-specific purity. A correction factor of 1.243 was used.

c Group 1 received vehicle control article only (0.5% (w/v) methylcellulose (4000 cps) in reverse osmosis water).

Results

Mortality

A total of 8 males and 3 females were euthanized in moribund condition during the dosing phase (See Table 28). Two HD males presented clinical signs of vomitus, abnormal feces, hypoactivity, dehydration, excessive salivation, and clenched teeth along with significant body weight loss and reduced food consumption during Week 2. Canned food supplementation was offered, and dosing holiday started on Day 15. These dogs did not recover and were euthanized on Day 19. Canned food supplementation was offered to the 10 mg/kg/day starting on Day 23.

Table 28 Incidence of Glasdegib-related Mortality in the 39-W Dog Study

Glasdegib (mg/kg/day)	Sex	Study Day 19	112	114	121	126	138	141	155	Total # of Early Decedents
1	Males			1						1
	Females					1				1
5	Males		1		1		1		1	4
	Females						1	1		2
10	Males	2							1	3
	Females									0

The remaining unscheduled moribund sacrifices occurred during Weeks 16-23 and were associated with significant body weight loss, reduced or no food consumption, poor body condition (thin appearance), and/or evidence of dehydration; hypoactivity;

periodic vomitus; abnormal feces (liquid, mucoid); thinning hair coat; discolored (red) skin around the head, feet, muzzle, or legs; and cold to touch. All early decedents went through a dosing holiday for one or more days.

Glasdegib target organs of toxicity in early decedents included the kidney and liver with corresponding clinical pathology findings of azotemia and glucosuria.

Clinical Signs

In addition of the glasdegib-related and adverse clinical signs described for early decedents, other clinical signs included:

- At ≥1 mg/kg/day, skin and pelage observations (discolored red or blue skin; dry skin; alopecia; thinning hair coat on the entire body, muzzle, ears, eyes, lips, feet, legs, ventral cervical or thorax, perioral, or periorbital areas; or oily hair coat), evidence of dehydration, clear or cloudy ocular discharge. Skin findings were less prominent over the course of the dosing phase and did not present microscopic correlates for the skin/subcutis except for atrophy of the hair shafts, which the Applicant claims is a pharmacological effect.
- At ≥5 mg/kg/day, hypoactivity, abnormal (liquid, mucoid, discolored orange) feces, and cold to touch (5 mg/kg/day females).
- At 10 mg/kg/day, excessive salivation and vomitus.

The incidence and severity of most of glasdegib-related clinical signs were reduced over the course of the 16-W recovery phase.

Body Weights

Glasdegib-related body weight loss occurred starting on Week 2 of the dosing phase for 10 mg/kg/day dogs and later for ≥1 mg/kg/day individual dogs that corresponded with reduced food consumption and often unscheduled euthanasia. By the end of the 16-W recovery phase, body weight of 10 mg/kg/day dogs remained stable while control dogs gained weight.

Feed Consumption

Glasdegib-related reduced food consumption occurred starting on Week 2 of the dosing phase for 10 mg/kg/day dogs and later for ≥1 mg/kg/day individual dogs that corresponded with body weight loss and often unscheduled euthanasia. Canned food supplementation was offered at different starting dates to all dogs including control dogs and quantitative food consumption was not conducted.

Ophthalmoscopy

No glasdegib-related ophthalmic findings occurred during the dosing phase and were not conducted during the recovery phase.

ECG

Changes of small magnitude occurred in 1 mg/kg/day dogs:

- statistically decreased mean PR in females predose and 1-2 hours postdose on Day 135 and predose on Day 268 of the dosing phase compared with controls,

- statistically decreased mean QTc in males predose on Day 268 of the dosing phase compared with controls.

All the electrocardiograms evaluated in this study were qualitatively and quantitatively considered normal for the canine. No arrhythmias were found. No glasdegib-related abnormal electrocardiographic findings occurred.

Hematology

The only consistent glasdegib-related hematology effect in the 11 dogs euthanized at an unscheduled interval was increased red cell mass that corresponded with dehydration. Hematocrit values were ≥1.6X in early decedents. Increased fibrinogen concentrations (2.8X and 2.3X baseline, respectively) indicative of inflammation occurred only in the two 10 mg/kg/day males euthanized on Day 19 (See Table 28).

Clinical Chemistry

Glasdegib-related serum chemistry effects in the 11 animals euthanized at an unscheduled interval included increased urea nitrogen (≥1.31X) and creatinine concentrations (≥1.7X) in 10 mg/kg/day dogs, and increased ALT (up to 3.7X baseline on Days 90 and/or 160 in 1 or 5 mg/kg/day males), AST (up to 3.7X baseline), ALP, and GGT activities as well as increased cholesterol (up to 1.7X baseline on Days 21 and 90 in 5 mg/kg/day males and 10 mg/kg/day females). One male early decedent at 10 mg/kg/day had ≥10X increased urea nitrogen and creatinine concentration that was accompanied with the highest inorganic phosphorus concentration and lowest potassium and chloride concentrations.

Urinalysis

Glasdegib-related urinalysis effect in early decedents included urine glucose (which is a rare event in dogs) in 7/11 dogs and the presence granular casts in the urine sediment of 2/11 dogs. These findings indicated effects on kidney function and integrity. Glasdegib-related urinalysis effect in surviving dogs included the presence of granular casts in urine sediment in one 5 mg/kg/day male and one 10 mg/kg/day female. In all cases, granular cases, corresponded with the kidney microscopic findings of tubular degeneration/necrosis and granular/cellular casts. Granular casts were not present in the urine sediment of 10 mg/kg/day dogs at the end of the recovery sacrificed.

Gross Pathology

Glasdegib-related macroscopic observations in early decedents and surviving dogs at terminal sacrifice included discolored kidney in ≥5 mg/kg/day dogs with microscopic correlates of tubular degeneration/necrosis and regeneration; alopecia that corresponded with microscopic findings of hair shaft atrophy; discoloration (red, dark red, black, or brown) of 1 or more segments of the gastrointestinal tract (stomach, duodenum, jejunum, ileum, and colon) at ≥1 mg/kg/day. All macroscopic findings were resolved at the end of the recovery sacrificed.

Organ Weights

Organ weights were collected at the terminal euthanasia on Day 274 of the dosing phase (Terminal Euthanasia 2) for designated control and surviving 1 mg/kg/day dogs (Group 2) and at the recovery euthanasia on Day 113 of the recovery phase for remaining control and surviving 10 mg/kg/day dogs (Group 4).

Adrenals to brain and body weight ratios were significantly increased 1.33X and 1.44X, respectively, in 1 mg/kg/day males at the terminal euthanasia. The brain weight/body weight ratio was significantly decreased, 0.84X, in 1 mg/kg/day males. The significance of the brain weight changes is uncertain in the absence of terminal euthanasia for the MD and HD data.

Histopathology

Adequate Battery: Yes
Peer Review: Yes
Histological Findings
Early mortality of 5 or 10 mg/kg/day dogs resulted in the cessation of dosing and the necropsy on Week 24. A subset of 10 mg/kg/day dogs underwent a 16-week recovery phase. Dosing continued for 39 weeks for surviving 1 mg/kg/day dogs.

Glasdegib-related microscopic findings in early decedents and at 5 or 10 mg/kg/day terminal sacrifice occurred in the kidney, liver, skin/subcutis, gut-associated lymphoid tissue (GALT)/Peyer's Patch, thymus, mesenteric lymph node, pancreas, and prostate (See Table 29). Additionally, vascular/perivascular inflammation in the stomach, uterus, and oviduct of one female administered 5 mg/kg/day and euthanized in moribund condition was considered most likely secondary to glasdegib-related kidney failure/uremia. Glasdegib-related microscopic findings in 1 mg/kg/day dogs and euthanized on Day 274 of the dosing phase occurred in the liver (See Table 30).

Table 29 Incidence and Severity of Glasdegib-related Microscopic Findings in the 39-W Dog Study - Terminal Necropsy 5 and 10 mg/kg/day

(Excerpted from Submission)

Tissue/ Observation	Group/Sex: Number of Animals:	3/M 2	4/M 0	3/F 4	4/F 3
Brain	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	1
Dilatation, ventricles					
	finding not present -	2	0	4	2
	slight 2	0	0	0	1
	Total Incidence:	0	0	0	1
Infiltrate, mononuclear cell, perivascular					
	finding not present -	2	0	3	2
	minimal 1	0	0	1	1
	Total Incidence:	0	0	1	1
Cecum	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	3
Hemorrhage, mucosa					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
Cervix	Number Examined:	0	0	4	3
	Unremarkable:	0	0	4	3
Colon	Number Examined:	2	0	4	3
	Unremarkable:	2	0	4	3
Duodenum	Number Examined:	2	0	4	3
	Unremarkable:	1	0	2	1
Congestion					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Ectasia, crypt/gland					
	finding not present -	1	0	2	1
	minimal 1	1	0	2	2
	Total Incidence:	1	0	2	2
Gall Bladder	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	3
Infiltrate, mononuclear cell					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
GALT/Peyer's Patch	Number Examined:	2	0	4	3
	Unremarkable:	1	0	2	2
Hemorrhage					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Lymphoglandular complex, increased					
	finding not present -	1	0	2	2
	minimal 1	1	0	2	1
	Total Incidence:	1	0	2	1
Heart	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	3
Degeneration, myofiber					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Hemorrhage					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
Infiltrate, mononuclear cell					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
Ileum	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	3
Ectasia, crypt/gland					
	finding not present -	2	0	3	3
	slight 2	0	0	1	0
	Total Incidence:	0	0	1	0
Jejunum	Number Examined:	2	0	4	3
	Unremarkable:	1	0	4	3
Congestion					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Kidney	Number Examined:	2	0	4	3
	Unremarkable:	0	0	0	0
Cast, granular/cellular					
	finding not present -	2	0	4	2
	moderate 3	0	0	0	1
	Total Incidence:	0	0	0	1
Cast, proteinaceous					
	finding not present -	2	0	4	2
	minimal 1	0	0	0	1
	Total Incidence:	0	0	0	1
Degeneration/necrosis, tubule					
	finding not present -	0	0	4	1
	minimal 1	2	0	0	1
	moderate 3	0	0	0	1
	Total Incidence:	2	0	0	2
Dilatation, tubule(s)					
	finding not present -	2	0	4	2
	slight 2	0	0	0	1
	Total Incidence:	0	0	0	1
Infiltrate, mononuclear cell					
	finding not present -	2	0	3	2
	minimal 1	0	0	1	0
	slight 2	0	0	0	1
	Total Incidence:	0	0	1	1
Lipid, glomerulus					
	finding not present -	2	0	4	2
	Present	0	0	0	1
Mineralization, tubule					
	finding not present -	0	0	1	1
	minimal 1	2	0	3	2
	Total Incidence:	2	0	3	2

Continued…

Tissue/ Observation	Group/Sex: / Number of Animals:	3/M 2	4/M 0	3/F 4	4/F 3
Kidney	Number Examined:	2	0	4	3
	Unremarkable:	0	0	0	0
Pigment, tubule cell					
	finding not present -	1	0	2	2
	minimal 1	1	0	2	1
	Total Incidence:	1	0	2	1
Regeneration, tubule cell					
	finding not present -	0	0	4	0
	minimal 1	1	0	0	2
	slight 2	1	0	0	0
	moderate 3	0	0	0	1
	Total Incidence:	2	0	0	3
Vacuolation, tubule cell					
	finding not present -	2	0	4	1
	minimal 1	0	0	0	2
	Total Incidence:	0	0	0	2
Liver	Number Examined:	2	0	4	3
	Unremarkable:	0	0	0	0
Hypertrophy, Ito cell					
	finding not present -	2	0	4	1
	minimal 1	0	0	0	2
	Total Incidence:	0	0	0	2
Infiltrate, mononuclear cell					
	finding not present -	2	0	0	0
	minimal 1	0	0	4	3
	Total Incidence:	0	0	4	3
Inflammation, mixed cell, centrilobular/midzonal					
	finding not present -	0	0	3	1
	minimal 1	0	0	1	0
	slight 2	0	0	0	1
	moderate 3	2	0	0	1
	Total Incidence:	2	0	1	2
Pigment, Kupffer cell/macrophage					
	finding not present -	0	0	2	1
	minimal 1	0	0	2	1
	slight 2	2	0	0	1
	Total Incidence:	2	0	2	2
Single cell necrosis, hepatocyte					
	finding not present -	0	0	4	1
	minimal 1	2	0	0	1
	slight 2	0	0	0	1
	Total Incidence:	2	0	0	2
Vacuolation, hepatocyte					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
Vacuolation, hepatocyte, centrilobular					
	finding not present -	1	0	4	2
	minimal 1	0	0	0	1
	slight 2	1	0	0	0
	Total Incidence:	1	0	0	1

Tissue/ Observation	Group/Sex: / Number of Animals:	3/M 2	4/M 0	3/F 4	4/F 3
Lung	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	1
Hemorrhage					
	finding not present -	2	0	3	3
	moderate 3	0	0	1	0
	Total Incidence:	0	0	1	0
Hyperplasia, epithelium, terminal bronchial					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Infiltrate, macrophages, alveolus					
	finding not present -	2	0	3	2
	minimal 1	0	0	0	1
	slight 2	0	0	1	0
	Total Incidence:	0	0	1	1
Infiltrate, mixed cell					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Inflammation					
	finding not present -	2	0	3	2
	slight 2	0	0	0	1
	marked 4	0	0	1	0
	Total Incidence:	0	0	1	1
Lymph Node, Mesenteric	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	1
Erythrocytes, sinus					
	finding not present -	1	0	3	1
	minimal 1	1	0	0	2
	slight 2	0	0	1	0
	Total Incidence:	1	0	1	2
Lymph Node, Popliteal	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	2
Erythrocytes, sinus					
	finding not present -	2	0	3	2
	minimal 1	0	0	0	1
	slight 2	0	0	1	0
	Total Incidence:	0	0	1	1
Mammary Gland	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	2
Ectasia, duct					
	finding not present -	2	0	3	2
	minimal 1	0	0	1	0
	slight 2	0	0	0	1
	Total Incidence:	0	0	1	1

Continued…

Tissue/ Observation	Group/Sex: / Number of Animals:	3/M 2	4/M 0	3/F 4	4/F 3
Mandibular Salivary Gland	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	2
Infiltrate, mononuclear cell					
	finding not present -	1	0	3	2
	minimal 1	0	0	1	1
	slight 2	1	0	0	0
	Total Incidence:	1	0	1	1
Skin/Subcutis	Number Examined:	2	0	4	3
	Unremarkable:	0	0	1	1
Acanthosis					
	finding not present -	1	0	4	2
	minimal 1	1	0	0	0
	slight 2	0	0	0	1
	Total Incidence:	1	0	0	1
Atrophy, hair shaft					
	finding not present -	0	0	1	1
	slight 2	2	0	3	2
	Total Incidence:	2	0	3	2
Ectasia, follicular					
	finding not present -	1	0	2	2
	minimal 1	1	0	1	1
	slight 2	0	0	1	0
	Total Incidence:	1	0	2	1
Erosion/ulcer					
	finding not present -	2	0	4	2
	slight 2	0	0	0	1
	Total Incidence:	0	0	0	1
Granuloma, hair shaft					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Infiltrate, mononuclear cell					
	finding not present -	0	0	3	2
	minimal 1	2	0	1	1
	Total Incidence:	2	0	1	1
Inflammation					
	finding not present -	2	0	4	2
	slight 2	0	0	0	1
	Total Incidence:	0	0	0	1
Spinal Cord	Number Examined:	2	0	4	3
	Unremarkable:	2	0	4	3
Spleen	Number Examined:	2	0	4	3
	Unremarkable:	1	0	1	3
Pigment					
	finding not present -	1	0	1	3
	minimal 1	1	0	3	0
	Total Incidence:	1	0	3	0
Sternum	Number Examined:	2	0	4	3
	Unremarkable:	2	0	4	3
Stomach	Number Examined:	2	0	4	3
	Unremarkable:	2	0	4	3
Thymus	Number Examined:	2	0	4	3
	Unremarkable:	0	0	1	0
Cyst					
	finding not present -	1	0	3	2
	minimal 1	1	0	0	1
	slight 2	0	0	1	0
	Total Incidence:	1	0	1	1
Decreased cellularity, lymphocytes					
	finding not present -	1	0	1	0
	minimal 1	0	0	1	1
	slight 2	1	0	2	1
	moderate 3	0	0	0	1
	Total Incidence:	1	0	3	3
Hemorrhage					
	finding not present -	0	0	2	3
	minimal 1	1	0	2	0
	slight 2	1	0	0	0
	Total Incidence:	2	0	2	0
Thyroid	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	0
Cyst, follicle					
	finding not present -	2	0	3	2
	minimal 1	0	0	1	1
	Total Incidence:	0	0	1	1
Decreased, colloid					
	finding not present -	2	0	4	1
	minimal 1	0	0	0	2
	Total Incidence:	0	0	0	2
Hyperplasia, C-cell					
	finding not present -	1	0	4	1
	minimal 1	0	0	0	2
	slight 2	1	0	0	0
	Total Incidence:	1	0	0	2

Table 30 Incidence and Severity of Glasdegib-related Microscopic Findings in the 39-W Dog Study - Terminal Necropsy 0 and 1 mg/kg/day

(Excerpted from Submission)

Tissue/ Observation	Group/Sex: Number of Animals:	1/M 4	2/M 3	1/F 4	2/F 3
Adrenal, Cortex	Number Examined:	4	3	4	3
	Unremarkable:	2	3	3	0
Vacuolation, zona fasciculata					
	finding not present -	4	3	3	0
	minimal 1	0	0	1	3
	Total Incidence:	0	0	1	3
Liver	Number Examined:	4	3	4	3
Inflammation, mixed cell, centrilobular/midzonal					
	finding not present -	4	1	4	3
	minimal 1	0	2	0	0
	Total Incidence:	0	2	0	0
Pigment, Kupffer cell/macrophage					
	finding not present -	4	0	4	3
	minimal 1	0	3	0	0
	Total Incidence:	0	3	0	0
Vacuolation, hepatocyte					
	finding not present -	3	3	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Vacuolation, hepatocyte, centrilobular					
	finding not present -	4	1	4	3
	minimal 1	0	2	0	0
	Total Incidence:	0	2	0	0
Lung	Number Examined:	4	3	4	3
	Unremarkable:	3	2	3	2
Foreign material					
	finding not present -	4	2	4	3
	Present	0	1	0	0
Infiltrate, macrophages, alveolus					
	finding not present -	4	3	3	2
	minimal 1	0	0	1	1
	Total Incidence:	0	0	1	1
Inflammation, granulomatous					
	finding not present -	4	2	4	3
	minimal 1	0	1	0	0
	Total Incidence:	0	1	0	0
Skin/Subcutis	Number Examined:	4	3	4	3
	Unremarkable:	4	2	3	3
Acanthosis					
	finding not present -	4	2	4	3
	slight 2	0	1	0	0
	Total Incidence:	0	1	0	0
Crust, serocellular					
	finding not present -	4	2	4	3
	minimal 1	0	1	0	0
	Total Incidence:	0	1	0	0
Erosion/ulcer					
	finding not present -	4	2	4	3
	moderate 3	0	1	0	0
	Total Incidence:	0	1	0	0

Special Evaluation

None

Toxicokinetics

Due to early termination, blood samples were collected and toxicokinetic parameters calculated for LD, MD and HD on Day 1, during Week 13 and on Day 160 and for the LD during Week 39. Plasma preparations were analyzed with a validated bioanalytical method under Study 6348-660 "Method Validation for Glasdegib Dog Plasma using LC/MS/MS." The calibration standard data, quality control (QC) sample data, incurred sample reanalysis (ISR) data, and chromatograms indicate the method performed acceptably during the sample analysis phase. Systemic exposure, as assessed by C_{max} and AUC_{0-24} increased with increasing dose although the increase was generally greater than dose proportional at all intervals. There were no apparent gender-related differences in exposure or accumulation of glasdegib over time after repeated exposure. Mean T_{max} ranged from 0.92 to 1.3 hours on Day 1, during Week 13, and on Day 160.

Table 31 Mean Toxicokinetic Parameters for Glasdegib in Combined Sex in the 39-W Dog Study: Day 1 and Week 13

(Excerpted from Submission)

Interval	Dose Group	Dose Level (mg/kg/day)		C_{max} (ng/mL)	T_{max} (hr)	AUC_{24} (ng·hr/mL)	AUC_{24}/Dose [(ng·hr/mL)/(mg/kg/day)]
Day 1	2	1	Mean	137	0.94	692	692
			SD	18.7	0.50	236	236
			N	8	8	8	8
	3	5	Mean	1830	0.92	9060	1810
			SD	273	0.42	2740	548
			N	12	12	12	12
	4	10	Mean	5050	1.0	35200	3520
			SD	892	0.0	10900	1090
			N	12	12	12	12
Week 13	2	1	Mean	139	1.1	834	834
			SD	29.9	0.78	327	327
			N	8	8	8	8
	3	5	Mean	2340	1.2	13000	2600
			SD	892	0.39	6020	1200
			N	12	12	12	12
	4	10	Mean	4560	1.2	20100	2010
			SD	1430	0.63	15300	1530
			N	10	10	10	10

AUC_{24}	Area under the concentration-time curve from hour 0 to hour 24.
AUC_{24}/Dose	Area under the concentration-time curve from hour 0 to hour 24 divided by dose level.
C_{max}	Maximum concentration in plasma.
N	Number of values in calculation.
SD	Standard deviation.
T_{max}	Time to maximum plasma concentration.

Table 32 Mean Toxicokinetic Parameters for Glasdegib in Combined Sex in the 39-W Dog Study: Day 160 and Week 39

(Excerpted from Submission)

Interval	Dose Group	Dose Level (mg/kg/day)		C_{max} (ng/mL)	T_{max} (hr)	AUC_{24} (ng·hr/mL)	AUC_{24}/Dose [(ng·hr/mL)/ (mg/kg/day)]
Day 160	2	1	Mean	112	0.92	381	381
			SD	22.7	0.59	65.3	65.3
			N	6	6	6	6
	3	5	Mean	1400	1.3	6300	1260
			SD	498	0.52	3390	678
			N	6	6	6	6
	4	10	Mean	4070	1.2	20000	2000
			SD	606	0.62	13200	1320
			N	9	9	9	9
Week 39	2	1	Mean	95.9	0.67	372	372
			SD	39.0	0.26	110	110
			N	6	6	6	6

AUC_{24}	Area under the concentration-time curve from hour 0 to hour 24.
AUC_{24}/Dose	Area under the concentration-time curve from hour 0 to hour 24 divided by dose level.
C_{max}	Maximum concentration in plasma.
N	Number of values in calculation.
SD	Standard deviation.
T_{max}	Time to maximum plasma concentration.
Note:	Groups 3 and 4 were sacrificed prior to the Week 39 TK collection.

Dosing Solution Analysis

All dose formulations from the Day 1 (0.2 and 2 mg/mL) and Day 267 (0.2 mg/mL) met specifications and were considered homogeneous. The RSD ranged from 1.1%-4.7%. All dose formulations from the Day 1 and Weeks 10, 20, 30, and 39 preparations (Days 1, 64, 134, 204 and 267, respectively) were within +10% of the target concentrations (mean values ranging from 97.7%-106.5% of theoretical). No glasdegib was detected in the control vehicle.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Assay with a Confirmatory Assay

Study no.:	6348-654 (08GR352)
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 5, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; GR01590 121657-119-8; 99.8%

Key Study Findings

- Using a plate incorporation method with *Salmonella* strains (TA98, TA100, TA1535, and TA1537) and *E. coli* tester strain WP2uvrA(pKM101), glasdegib did not induce genotoxic responses with or without S9 metabolic activation. This study used the highest level recommended by ICH S2(R1) (5.0 mg/plate). The results are considered valid and adequate.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Genetic Toxicology Human Lymphocyte Assay of PF-04449913-01 (Clastogenicity Assay)

Study no.:	09GR016
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	Pfizer Global Research & Development Safety Sciences Genetic Toxicology Groton, CT
Date of study initiation:	February 2, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913-01; GLASDEGIB-01-0014; 99.04% (Potency 77.6%)

Key Study Findings

- Using cultured primary human lymphocytes, glasdegib did not induce chromosomal aberrations or increases of numerical chromosome changes with or without S9 metabolic activation when tested up to concentrations that

produced marked mitotic suppression (≥58%). The results are considered valid and adequate.

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

Study title: 1-Month Oral Toxicity Study of PF-04449913 in Rats with 1-Month Recovery with Micronucleus Assessment

(Parts were adapted from IND 105453 Dr. T. Kropp review)

Study no.:	6348-650 (08LJ094)
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 26, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; 121657-119-8; 98% (Potency factor of 0.776)

Key Study Findings

Glasdegib was negative for inducing micronuclei in the rat bone marrow.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Not conducted

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not conducted

9.2 Embryonic Fetal Development

Study title: A Preliminary Embryo-Fetal Development Study of PF-04449913 by Oral Gavage in Rats

Study no.:	20069225 (15GR056)
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 10, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; E010015565; Chiral potency factor 0.761.

Key Study Findings

- Glasdegib produced fetal toxicity in rats that included increased post-implantation loss at ≥10 mg/kg/day and lower embryo-fetal survival and increased post-implantation loss at ≥50 mg/kg/day at maternal exposures approximately 0.6-times the human exposure at the recommended dose based on total C_{max}.
- Glasdegib is a teratogen in rats based on a higher incidence of skeletal anomalies at ≥10 mg/kg/day, and higher incidences of external, visceral, and skeletal malformations and variations at ≥50 mg/kg/day.
- Teratogenicity in rats occurred in the absence of maternal toxicity at maternal exposures of approximately 0.6-times the human exposure at the recommended dose based on total plasma concentrations at 10 mg/kg/day of 726 ng/mL.

Methods

Doses:	10, 50, or 100 mg/kg/day on GD 7 though GD 17. Doses based on 13-w repeat-dose toxicology study 8299265 (14LJ055) in rats.
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose (4000 cps) in reverse osmosis water
Species/Strain:	Crl:CD(SD) Sprague Dawley rat
Number/Sex/Group:	8 time-mated females/group
Satellite groups:	None
Study design:	Standard. Cesarean section conducted on GD 21. Plasma concentration analysis on GD 17 4 hours after dosing.
Deviation from study protocol:	None that had an impact on study data or interpretation of results.

Observations

Table 33 Experimental Design of EFD in Rats

(Excerpted from Submission)

Group No.	Test Article	PF-04449913 Doses (mg/kg/day)[a]	PF-04449913 Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Rats (Assigned Female Rat Numbers)
1	Vehicle Control Article	0	0	10	8 (161-168)
2	PF-04449913	10	1	10	8 (169-176)
3	PF-04449913	50	5	10	8 (177-184)
4	PF-04449913	100	10	10	8 (185-192)

[a] The potency of PF-04449913 was 0.761. Dose calculations were corrected for potency.

Results

Mortality

All rats survived to scheduled euthanasia on GD 21.

Clinical Signs

Single 100 mg/kg dams presented an increase in activity and abnormal breathing sounds on GD 9. Vaginal discharge occurred in two 100 mg/kg dams on GD 19 and 20.

Body Weight

Glasdegib dose-related decrease in body weight gain occurred at 50 mg/kg (↓0.48x) and 100 mg/kg (↓0.65x) for the interval GD 7 through GD 21 that was attributed to significant lower gravid uterus weight (↓0.82x and 0.95x, respectively).

Table 34 Maternal Body Weight Gains in Pregnant Rats

(Excerpted from Submission)

Bodyweight Gain (Interval)

Sex: Female		Day(s) Relative to Mating (L)						
		7 → 10	10 → 12	12 → 15	15 → 18	7 → 18	18 → 21	7 → 21
0 mg/kg/day	Mean	10.6	13.1	19.3	36.3	79.3	52.4	131.7
	SD	3.3	4.6	6.0	6.0	10.3	10.2	19.3
	N	7	7	7	7	7	7	7
10 mg/kg/day	Mean	15.0	13.0	21.3	39.0	88.3	53.1	141.4
	SD	2.8	2.7	2.5	4.4	7.0	7.7	8.2
	N	8	8	8	8	8	8	8
50 mg/kg/day	Mean	14.8	14.0	15.4	16.6	60.8	7.8	68.5
	SD	6.0	3.8	5.2	6.5	10.6	4.1	11.4
	N	8	8	8	8	8	8	8
100 mg/kg/day	Mean	8.5	16.4	10.9	13.3	49.0	-2.8	46.3
	SD	5.3	4.4	4.1	2.6	8.1	8.0	9.2
	N	8	8	8	8	8	8	8

Feed Consumption

Mean maternal absolute food consumption in the 100 mg/kg/day dose group was lower than the control group mean for the interval of GD 7 to 8 (↓0.46x), GD 7 to 10 (↓0.12x) and GD 20 to 21 (↓0.24x). The lower mean food consumption was not considered an adverse effect of glasdegib because food consumption values were similar to the control group mean for the remainder of the dose period and there was no overall effect on mean food consumption for the interval of GD 7 to 21.

Toxicokinetics

A single blood sample was collected from the jugular vein at 4 hours postdose on GD 17, from the first three available females in the control group and the first five available females in glasdegib-groups. Plasma samples were analyzed for concentration of glasdegib using a validated analytical procedure (b) (4) Method No. P13RPP (See Table 35).

Table 35 Summary of Glasdegib Plasma Concentrations (ng/mL) on GD 17 in Pregnant Rats

(Excerpted from Submission)

Time Point: 4 hours postdose	Dose (mg/kg/day)		
	10	50	100
Mean	726	4502	7136
SD	359	1371	2224
n	5	5	5

The Applicant calculated the total plasma concentration of 726 ng/mL and 4502 ng/mL in rats at 10 mg/kg/day and 50 mg/kg/day, respectively. In previously untreated patients with AML or high-risk MDS, exposure of glasdegib at 100 mg QD in combination with LDAC, expressed as total C_{max}, was 1252 ng/mL. Fetal toxicity and teratogenicity occurred at maternal exposure of approximately 0.6-times the human exposure based on total plasma concentrations in rats at 10 mg/kg/day of 726 ng/mL.

Dosing Solution Analysis

The relative standard deviation of each concentration was ≤ 10% and the mean sample concentration results were within ± 15% of the theoretical concentration, meeting specifications for homogeneity and concentration verification. No significant detection of glasdegib occurred in the vehicle control samples.

Necropsy

No glasdegib-related maternal macroscopic observations. Placenta exams were normal.

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

The primary glasdegib-related effects were lower embryo-fetal viability and mean fetal body weights and higher incidences of fetal external, visceral, and/or skeletal malformations and/or variations. Glasdegib-related and significant effects occurred in all ovary and uterus parameters examined at 50 and 100 mg/kg/day (See Table 36). Overall, the higher number of resorptions resulted in higher mean postimplantation loss at 50 and 100 mg/kg/day (88.36% and 100%, respectively, compared with 4.72% in controls). No live fetuses were available for evaluations at 100 mg/kg/day due to total litter loss in all dams. Mean fetal body weights (male, female, and sexes combined) in the 50 mg/kg/day dose group were lower (0.62x) than control group means (See Table 37).

Table 36 Summary of Laparohysterectomy Evaluations in Rats

Glasdegib Dose (mg/kg/day)	0	10	50	100
Number of females tested	8	8	8	8
Number of females pregnant	7	8	8	8
Unscheduled euthanasia	0	0	0	0
Early delivery	0	0	0	0
Number of litters examined	7	8	5	0
Total number of resorptions N	0.6	0.9	8.0	12.5
Mean number Corpora lutea per liter	13.1	14.4	12.4	13.3
Mean number of Implantations	13.0	13.5	11.0	12.5
Mean Percent pre-implantation loss	1.0	4.8	11.9	3.7
% Live fetuses	12.4	12.6	1.1	0

Glasdegib Dose (mg/kg/day)	0	10	50	100
Post-implantation loss				
Mean Early resorptions/litter	0.6	0.9	8.0	12.5
Mean Late resorptions/litter	0.0	0.0	1.9	0.0
Mean Percent post-implantation loss	4.7	6.4	88.4	100.0

Percent Post-implantation loss = [(# implantations - # live fetuses) / # implantations] x 100

Table 37 Summary of Litter Observations in Rats

Glasdegib Dose (mg/kg/day)	0	10	50	100
Number of females pregnant	7	8	8	8
% Live fetuses	12.4	12.6	1.1	0
Sex distribution				
Mean # of Males	5.7	5.6	0.2	
Mean # of Females	6.7	6.9	1.6	
Mean fetal body weight (g) per litter	5.784	5.955	3.611	
Male fetuses	5.987	6.148	3.910	
Female fetuses	5.609	5.809	3.567	

Fetal Evaluations

Fetal observations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development).

Fetal evaluations were based on 87, 101, and 9 viable GD 21 cesarean-delivered fetuses in 7, 8, and 5 litters in the 0, 10, and 50 mg/kg/day groups, respectively. Each of these fetuses was examined for external, visceral (fixed head and/or fresh Staples), and skeletal (intact or body only) abnormalities. Total glasdegib-related litter loss at 100 mg/kg/day precluded fetal external, visceral, and skeletal examinations at this dose.

Fetal Examinations (External, Visceral and Skeletal)

No glasdegib-related external malformations or variations at 10 mg/kg/day, but at skeletal examinations there were delays in skeletal ossification, structural changes in the ribs, and one fetus had a few skeletal malformations (absent ribs, a fused lumbar vertebra and a fused thoracic vertebra) that were similar to findings noted at 50 mg/kg/day. The skeletal malformations noted at 10 mg/kg/day were considered glasdegib-related since these findings were considered part of the larger spectrum of anomalies at 50 mg/kg/day.

All 9 viable fetuses from 5 litters in the 50 mg/kg/day group were available for evaluation, and each fetus had multiple external malformations and/or variations (See Table 37).

- The most frequently observed glasdegib-related external malformations, which occurred in 89% to 100% of the fetuses available for examination in the 50 mg/kg/day group, consisted of small mouth, misshapen snout, short fore- and/or hindlimbs, absent fore and/or hindpaw digits, short fore and/or hindpaw digits, and a short tail. Additional malformations consisted of malformed eyes (malpositioned, open, depressed eye bulge), face (small mouth, snout misshapen), trunk (short trunk), and tail (narrow, thread-like). Most of the external malformations were confirmed to be the result of underlying skeletal dysmorphogenesis.
- The most frequently observed glasdegib-related visceral malformations consisted of severe dilation of the lateral ventricles of the brain, a malpositioned eye, a misshapen head (rhinocephaly), absent palatal rugae, a small tongue, an absent tooth (upper incisors), an interrupted nasopharynx, malformed adrenals (absent, large, small, and/or malpositioned), a malpositioned aorta, a malformed esophagus (atretic or narrow), large ovaries, persistent truncus arteriosus, a ventricular septum defect, malformed kidneys (absent, malpositioned, small), a misshapen liver, a malformed liver (absent, small), a narrow pulmonary trunk, a retroesophageal subclavian artery, an absent trachea, an absent ureter, and an absent urinary bladder.
- The most frequently observed glasdegib-related skeletal malformations, which occurred in 78% to 100% of the fetuses available for examination in the 50 mg/kg/day dose group, consisted of multiple rib or vertebral abnormalities, absent fore- and/or hindpaw phalanges, short long bones (humerus, radius, ulna, femur, tibia), an unossified fibula, a small scapula body, and absent metatarsals. Additional malformations consisted of fused skull bones (mandible and maxilla), a palatine cleft, an absent premaxilla, and a bent clavicle.

Table 38 Summary of Glasdegib-Related Abnormalities in Rats

Glasdegib Dose (mg/kg)		0	10	50
Fixed Head				
Total number of litters examined		7	8	5
Total number of fetuses examined		42	48	3
M- Brain: Severe dilated lateral ventricle	Litter incidence (%)	-	-	2 (66.7)
	Fetal incidence (%)	-	-	2 (66.7)
V- Brain: Moderate dilated lateral ventricle	Litter incidence (%)	-	-	1(33.3)
	Fetal incidence (%)	-	-	1(33.3)
M- Eye: Malpositioned	Litter incidence (%)	-	-	1(33.3)
	Fetal incidence (%)	-	-	1(33.3)
M- Head/neck: Head, misshapen	Litter incidence (%)	-	-	3(100.0)
	Fetal incidence (%)	-	-	3(100.0)
M- Mouth: Palatal rugae, Tongue absent, small	Litter incidence (%)	-	-	3(33.3)
	Fetal incidence (%)	-	-	3(33.3)
M- Mouth: Tooth, absent	Litter incidence (%)	-	-	3(100.0)
	Fetal incidence (%)	-	-	3(100.0)

M- Nasopharynx, interrupted	Litter incidence (%)	-	-	3(100.0)
	Fetal incidence (%)	-	-	3(100.0)
Fresh Visceral Body				
Total number of litters examined		7	8	5
Total number of fetuses examined		87	101	9
M- Adrenal Gland: absent, large, malpositioned	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	1(11.1)
M- Adrenal Gland: small	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	5(55.6)
M- Carotid artery: origin, malpositioned	Litter incidence (%)	-	-	2(40.0)
	Fetal incidence (%)	-	-	3(33.3)
M- Vessels : Truncus Arteriosus, persistent	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	1(11.1)
M- Esophagus: narrow	Litter incidence (%)	-	-	2(40.0)
	Fetal incidence (%)	-	-	2(22.2)
M- Gonad: Ovary large	Litter incidence (%)	-	-	2(40.0)
	Fetal incidence (%)	-	-	(2(22.2)
M- Heart: Ventricular Septum, defect	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	7(77.8)
V- Innominate Artery: absent	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	5(55.5)
M- Kidneys: absent	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	3(33.3)
M- Kidneys: small	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	2(22.0)
V- Kidneys: malpositioned	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	3(33.3)
M- Liver: misshapen	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	1(11.1)
M- Lung: absent	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	6(66.7)
M- Lung: small	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	5(55.6)
M- Pulmonary trunk: narrow	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	6(66.7)
V- Spleen: large	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	4(44.4)
M- Subclavian Artery: retroesophageal	Litter incidence (%)	-	-	2(40.0)
	Fetal incidence (%)	-	-	3(33.3)
V- Subclavian Artery: malpositioned	Litter incidence (%)	-	-	5(100.0)
	Fetal incidence (%)	-	-	7(77.8)
M-Trachea: absent	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	5(55.6)
M- Ureter: absent	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	3(33.3)
M- Urinary Bladder: absent	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	3(33.3)
Skeletal[1]				
Total number of litters examined		7	8	5
Total number of fetuses examined		87	101	9
M- Mandible: fused	Litter incidence (%)			4(80.0)

	Fetal incidence (%)			5(83.3)
M- Maxilla: fused	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			6(100.0)
M- Palatine: cleft	Litter incidence (%)			1(20.0)
	Fetal incidence (%)			1(16.7)
M- Premaxilla: absent	Litter incidence (%)			4(80.0)
	Fetal incidence (%)			5(83.3)
M- Forepaw phalanges: absent	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Humerus: bent	Litter incidence (%)			1(20.0)
	Fetal incidence (%)			1(11.1)
M- Humerus: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Metacarpal: absent	Litter incidence (%)			3(60.0)
	Fetal incidence (%)			4(44.4)
V- Metacarpal: unossified	Litter incidence (%)		1(12.5)	5(100.0)
	Fetal incidence (%)		1(1.0)	8(88.9)
M- Radius: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Ulna: unossified	Litter incidence (%)			1(20.0)
	Fetal incidence (%)			1(11.1)
M- Ulna: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0
M- Femur: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			8(88.9)
M- Fibula: unossified	Litter incidence (%)			4(80.0)
	Fetal incidence (%)			7(77.8)
M- Fibula: short	Litter incidence (%)			2(40.0)
	Fetal incidence (%)			3(33.3)
M- Hindpaw phalanges: absent	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Metatarsal: absent	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Tibia: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			8(88.9)
M- Tibia: unossified	Litter incidence (%)			1(20.0)
	Fetal incidence (%)			1(11.1)
V- Ischium: incomplete ossification	Litter incidence (%)		1(12.5)	4(80.0)
	Fetal incidence (%)		1(1.0)	7(77.8)
V- Pubis: incomplete ossification	Litter incidence (%)		1(12.5)	4(80.0)
	Fetal incidence (%)		1(1.0)	7(77.8)
M- Rib: multiple ribs	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
V- Rib: nodulated	Litter incidence (%)		2(25.0)	
	Fetal incidence (%)		2(2.0)	
V- Rib: wavy	Litter incidence (%)		1(12.5)	
	Fetal incidence (%)		1(1.0)	
V- Rib: incomplete ossification	Litter incidence (%)		4(50.0)	
	Fetal incidence (%)		5(5.0)	
V- Sternebra: asymmetric	Litter incidence (%)		1(12.5)	
	Fetal incidence (%)		1(1.0)	
M- Scapula body: small	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			8(88.9)

[1] Numerous skeletal variations occurred. Only skeletal malformations are listed except for few cases to document similar variations occurrence at 10 mg/kg and 50 mg/kg

Study title: A Preliminary Embryo-Fetal Development Study of PF-04449913 by Oral Gavage in Rabbits

Study no.:	20069230 (15GR057)
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 29, 2015
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	PF-04449913; E010015565; Chiral potency factor 0.761.

Key Study Findings

- Glasdegib produced maternal toxicity in rabbits based on maternal body weight losses and lower food intake at ≥5 mg/kg/day and abortions and elective euthanasia at ≥10 mg/kg/day.
- Glasdegib produced fetal toxicity and teratogenicity in rabbits at 5 mg/kg/day based on lower embryo-fetal survival, increased post-implantation loss, lower mean fetal body weights, and higher incidences of external, visceral, and skeletal malformations and variations at maternal exposures of approximately 2.5-times the human exposure at the recommended dose based on total C_{max} at 5 mg/kg/day of 3120 ng/mL.

Methods

Doses:	5, 10, 50, or 100 mg/kg/day on GD 7 though GD 19. Doses based on 13-w repeat-dose toxicology study 8299265 (14LJ055) in rats.
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose (4000 cps) in reverse osmosis water
Species/Strain:	New Zealand White [Crl:KBL(NZW)] rabbits
Number/Sex/Group:	8 time-mated females/group
Satellite groups:	None
Study design:	Standard. Cesarean section conducted on GD 29.
Deviation from study protocol:	None that had an impact on study data or interpretation of results.

Observations

Table 39 Experimental Design of EFD in Rabbits

(Excerpted from Submission)

Group No.	Test Article	PF-04449913 Dose (mg/kg/day)	PF-04449913 Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Rabbits (Assigned Rabbit Numbers)
1	Vehicle Control Article	0	0	10	8 (4901-4908)
2	PF-04449913	5	0.5	10	8 (4909-4916)
3	PF-04449913	10	1	10	8 (4917-4924)
4	PF-04449913	50	5	10	8 (4925-4932)
5	PF-04449913	100	10	10	8 (4933-4940)

Results

Mortality

None of the rabbits in the 10, 50, and 100 mg/kg/day dose groups survived to scheduled C-section.

Table 40 Summary of Glasdegib-Related Mortality in Rabbits

Glasdegib (mg/kg/day)	Number	GD	Reason
0	1	21	Aborted ↓BW/FC. Litter: 3 early/1 late resorptions, 2 live fetuses
5	1	12	Euthanized acute paralysis. ↓BW/FC. Litter: 6 live fetuses
	1	23	Aborted ↓BW/FC. Litter: 6 late resorptions, 2 live fetuses
10	1	19	Found dead. Red gelatinous material present in the thoracic cavity and a perforation in the right diaphragmatic lobe of the lung. Litter: 62.5% post-implantation loss.
	5	19-21	Aborted. ↓BW/FC. Litter: 100% early resorptions.
	2	23	Euthanized ↓BW and adverse clinical signs. One dam no pregnant. Litter: 83.3% post-implantation loss.
50	1	18	Euthanized. ↓BW and adverse clinical signs. Litter: 100% early resorptions.
	3	18-20	Aborted. Litter: 100% early resorptions.
	4	19-20	Euthanized ↓BW and adverse clinical signs. Litter: 100% early resorptions.
100	1	14	Euthanized ↓BW and adverse clinical signs. Litter: 100% early resorptions.
	7	16-17	Euthanized ↓BW and adverse clinical signs. Litter: 100% early resorptions.

Clinical Signs

Glasdegib (mg/kg/day)	Common Clinical Signs
0	None
5	Unknown etiology for the acute paralysis. Ungroomed fur and limited usage of the left and right hindlimbs

10	Soft feces, decreased fetal output, red discharge from the vagina (aborted tissue, liquid material), ungroomed fur, and fur loss
50	Absent feces/decreased fecal output, ungroomed coat, fur loss, and red aborted tissue and red liquid material.
100	Absent feces/decreased fecal output, ungroomed coat, fur loss, and red discharge from the vagina and red liquid material.

Body Weight

Glasdegib-related and adverse lower maternal body weight gain occurred at all doses. The mean maternal body weight gain in the 5 mg/kg/day surviving dams was 0.15x control for the interval of GD 7 to 29.

Table 41 Maternal Body Weight Gains in Pregnant Rabbits

(Excerpted from Submission)

Sex: Female		Day(s) Relative to Mating (L)			
		7 → 10	10 → 13	13 → 16	16 → 20
0 mg/kg/day	Mean	55.84	-5.95	22.19	-1.41
	SD	29.09	55.51	55.59	100.61
	N	8	8	8	8
5 mg/kg/day	Mean	-23.95	31.40	-26.57	-20.36
	SD	44.65	45.99	56.14	106.80
	N	8	7	7	7
10 mg/kg/day	Mean	68.69	27.23	-39.41	-207.04
	SD	59.44	67.68	14.48	158.98
	N	7	7	7	5
50 mg/kg/day	Mean	33.73	-38.95	-131.01	-108.03
	SD	85.84	100.27	51.88	175.01
	N	8	8	8	3
100 mg/kg/day	Mean	-37.36	-177.18	-200.87	-
	SD	64.07	93.57	46.82	-
	N	8	8	7	-

7 → 20	20 → 24	24 → 29	20 → 29	7 → 29
70.66	22.33	27.70	50.03	108.51
121.43	61.41	73.03	98.97	134.05
8	7	7	7	7
-28.56	-34.18	41.23	7.05	15.92
150.65	100.67	54.19	125.20	209.80
7	6	6	6	6
-157.54	-	-	-	-
109.75	-	-	-	-
5	-	-	-	-
-219.80	-	-	-	-
357.13	-	-	-	-
3	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-

Feed Consumption

Glasdegib-related and adverse lower maternal food consumption occurred at all doses. The mean maternal food consumption in the 5 mg/kg/day group was 0.71x control for the interval of GD 7 to 20. The mean food consumption improved during the postdose phase GD 20 to 29.

Table 42 Maternal Food Consumption in Pregnant Rabbits (g/animal/day)

(Excerpted from Submission)

Group 1 - Vehicle Control Article
Group 2 - PF-04449913 5 mg/kg/day
Group 3 - PF-04449913 10 mg/kg/day
Group 4 - PF-04449913 50 mg/kg/day
Group 5 - PF-04449913 100 mg/kg/day

Group / Sex		Day (From/To) 7/20	20/29	7/29
1F	Mean	117.1	83.1	101.0
	SD	29.3	33.0	25.5
	N	8	7	7
2F	Mean	82.8	92.1	91.7
	SD	35.8	40.1	28.3
	N	7	6	6
3F	Mean	109.2	-	-
	SD	42.0	-	-
	N	4	-	-
4F	Mean	43.9	-	-
	SD	31.6	-	-
	N	2	-	-
5F	Mean	-	-	-
	SD	-	-	-
	N	-	-	-

Toxicokinetics

Blood samples were collected at approximately 1, 4, 7, and 24 hours postdose on GD 19 except for 100 mg/kg/day rabbits due to early elective euthanasia. Plasma samples were analyzed for concentration of glasdegib using a validated analytical procedure ([(b) (4)] Method No. PF44BPP, validated under [(b) (4)] 8325312). Toxicokinetic parameters were estimated using WinNonlin and fitted to a non-compartmental approach consistent with the oral route of administration (See Table 43).

Table 43 Summary of Toxicokinetic Parameters of Glasdegib in Pregnant Rabbits

(Excerpted from Submission)

Dose (mg/kg/day)		C_{max} (ng/mL)	T_{max} (hr)	AUC_{24} (ng·hr/mL)
5	Mean	3120	2.0	43200
	SD	1030	1.7	40400
	N	3	3	3
10	Mean	22700	1.0	262000
	SD	NA	NA	NA
	N	2	2	1
50	Mean	79800	5.0	1790000
	SD	5460	3.5	NA
	N	3	3	2

AUC_{24} Area under the concentration-time curve from hour 0 to hour 24.
C_{max} Maximum concentration in plasma.
N Number of values in calculation.
NA Not applicable.
SD Standard deviation.
T_{max} Time to maximum plasma concentration.
Note: All animals in the 100 mg/kg/day group were euthanized prior to GD 19.

The Applicant calculated the total C_{max} of 3120 ng/mL in rabbits at 5 mg/kg/day. In previously untreated patients with AML or high-risk MDS, exposure of glasdegib at 100 mg QD in combination with LDAC, expressed as total C_{max}, was 1252 ng/mL. Fetal toxicity and teratogenicity occurred at maternal exposure of approximately 2.5-times the human exposure at the recommended dose based on total C_{max} at 5 mg/kg/day of 3120 ng/mL.

Dosing Solution Analysis

The relative standard deviation of each concentration was ≤ 10% and the mean sample concentration results were within ± 15% of the theoretical concentration, meeting specifications for homogeneity and concentration verification. No significant detection of glasdegib occurred in the vehicle control samples.

Necropsy

Macroscopic observations in early decedents were summarized in Table 40 under mortality. No other glasdegib-related maternal macroscopic observations were detected at necropsy examination in the rabbits that survived to scheduled euthanasia.

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

All rabbits that survived to scheduled euthanasia in the 0 and 5 mg/kg/day dose groups were pregnant and were examined for ovarian and uterine contents on GD 29. Glasdegib-related and significant effects occurred at 5 mg/kg/day including reduced number of live fetuses, increased late resorptions and mean percent post-implantation loss (See Table 44).

Table 44 Summary of Laparohysterectomy Evaluations in Rabbits

Glasdegib Dose (mg/kg/day)	0	5	10	50	100
Number of females tested	8	8	8	8	8
Number of females pregnant	8	8	7	8	8
Females with live fetuses	8	8	0	0	0
Females with dead or resorbed	0	0	7	8	8
Unscheduled euthanasia	1	2	8	8	8
Females aborted	1	1	5	3	0
Number of litters	7	6			
Mean number Corpora lutea	11.9	11.5			
Mean number of Implantations	10.0	8.3			
Mean Percent pre-implantation loss	16.0	28.6			
Number of live fetuses (mean)	68(9.7)	22(3.7)			
Post-implantation loss Mean Early resorptions Mean Late resorptions Mean Percent post-implantation loss	 0.3 0.0 3.0	 0.3 4.3 47.2			

Percent Post-implantation loss = [(# implantations - # live fetuses) / # implantations] x 100

Table 45 Summary of Litter Observations in Rabbits

Glasdegib Dose (mg/kg/day)	0	5
Number of females pregnant	8	8
Number of live fetuses	9.7	3.7
Sex distribution Mean # of Males (%) Mean # of Females	 5.6(57.5) 4.1	 1.5(40.7) 2.0
Mean fetal body weight (g) Male fetuses Female fetuses	38.9 39.8 37.7	29.2 30.7 29.2

Fetal Evaluations

Fetal observations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development).

Fetal evaluations were based on 68 and 22 viable GD 29 cesarean-delivered fetuses in 7 and 6 litters in the 0 and 5 mg/kg/day dose groups, respectively. Each of these fetuses was examined for external, visceral, and skeletal abnormalities.

Fetal Examinations (External, Visceral and Skeletal)

- The most frequently observed glasdegib-related external malformations, which occurred in 54.5% to 63.6% of the fetuses available for examination in the 5 mg/kg/day group, consisted of domed head, absent teeth, and hyperextension of the forepaws. Additional malformations included ears (malpositioned), eyes (malpositioned, open, absent eye bulge, depressed eye bulge), face (proboscis, small mouth, fused nares, malpositioned nares, absent naris, cleft palate, a misshapen palate, misshapen snout, and short snout), head (cranial meningocele), limbs (malrotated fore- and hindlimbs and short fore- and hindlimbs), mouth (a protruding tongue, absent teeth), paws (hyperflexion of the hindpaw), trunk (omphalocele, short trunk, a herniated umbilicus), and tail (bent and short). Most of the external malformations were confirmed to be the result of underlying skeletal dysmorphogenesis.
- The most frequently observed glasdegib-related visceral malformations, which occurred in 36.4% to 68.2% of the fetuses available for examination in the 5 mg/kg/day group, consisted of a diaphragmatic hernia, a malpositioned atrium, absent chordae tendinae, absent papillary muscles, and a retroesophageal subclavian artery. Additional malformations included a malpositioned aorta, malformed eyes (absent or small), persistent truncus arteriosus, an absent ventricular septum, a ventricular septum defect, malpositioned intestines, malpositioned kidneys, absent kidneys, small lungs, and an absent trachea.
- The most frequently observed glasdegib-related skeletal malformation, which occurred in 77.3% of the fetuses available for examination in the 5 mg/kg/day group consisted of short maxilla. Additional malformations consisted of bent clavicles, absent skull bones (exoccipital, palatine, premaxillae, squamosals), fused skull bones (mandibles, maxillae), absent vertebrae (caudal, cervical, lumbar), fused centra in one or more vertebrae (cervical, sacral), fused sacral vertebrae, and multiple interrelated vertebral abnormalities (including absent, fused, misshapen, small, unilaterally ossified, misaligned, bipartite ossification, unossified or incompletely ossified cervical, thoracic, lumbar, sacral, and/or caudal arches, centra and/or vertebrae).

Table 46 Summary of Glasdegib-Related Abnormalities in Rabbits

Glasdegib Dose (mg/kg)	0	5
External		
Total number of litters examined	7	6
Total number of fetuses examined	68	22

M- Ear: Pinna, malpositioned	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	2(9.1)
M- Eye: malpositioned	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	3(13.6)
M- Eye: open	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	2(9.1)
M- Eye: Bulge absent /depressed	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Face: probosis	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Mouth: small	Litter incidence (%)	-	2(9.1)
	Fetal incidence (%)	-	1(16.7)
M- Nares: fused	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	6(27.3)
M- Nares: malpositioned/absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Palate: cleft	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Palate: misshapen	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Snout: short/ misshapen	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Head: Domed	Litter incidence (%)	-	5(83.3)
	Fetal incidence (%)	-	12(54.5)
M- Neck: cranial meningocele	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Forelimb: malrotated, short	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Hindlimb: malrotated, short	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Tongue: protruding	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	2(9.1)
M- Tooth: absent	Litter incidence (%)	-	5(83.3)
	Fetal incidence (%)	-	13(59.1)
M- Forepaw: hyperextension	Litter incidence (%)	-	6(100.0)
	Fetal incidence (%)	-	14(63.6)
M- Hindpaw: hyperextension/ hyperflexion	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Trunk: omphalocele/ short/ Umbilicus	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Tail: bent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Tail: short	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	2(9.1)
Fresh Visceral			
Total number of litters examined		7	6
Total number of fetuses examined		68	22
M- Aorta: malpositioned	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Diaphragm: hernia	Litter incidence (%)	-	5(83.3)

	Fetal incidence (%)	-	15(68.2)
M- Truncus arteriosus: persistent	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	3(16.7)
M-Heart: Atrium malpositioned	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	8(36.4)
M-Heart: Chordae tendinae absent	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	8(36.4)
M-Heart: Papillary muscle absent	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	8(36.4)
M-Heart: Ventricular septum absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M-Heart: Ventricular septum defect	Litter incidence (%)	-	4(66.7)
	Fetal incidence (%)	-	4(18.2)
M- Innominate artery: absent	Litter incidence (%)	-	6(100.0)
	Fetal incidence (%)	-	10(45.5)
M- Intestine: malpositioned	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	2(9.1)
M- Kidney: malpositioned absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Subclavian artery: retroesophageal/ malpositioned	Litter incidence (%)	-	6(100.0)
	Fetal incidence (%)	-	9(40.9)
M- Trachea: absent	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	4(18.2)
Skeletal			
Total number of litters examined		7	6
Total number of fetuses examined		68	22
M- Clavicle: bent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Skull: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Mandible: fused	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Maxilla: fused	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Maxilla: short	Litter incidence (%)	-	6(100.0)
	Fetal incidence (%)	-	17(77.3)
M- Palatine: absent	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Premaxilla: absent	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Squamosal: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M-Caudal vertebrae: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Cervical centrum: fused	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	4(18.2)
M- Cervical vertebrae: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Lumbar vertebrae: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	2(9.1)
M- Sacral centrum: fused	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	2(9.1)

M- Sacral vertebrae: fused	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Vertebrae general: multiple abnormalities	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	3(13.6)

9.3 Prenatal and Postnatal Development

No studies were conducted.

10 Special Toxicology Studies

(The following studies were not completely review; Summary Adapted from Submission)

Study No. 16GR159: Single Dose Intravenous and Perivascular Study of PF-04449913 in Female New Zealand White Rabbits. Non-GLP.

Glasdegib (Lot No. 00706780-0036-001) and its associated vehicle were tolerated when administered as a single intravenous and perivascular dose at 1 and 0.05 mg, respectively, followed by a 3-day observation period. No glasdegib-, vehicle- or saline-related local irritation, macroscopic, or microscopic findings occurred in this study.

Study No. 16LJ129: A Multiple Dose Phototoxicity Study to Determine the Effects of Oral Gavage Administration of PF-04449913 on Eyes and Skin in Pigmented Rats. GLP-compliant.

Glasdegib (Lot No. GR10340 (also known as E010015971)) was administered once daily by oral gavage once daily for 3 consecutive days at doses of 0, 10, 30 or 100 mg/kg/day on the eyes and skin of Crl:LE (Long-Evans) pigmented rats, followed by exposure to ultraviolet B, ultraviolet A and visible light from a xenon lamp. In addition, the toxicokinetic characteristics of glasdegib were determined.

No glasdegib-related effects occurred on survival, clinical signs, skin reaction observations in pigmented and non-pigmented skin, body weights or ophthalmology observations. Glasdegib was not phototoxic based on the absence of effects in pigmented and non-pigmented skin and eyes under the conditions of the study. Mean exposure to glasdegib on Day 3 at 100 mg/kg/day was C_{max} of 14200 ng/mL and AUC_{24} of 137000 ng•hr/mL.

Study No. 17GR182: 1-Day In Vitro Hemolysis Compatibility Study of PF-04449913 with Human Blood. Non-GLP.

Glasdegib (Lot No. 00711282-0063-001) was combined with heparinized whole blood and plasma in a series of compound (0.01-1.0 mg/mL) and vehicle dilutions. The samples were then incubated and centrifuged. At predetermined time intervals, the tubes were observed and recorded for hemolysis and precipitation. The extent of hemolysis from each concentration of whole blood and vehicle was determined visually using the positive control and negative control (normal saline) as the baseline. The extent of precipitation from each concentration of plasma and vehicle was determined visually using the negative control (normal saline) as the baseline.

Glasdegib does not cause hemolysis in human whole blood under the study conditions and it is compatible with human donor plasma.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

PEDRO L DELVALLE
10/26/2018
This revised version of the nonclinical review was necessary based on amended information submitted by the Applicant, on embryofetal development studies, that prompted updates to the labeled exposure multiples

CHRISTOPHER M SHETH
10/26/2018
I concur

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:	210656
Supporting document/s:	1
Applicant's letter date:	April 27, 2018
CDER stamp date:	April 27, 2018
Product:	DAURISMO (glasdegib)
Indication:	(b) (4)
Applicant:	Pfizer, Inc
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer:	Pedro L. Del Valle, PhD
Supervisor/Team Leader:	Christopher M. Sheth, PhD
Division Director:	John Leighton, PhD Ann Farrell, MD (DHP)
Project Manager:	Thomas Iype, PharmD, RPh

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 210656 are owned by Pfizer or are data for which Pfizer has obtained a written right of reference. Any information or data necessary for approval of NDA 210656 that Pfizer 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 210656.

TABLE OF CONTENTS

Table of Tables

Table of Figures

1 Executive Summary

1.1 Introduction

Glasdegib (DAURISMO) is an orally administered smoothened (SMO) antagonist, a transmembrane protein that activates the Hedgehog (Hh) signaling transduction pathway. (b) (4)

Based on clinical trials submitted to support the use of DAURISMO in combination with low-dose cytarabine in the intended patient population, the recommended dose is 100 mg daily resulting in an average C_{max} of 1158 ng/mL and an AUC_{last} of 15 µg*hr/mL.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical pharmacology, pharmacokinetic and toxicology studies have been submitted to support the approval of glasdegib for the proposed indication. Pharmacology studies provided evidence that glasdegib inhibits the transmembrane protein SMO and attenuates Hh signal transduction. In an in vitro competition binding assay with a radiolabeled human SMO chemical ligand, glasdegib showed an IC_{50} of 7.7 nM. Gli1 is one of three transcription factors in the Gli family that mediate Sonic Hedgehog (Shh)-induced pathway activation. Glasdegib showed an IC_{50} of 5.2 nM in a cell-based reporter gene assay used to measure the potency to inhibit SMO-induced Hh signaling via the Gli1 transcription factor. The upregulation of Gli1 mRNA levels were stimulated in normal human fibroblasts with a recombinant human Shh. Glasdegib inhibited the Shh-induced Gli1 levels at 40 nM assessed through quantitative PCR. In vivo, the combination of glasdegib and low dose cytarabine (LDAC) significantly improved the anti-tumor effect over LDAC alone in AML (AML-183) patient-derived xenotransplanted (PDX) models. The established pharmacological class for glasdegib is a hedgehog pathway inhibitor.

Glasdegib was evaluated for its off-target activity across a panel of more than 50 receptors, enzymes, ion channels, and transporters. Glasdegib only demonstrated activity at potentially clinically relevant concentrations for human adenosine A1, histamine H1 and sodium channel site 2 (Nav 1.5).

In safety pharmacology studies in rats, glasdegib did not adversely affect Irwin screen or pulmonary parameters. Glasdegib had effects on grip strength and locomotor activity that occurred at toxic (≥50 mg/kg/day) doses in rats, which may be secondary to animal health issues that occurred in the 26-W rat study. Glasdegib increased heart rate, and QT, QTc and QRS intervals in the dog cardiovascular (CV) study and produced concentration-dependent inhibition of the Nav1.5 and hERG currents. Glasdegib systemic exposures in the dog CV study were approximately 0.9 to 3.5-fold the clinically relevant exposure at the recommended dose of 100 mg QD. A QTc prolongation effect of glasdegib (single dose of 150 mg and 300 mg) was also detected in trial B1371023 and the effect was dose/concentration-dependent.

In general, oral bioavailability was higher in fasted rats (35%) than in fed rats (19%), and glasdegib was 68% bioavailable in dogs. Glasdegib was bound to plasma proteins (86% to 98%) in rat, dog and human samples, and binding was independent of drug concentration. Glasdegib was distributed throughout the body with the highest concentration in the uveal tract, liver, kidney and renal structures, adrenal gland, and pancreas but had minimal distribution across the blood brain barrier.

Oxidative metabolite profiles were qualitatively similar in all species. One primary and two secondary N-glucuronide metabolites were identified in human, but not rat or dog, plasma and urine. Overall, the major metabolic pathways of glasdegib were oxidation (N-demethylation, hydroxylation, and dehydrogenation), primary glucuronidation, and secondary N-glucuronidation of oxidative metabolites. The average elimination half-life was 1.3 hours in rats and 2.3 hours in dogs.

Repeat-dose toxicology studies were conducted of 1-month (M), 13-week (W) and 26-W in rats with doses up to 100 mg/kg/day, and 1-M, 13-W and 39-W in dogs with doses up to 30 mg/kg/day. The 1-M toxicology studies in rats and dogs were used to support the FIH glasdegib dose of 5 mg/day. Doses for the toxicity studies of 26-W in rats and 39-W in dogs were selected based on the results of the 13-W toxicology studies in each species. Body weights and food consumption were decreased in rats and dogs treated with glasdegib. In both species, the major target organs of toxicity were the kidney, liver, skin, and bone. Species specific target organs included testis and peripheral nerve in the rat, and the adrenal, gastrointestinal tract, lymph nodes, and lung in dogs. Clinical pathology effects corresponded to the target organ toxicities. The primary toxicity was azotemia [abnormally high levels of nitrogen-containing compounds (such as urea, creatinine, and nitrogenous waste) in the blood] with subsequent damage to the kidneys (e.g., necrosis, mineralization, and evidence of inflammation in the renal tubules). Azotemia lead to the moribund sacrifice of rats and dogs.

In rats, glasdegib caused bone and teeth effects that are characteristic of SMO inhibitors, such as epiphysis alterations (decrease in number of chondrocytes and disorganization of chondrocytes) and decreased medullary trabeculae of femur and sternum bone sections with marrow hypercellularity. Teeth abnormalities consisted of missing or broken teeth (incisors) with histopathological atrophy leading to decreased food consumption and body weight loss. The adverse effect of glasdegib on teeth was severe enough to warrant the early sacrifice of some rats in the 13-W and 26-W toxicity studies. The effects on bones and teeth may have little significance for the intended adult patient population but they are of potential relevance for pediatric use of DAURISMO. Additional findings included alopecia and muscle tremors/twitching. These effects occurred in both rats and dogs at high doses with overt toxicity and/or moribund sacrificed animals.

Glasdegib-related gastrointestinal tract toxicities in dogs included hemorrhage in the gut-associated lymphoid tissue (GALT)/Peyer's patch, ectasia in the ileum, and congestion in the jejunum. Liver toxicity in both rats and dogs consisted of elevated cholesterol, increased alanine aminotransferase (ALT), and decreased alkaline

phosphatase (ALP) with microscopic findings of hypertrophy, inflammation, single cell necrosis and pigment in macrophage and Kupffer cells (iron). In dogs, administration of glasdegib also resulted in adrenal vacuolation with corresponding increased organ weight ratio. Alterations of serum chemistry parameters occurred in early decedents in the 39-W study in dogs.

Glasdegib was not mutagenic in vitro in the bacterial reverse mutation assay (Ames test) and was not clastogenic or aneugenic in an in vitro human chromosome aberration assay or in vivo rat bone marrow micronucleus test.

Women will be advised to not breastfeed during treatment with DAURISMO and for at least 30 days after the last dose. Toxicity in the testis in rats at ≥50 mg/kg/day included degeneration of seminiferous tubules and hypospermatogenesis which showed lack of recovery after cessation of dosing. This finding was included in the DAURISMO label. Preliminary embryofetal development studies were conducted in rats and rabbits at doses up to 100 mg/kg/day. Glasdegib induced embryofetal lethality in rats and rabbits at approximately 4-times and 0.6-times, respectively, the human exposure at the recommended dose based on unbound C_{max}. Glasdegib was clearly teratogenic in rats at ≥10 mg/kg/day and in rabbits at ≥5 mg/kg/day. A warning for embryo-fetal risk was recommended for DAURISMO and a black box warning for embryo-fetal toxicity is included in the label. Females and males of reproductive potential will be advised to avoid pregnancy and to use effective contraception during treatment with DAURISMO and for at least 30 days after the last dose.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, approval for glasdegib (DAURISMO) is recommended.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

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

2 Drug Information

2.1 Drug

CAS Registry Number
2030410-25-2

Generic Name (INN; USAN)
Glasdegib

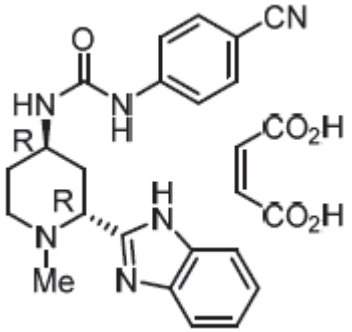
Code Name
PF-04449913-11

Chemical Name
CAS: N-[(2R,4R)-2-(1H-benzimidazol-2-yl)-1-methyl-4-piperidinyl]-N'-(4-cyanophenyl)-urea, (2Z)-2-butenedioic acid (1:1)
IUPAC: 1-((2R,4R)-2-(1H-benzo[d]imidazol-2-yl)-1-methylpiperidin-4-yl)-3-(4-cyanophenyl)urea maleate

Molecular Formula
$C_{25}H_{26}N_6O_5$
$C_{21}H_{22}N_6O$ (drug) $C_4H_4O_4$ (maleate)

Molecular Weight
374.5 Daltons (free drug)
490.51 Daltons (as the maleate)

Structure or Biochemical Description



Chiral centers are indicated in the structure. Glasdegib has two asymmetric centers, giving four possible stereoisomers. The absolute configuration is 2R, 4R.

Pharmacologic Class
Hedgehog pathway inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 105453

2.3 Drug Formulation

Glasdegib maleate (DAURISMO) is formulated as film-coated tablets provided at 25 and 100 mg strengths. The 25 mg tablets are presented as round, yellow film-coated tablets debossed with “Pfizer” on one side and “GLS” over “25” on the other side. The 100 mg tablets are presented as round, pale orange film-coated tablets debossed with “Pfizer” on one side and “GLS” over “100” on the other side. The drug product is packaged in (b) (4) high density polyethylene bottles. The detail composition of glasdegib maleate 25 and 100 mg film-coated tablets are provided in the following tables.

Table 1 Composition of Glasdegib Maleate 25 mg Film-Coated Tablet

(Excerpted from Submission)

Name of Ingredients	Reference to Standards	Function	Unit Formula (mg/tablet)
Glasdegib maleate	Pfizer	Active Ingredient	(b) (4)
Microcrystalline cellulose	NF/Ph.Eur./JP		(b) (4)
Dibasic calcium phosphate anhydrous	USP/Ph.Eur./JP		
Sodium starch glycolate (b) (4)	NF/Ph.Eur./JP		
Magnesium stearate (b) (4) (b) (4)	NF/Ph.Eur./JP (b) (4		
Opadry® II yellow (33G120011)	Pfizer (b) (4		

Note: NF = National Formulary; USP = United States Pharmacopeia; Ph.Eur. = European Pharmacopoeia, JP = Japanese Pharmacopoeia

Table 2 Composition of Glasdegib Maleate 100 mg Film-Coated Tablet

(Excerpted from Submission)

Name of Ingredients	Reference to Standards	Function	Unit Formula (mg/tablet)
Glasdegib maleate	Pfizer	Active Ingredient	(b) (4)
Microcrystalline cellulose	NF/Ph.Eur./JP		
Dibasic calcium phosphate anhydrous	USP/Ph.Eur./JP		
Sodium starch glycolate (b) (4)	NF/Ph.Eur./JP		
Magnesium stearate (b) (4) (b) (4)	NF/Ph.Eur./JP (b) (4)		
Opadry® II beige (33G170003)	Pfizer (b) (4)		

Note: NF = National Formulary; USP = United States Pharmacopeia; Ph.Eur. = European Pharmacopoeia, JP = Japanese Pharmacopoeia

Table 3 Composition of Opadry® II Yellow (33G120011)

(Excerpted from Submission)

Name of Ingredients	Reference to Standards	Unit Formula (% w/w)
(b) (4)		

Table 4 Composition of Opadry® II Beige (33G170003)

(Excerpted from Submission)

Name of Ingredients	Reference to Standards	Unit Formula (% w/w)
(b) (4)		

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

All impurities were either within acceptable limits according to ICHQ3A and ICHQ3B or qualified in nonclinical studies.

2.6 Proposed Clinical Population and Dosing Regimen

(b) (4)

The recommended dose and schedule of glasdegib (DAURISMO) is 100 mg, taken orally once per day. The summary of PK evaluations for 100 mg QD alone (Cycle 1 Day 21) and in combination with LDAC (Cycle 1 Day 10) tested in patients with previously untreated AML or high-risk MDS is provided in the following table as reference for exposure margins with pharmacology and animal TK data.

Table 5 Summary of Glasdegib Plasma Pharmacokinetic Parameters (Dose Compliant) - Phase 1b Arm A (Study B1371003)

(Excerpted from Submission)

Parameters (Units)	Cycle 1 Day 10 (N = 13)[a] Glasdegib 100 mg +LDAC	Cycle 1 Day 21 (N = 8)[b] Glasdegib 100 mg
C_{max} (ng/mL)	1074 (63)	1242 (56)
T_{max} (hr)	1.8 (0.75-24)	1.3 (0.53-2.0)
AUC_{tau} (ng•hr/mL)[c]	15020 (49)	16660 (43)
AUC_{last} (ng•hr/mL)	13320 (71)	16670 (43)
C_{avg} (ng/mL)	554 (71)	695 (43)
C_{trough} (ng/mL)	270 (73)	375 (55)

Source: Study B1371003 CSR Table 32.
Pharmacokinetic parameters are defined in Table 3.
Geometric mean (geometric %CV) for all except: median (range) for T_{max}.
%CV = percent coefficient of variation; LDAC = low-dose cytarabine; N = number of patients in each group; n = number of patients evaluable for the indicated PK parameters.
a. On Cycle 1/Day 10, n = 10 for AUC_{tau}. n = N otherwise.
b. On Cycle 1/Day 21, n = 7 for C_{trough}. n = N otherwise.
c. For AUC_{tau}, tau = 24 hours.

2.7 Regulatory Background

Glasdegib (code name PF-04449913-11) was investigated under IND 105453 for the treatment of (b) (4) AML. (b) (4)

3 Studies Submitted

3.1 Studies Reviewed

Study No.	Title
Primary and Secondary Pharmacology	
191616	Pharmacology Summary for PF-04449913 in Nonclinical Cancer Models In Vitro and In Vivo
075357	Anti-Tumor Efficacy of Glasdegib (PF-04449913) in Combination with Chemotherapy in AML PDX Models
7570637	In Vitro Pharmacology: Pfizer Tier 0 Profile
7571398	In Vitro Pharmacology and ADME-Tox: Pfizer Tier 1 Profile
Safety Pharmacology	
PF04449913NA15	Effect of PF-04449913 on Nav1.5 Sodium Current Stably Expressed in HEK293 Cells
PF4449913/ESD/ 1108/HERG	Effects of PF-04449913-01 on HERG Potassium Channels Stably Expressed in HEK.293 cells
08SN228	Neurofunctional Assessment of PF-04449913 in Male Rats
08SN229	Pulmonary assessment of PF-04449913 in male rats
08SN238	Cardiovascular assessment of PF-04449913 in Beagle dogs
Pharmacokinetics	
PF-04449913/ 14Nov08/143627	Single Dose Pharmacokinetics and Oral Bioavailability of PF-04449913 (b) (4) in Sprague-Dawley Rats Following Intravenous and Oral Administration

Study No.	Title
PF-04449913/ 14Nov08/143413	Single Dose Pharmacokinetics and Oral Bioavailability of PF-04449913 [redacted] (b) (4) in Beagle Dogs Following Intravenous and Oral Administration
PF-04449913/ 17Nov08/142017	Protein Binding of PF-04449913 in Rat, Dog, Mouse, and Human Plasma
PF-04449913 08Dec15-022737	Protein Binding of PF-04449913 in Rabbit Plasma
PF-04449913 23Feb16-104614	Protein Binding of PF-04449913 in Human Serum Albumin (HSA), and α1- Acid Glycoprotein (AAG)
PF-04449913/ 12Nov08/104333	Red Blood Cell to Plasma Partitioning of PF-04449913 in Human, Rat, and Dog Whole Blood
PF-04449913 08Dec15-031858	Red Blood Cell to Plasma Partitioning of PF-04449913 in Rabbit Whole Blood
Toxicology	
6348-650 (08LJ094)	1-Month Oral Toxicity Study of PF-04449913 in Rats with 1-Month Recovery with Micronucleus Assessment
8299121 (14LJ052)	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04449913 in Rats
8303065 (14LJ108)	26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with PF-04449913 in Rats with a 13-Week Recovery Phase
6348-651 (08LJ093)	1-Month Oral Toxicity and Toxicokinetic Study of PF-04449913 in Dogs with 1-Month Recovery
8299265 (14LJ055)	13-Week Oral Gavage Toxicity and Toxicokinetic Study with PF-04449913 in Dogs
8303066 (14LJ109)	39-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with PF-04449913 in Dogs with a 16-Week Recovery Phase
6348-654 (08GR352)	Bacterial Reverse Mutation Assay with a Confirmatory Assay
09GR016	Genetic Toxicology Human Lymphocyte Assay of PF-04449913-01 (Clastogenicity Assay)
20069225 (15GR056)	A Preliminary Embryo-Fetal Development Study of PF-04449913 by Oral Gavage in Rats
20069230 (15GR057)	A Preliminary Embryo-Fetal Development Study of PF-04449913 by Oral Gavage in Rabbits

3.2 Studies Not Reviewed

Study No.	Title
Pharmacology	
121934	Glasdegib (PF-04449913) Expanded Human Kinome Biochemical Selectivity
8850718	In Vitro Pharmacology - Study of PF-04449913-01
SP1008	Cell-based investigation into the functional activity of PF-04449913 at the μ opioid receptor using the DiscoveRx technology
17GR212	Assessment of the Activity of PF-04449913 on the Peak Nav1.5 Sodium Current
17GR318	Summary of the Isolated Tissue Studies with the Smo inhibitor PF-04449913
Safety Pharmacology	
16GR392	Effect of PF-04449913 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells
Pharmacokinetics	
6348-659	Method Validation for PF-04449913 Rat Plasma using LC/MS/MS
8361925	Validation of a Method for the Determination of PF-04449913 in Long Evans Rat Plasma by HPLC with MS/MS Detection

Study No.	Title
8325312	Validation of a Method for the Determination of PF-04449913 in New Zealand White Rabbit Plasma by HPLC with MS/MS Detection
6348-660	Method Validation for PF-04449913 Dog Plasma using LC/MS/MS
161811	Single Dose Pharmacokinetics and Excretion Evaluations of PF-04449913 in Sprague-Dawley Rats Following Intravenous and Oral Administration
8283508-144549	Quantitative Whole-Body Autoradiography of Male Rats Following Oral Administration of [^{14C}]PF-04449913
090256	Biotransformation of [^{14C}]PF-04449913 in Plasma and Excreta following Single Oral Administration to Rats
122431	Identification of Drug-Related Material Excreted in the Urine and Feces Following a Single 10 mg/kg Oral Dose of [^{14C}]PF-04449913 Administered to Male Long Evans Rats
090417	Biotransformation of [^{14C}]PF-04449913 in Plasma and Excreta following Single Oral Administration to Dogs
102952	Biotransformation of [^{14C}]PF-04449913 in Plasma and Excreta following Single Oral Administration to Healthy Male Human Volunteers
184200	Assessment of Circulating Metabolites of PF-04449913 in Human Plasma from Clinical Study Protocol B1371010
104634	Chiral Inversion Analysis of PF-04449913 in Human Plasma (Study Protocol B1371010)
153629	Investigation of the Glucuronide Conjugates of Glasdegib (PF-04449913) following Incubation with Human Liver Microsomes and Recombinant Human UDP-Glucuronosyltransferase (UGT)1A9 in Human Urine following Single Oral Administration
130612	Preliminary Assessment of the Biotransformation of PF-04449913
081555	Exploratory Reaction Phenotyping of PF-04449913 using Human Hepatocyte Relay Method
172724	In Vitro Cytochrome P450 Reaction phenotyping of PF-04449913
112959	Enzyme Kinetics and Identification of Cytochrome P450 Isoforms Involved in the In Vitro Metabolism of PF-04449913
050630	Enzyme Kinetics and Identification of UDP-Glucuronosyltransferase (UGT) Isoforms Involved in the In Vitro Metabolism of PF-04449913
163216	Preliminary In Vitro Recombinant UDP-Glucuronosyltransferase (UGT) Phenotyping of Glasdegib (PF-04449913)
8309182-084046	Determination of Routes of Excretion of [^{14C}]PF-04449913 in Sprague Dawley Rats After a Single Oral Dose
8309181-083752	Determination of Routes of Excretion of [^{14C}]PF-04449913 in Dogs After a Single Oral Dose
150827	In Vitro Assessment of Uptake and Biliary Excretion of Glasdegib using Sandwich Culture Human Hepatocytes (SCHH)
XT135086-115159	In Vitro Evaluation of PF-04449913 as an Inhibitor of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes
135232	An Investigation of the Potential for PF-04449913 to Induce CYP3A4 and CYP1A2 in Human Hepatocytes
XT133102-120525	In Vitro Investigation of the Potential for PF-04449913 and Rifampin to Induce Cytochrome P450 (CYP2B6 and CYP3A4) in Cultured Human Hepatocytes
152325	In Vitro Evaluation of PF-04449913 as an Inhibitor of UDP-Glucuronosyltransferase (UGT) Enzyme Activities in Human Liver Microsomes
131858	PF-04449913 Exploratory in Vitro PgP Substrate Profiling Determination in MDR-MDCK Cell Monolayers
101906	In Vitro Evaluation of PF-04449913 as a Substrate of BCRP (ABCG2) in Transfected MDCK Cells
1153561	In Vitro Renal Transporter Substrate of PF-04449913

Study No.	Title
154108	In Vitro Evaluation of Glasdegib (PF-04449913) as an Inhibitor of Major Drug Transporters
Toxicology	
09LJ058	7-Day Oral In Vivo Toleration Study of PF-05171171 and PF-04449913 in Rats
07GR162	In Vivo Toleration Study of PF-04449913 in Rats
08LJ052	7-Day Repeat Oral Dose In Vivo Toleration Study of PF-04449913 in Dogs
16GR159	Single Dose Intravenous and Perivascular Study of PF-04449913 in Female New Zealand White Rabbits
17GR041	1-Month Oral Toxicity Study of PF-04449913 in Sprague-Dawley Rats
16LJ129	A Multiple Dose Phototoxicity Study to Determine the Effects of Oral Gavage Administration of PF-04449913 on Eyes and Skin in Pigmented Rats
17GR182	1-Day In Vitro Hemolysis Compatibility Study of PF-04449913 with Human Blood

3.3 Previous Reviews Referenced

The Pharmacology/Toxicology and other discipline reviews for IND 105453.

4 Pharmacology

4.1 Primary Pharmacology

Study No. 191616: Pharmacology Summary for PF-04449913 in Nonclinical Cancer Models In Vitro and In Vivo.

A series of in vitro and in vivo assays were conducted to characterize glasdegib potency against SMO/Hh pathway signaling, including inhibition of Gli-1-associated endpoints. Table 6 shows a summary of data from the different assays described below.

Table 6 Summary of Key Pharmacological Properties of PF-04449913

(Excerpted from Submission)

In Vitro	
Inhibition of:	**Potency**
^{3}H-PF-03451358 interaction with SMO (181-787) (IC_{50})	7.7 nM
Shh Induced Gli-luciferase reporter in C3H10T1/2 (IC_{50})	5.2 nM
75% Inhibition of Shh-induced Gli-1 mRNA in Human Fibroblasts	40 nM
In Vivo	
Ptc+\-p53+\- medulloblastoma tumor growth inhibition (IC50)	73.2 nM[a] (6.8 nM free)
Gli-1 expression in tumor (IC50)	101.8 nM[a] (9.4 nM free)
Gli-1 expression in skin (IC50)	675.7 nM[a] (62.9 nM free)

IC_{50} = 50% inhibitive concentration; PF-03451358 = A cyclopamine-competitive SMO antagonist; Ptc = Patched; SMO = Smoothened; TGI = Tumor growth inhibition.
a. PF-04449913 molecular weight is = 374.4 Daltons; (X ng/mL/(374.4))*1000 = nM.

Competition Binding Assay

The ability of glasdegib to bind to the human SMO target was evaluated in a competition binding assay using a characterized cyclopamine-competitive SMO antagonist designated PF-03451358. This assay used membranes with surface expression of SMO 181-781 protein prepared from a stable cell line created in HEK293FlpIn-TetR cells (Invitrogen). The IC_{50} of glasdegib competing with the radiolabeled chemical ligand for binding to human SMO (amino acids 181-787) was of 7.7 ± 7.2 nM (n = 3).

Gli Reporter Assay and the Shh-induced Gli-Luciferase Reporter Assay

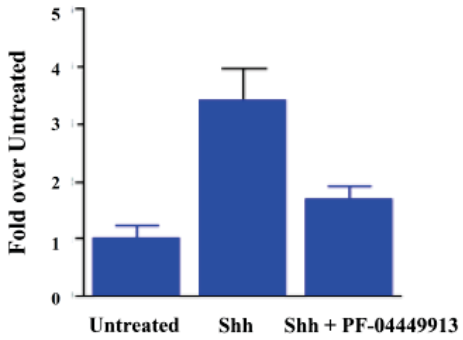
The Gli Reporter – cell line contains the firefly luciferase gene under the control of Gli responsive elements stably integrated into the cells. Luciferase expression correlates with activation of Hh signaling. To evaluate the functional effects of glasdegib on a ligand-stimulated Hh pathway, a mouse primary fibroblast cell line, C3H10T1/2, was used in transient transfection-based assays with a Gli responsive luciferase reporter. The IC_{50} of glasdegib inhibition of Shh-induced Gli reporter activity was 5.22 ± 1.65 nM (n = 12) under low serum (0.5% calf serum) conditions.

Inhibition of Shh Signaling in Normal Human Fibroblasts

Normal human fibroblasts were used to demonstrate the effects of glasdegib on an endogenous (non-engineered) Hh pathway signaling. Prepared Shh\PF-04449913 mix containing medium was added to cultured cells and incubated for 24 h prior to extraction and purification of Gli1 mRNA using a Qiagen RNA purification kit and detection with quantitative PCR. Shh stimulated the upregulation of Gli1 mRNA levels approximately three-fold in a 24-hour stimulation. Glasdegib at 40 nM inhibited the Shh-induced Gli1 levels assessed through quantitative PCR (See Figure 1).

Figure 1 Glasdegib Functional Effects in Normal Human Fibroblasts

(Excerpted from Submission)



Inhibition of the endogenous Hedgehog signaling pathway in normal human lung fibroblasts (4 replicates per group). PF-04449913 inhibits Shh-induced Gli1 mRNA levels assessed through quantitative PCR. mRNA = Messenger ribonucleic acid; PCR = Polymerase chain reaction; Shh = Sonic Hedgehog.

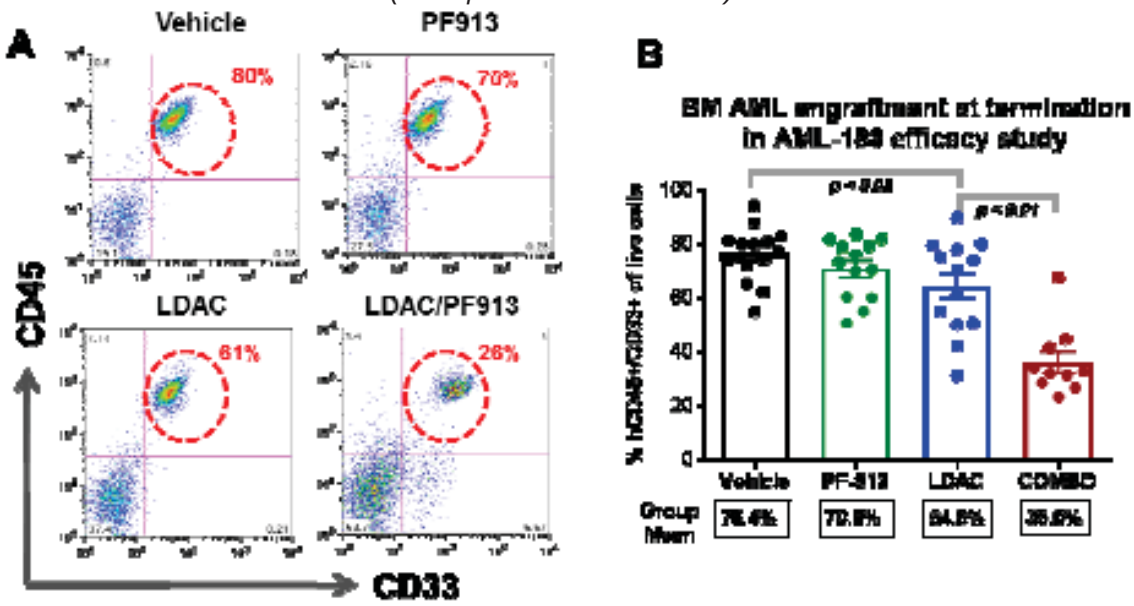
Study No. 075357: Anti-Tumor Efficacy of Glasdegib (PF-04449913) in Combination with Chemotherapy in AML PDX Models.

The studies were performed with two of the commonly used standard of care chemotherapies in AML patients: LDAC or high dose induction chemotherapy (daunorubicin and cytarabine or DA). Glasdegib showed enhanced activity when combined with LDAC in one (AML-183) of the two tested AML PDX models. In another

AML PDX model (BM2407), a combinatorial effect of glasdegib plus high dose induction chemotherapy was also observed.

Figure 2 The Combination of Glasdegib with LDAC in the AML-183 PDX Model

(Excerpted from Submission)



A. Representative image of CD45+/ CD33+ disease burden in BM following 56 days of treatment with vehicle, 100 mg/kg PO PF-913, 4 mg/kg SC LDAC, or the combination. **B.** Quantitative assessment and the mean values of the BM AML burden at study termination on day 56 post treatment. (N = 10 to 14 mice/group). AML = Acute myeloid leukemia; BM = Bone marrow; LDAC = Low dose cytarabine; PF913 = PF-04449913 = glasdegib; PO = oral; SC = Subcutaneous.

Glasdegib was tested in combination with LDAC in AML-183 xenotransplanted mice. The 4 mg/kg SC dose for cytarabine was selected based on exposure (AUC) in NSG mice, which matches the intended exposure in the unfit patient population. Mice that received oral glasdegib at 100 mg/kg exhibited minimal antileukemic activity (70.8% CD45+/CD33+ cells) compared to vehicle (76.4% CD45+/CD33+ cells), while LDAC treated mice had a significant decrease in CD45+/CD33+ cells compared to vehicle ($p < 0.05$). The combination of glasdegib and LDAC significantly (36.0% CD45+/CD33+; $p < 0.01$) improved the effect over LDAC alone (64.5% CD45+/CD33+ cells) (See Figure 2).

4.2 Secondary Pharmacology

Study No. 7570637: In Vitro Pharmacology: Pfizer Tier 0 Profile
Study No. 7571398: In Vitro Pharmacology and ADME-Tox: Pfizer Tier 1 Profile
These studies were conducted to investigate the effects of glasdegib in various in vitro receptor binding and enzyme assays screens at an initial concentration of 10 µM. Glasdegib demonstrated activity, defined by a response greater that 50% of a maximal response, against several targets (See Table 7).

Table 7 Summary of Glasdegib Off-Target Binding

Receptor	Assay type	Ki (nM)
human adenosine A1	Binding	370
human alpha 2a adrenoceptor	Binding	1900
human alpha 2c adrenoceptor	Binding	1600
human mu opioid	Binding	3500
human histamine H1	Binding	470
sodium channel site 2 (Nav 1.5)	Binding	470
Cell based functional assays	Result	Kb (nM)
human adenosine A1 receptor	Antagonist	550
mu opioid receptor	Antagonist	31600
histamine H1 receptor	Antagonist	40

The Applicant noted that all observed functional activities were at multiples greater than approximately 2-fold above the observed unbound C_{max} (300 nM or 113 ng/mL) at the 100 mg QD recommended clinical dose except for histamine H1 antagonism which was 7-fold lower. Centrally active histamine H1 antagonists are known to cause sedation in the clinic, and in studies in rats, have been shown to induce changes in locomotor activity. In the rat neurofunctional studies (Study 08SN228) no effect was observed on locomotion (up to 30 mg/kg), however, hypoactivity was observed in the 26-week repeat dose toxicity study in rats at doses with adverse effects (≥50 mg/kg/day). In addition, glasdegib was shown to be an antagonist of the adenosine A1 receptor in both a functional cell-based assay (Kb 550 nM) and in a radioligand binding assay (Ki 370 nM). Given a potential role for adenosine A1 antagonism in promoting natriuresis and diuresis, inhibition of this receptor at higher glasdegib exposures in animals may be a contributing factor to the dehydration, electrolyte changes and weight loss observed in the rat and dog toxicity studies.

4.3 Safety Pharmacology

(Parts were excerpted from Dr. T. Kropp's review of IND 105453)

The Applicant conducted the required safety pharmacology studies with glasdegib. Glasdegib did not cause any adverse effects in an Irwin screen nor in a pulmonary study in rats but did increase HR, QT, QTc and QRS in ECG measurements in dog CV study, small decreases in QTc at the low dose (LD) in the 39-W dog study, and concentration-dependent inhibition of the Nav1.5 and hERG currents. Glasdegib systemic exposures in the dog CV study ranged 0.9 to 3.5-fold the clinically relevant exposure at the recommended clinical dose of 100 mg QD.

Study title: Effect of PF-04449913 on Nav1.5 Sodium Current Stably Expressed in HEK293 Cells

Study no.:	PF04449913NA15
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer Global Research & Development Drug Safety Research & Development Eastern Point Road Groton, CT USA
Date of study initiation:	Not indicated
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	PF-04449913, Lot # PF-04449913-01-0001, impurity not indicated

Key Study Findings

- Glasdegib produced a concentration-dependent inhibition of the Nav1.5 current with an IC_{50} = 32.2 µM.

Table 8 Inhibition of Nav1.5 Sodium Currents by Glasdegib

(Excerpted from Submission)

Cell Number	% Inhibition 10 µM	% Inhibition 30 µM	% Inhibition 100 µM	% Inhibition 300 µM
1	19.2	52.1	87.2	100.6
2	14.4	54.5	87.7	99.9
3	5.9	40.3	73.1	94.3
4	30.5	56.5	82.2	Not tested
5	8.2	42.1	76.6	93.3
Mean ± SEM	15.6 ± 4.4 P <0.05	49.1 ± 3.3 P <0.05	81.3 ± 2.9 P <0.05	97.0 ± 1.9 P <0.05

SEM = Standard error of the mean.

Study title: Effects of PF-04449913-01 on HERG Potassium Channels Stably Expressed in HEK.293 cells.

Study no.:	PF4449913/ESD/1108/HERG
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer Global Research & Development Drug Safety Research & Development Eastern Point Road Groton, CT USA
Date of study initiation:	Not indicated
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	PF-04449913, Lot # PF-04449913-01-0001, impurity not indicated

Key Study Findings

- Glasdegib inhibited hERG currents in a concentration-dependent manner. The magnitude of inhibition was statistically significant at 1 µM (20.4 ± 6.4%), 3 µM (49.7 ±4.6%) and 10 µM (78.5 ± 3.7%), respectively. The IC_{50} value for hERG current inhibition by PF-04449913-01 is 3.1 µM.

Table 9 Effect of Glasdegib on hERG Current Amplitude

(Excerpted from Submission)

Observation	% Inhibition		
	1 µM	3 µM	10 µM
1	35.4	56.1	77.1
2	2.5	33.9	67.9
3	34.2	59.8	78.9
4	11.0	45.1	77.5
5	19.1	53.8	90.9
Mean ± SEM	20.4 ± 6.4*	49.7 ± 4.6**	78.5 ± 3.7*

Study title: Neurofunctional Assessment of PF-04449913 in Male Rats

Study no.:	08SN228
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer, Sandwich, Kent, UK
Date of study initiation:	September 23, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913-01, 121657-119-8, >99%

Key Study Findings

- No glasdegib-related effects up to high dose tested of 50 mg/kg.

Methods

Doses:	vehicle, 1, 5, or 50 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (aqueous)
Species/Strain:	Rat/Crl:CD(SD)IGS BR
Number/Sex/Group:	6 males/group
Age:	56-70 days
Weight:	271-320 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that had an impact on study data or interpretation of results.

Study title: Pulmonary assessment of PF-04449913 in male rats

Study no.:	08SN229
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer, Sandwich, Kent, UK
Date of study initiation:	September 17, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913-01, 121657-119-8, >99%

Key Study Findings

- No glasdegib-related effects on tidal volume, respiratory rate or minute volume in the conscious male rat at doses of 1, 5, and 50 mg/kg P.O.

Methods

Doses:	vehicle, 1, 5, or 50 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (aqueous)
Species/Strain:	Rat/Crl:CD(SD)IGS BR
Number/Sex/Group:	6 males/group
Age:	56-70 days
Weight:	271-320 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None that had an impact on study data or interpretation of results.

Study title: Cardiovascular assessment of PF-04449913 in Beagle dogs

Study no.:	08SN238
Study report location:	eCTD 4.2.1.3
Conducting laboratory and location:	Pfizer Global Safety Pharmacology Kent, UK
Date of study initiation:	September 12, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913-01, 121657-119-8, >99%

Key Study Findings

- Glasdegib-dose dependent increase in QT and QTc that did not resolve by the end of study (22-hour post-dose) in the high dose (30 mg/kg).
- Significant increases in HR and QRS in HD were also seen but resolved by 22-hour post dose.

Methods

Doses:	vehicle, 1, 5, or 30 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (aqueous)
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4 males
Age:	Not given
Weight:	14.6-16.9 kg
Satellite groups:	None
Unique study design:	Latin square design with 3-5-day washout. Hemodynamics and ECG from 0.5-6 hour, 7-14 hour, 14-22 hour.
Deviation from study protocol:	None that had an impact on study data or interpretation of results.

Results

- 1 mg/kg unremarkable
- 5 mg/kg
  - 7-14 hour
    - QTc ↑ 5 msec*
- 30 mg/kg
  - 0.5-6 hr
    - QRS ↑3 msec*; QT ↑12 msec*; QTc ↑18 msec*; HR ↑10 bpm*
  - 7-14 hour
    - QRS ↑2 msec*; QT ↑22 msec*; QTc ↑24 msec*;
  - 14-22 hour
    - QT ↑18 msec*; QTc ↑ 18 msec*
  - Emesis occurred between 0.6 to 1.2 h post-dose

- Plasma C_{max} 1972.5 ng/mL (range 680.0 to 2780.0 ng/mL; unbound 96 to 392 ng/mL) after dosing at 5 mg/kg occurred between 0.25-1-hour post-dose
- Glasdegib C_{max} at 5 mg/kg in this study ranged 0.9 to 3.5-fold the clinical relevant exposure at the 100 mg QD

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption

Study PF-04449913/14Nov08/143627. Single Dose Pharmacokinetics and Oral Bioavailability of PF-04449913 (b) (4) in Sprague-Dawley Rats Following Intravenous and Oral Administration.

The Applicant evaluated the PK and oral bioavailability of glasdegib in male Sprague Dawley rats after administration of a 1 mg/kg single IV bolus or oral dose of glasdegib

[redacted] (b) (4) salt. Following oral administration, glasdegib had an oral bioavailability of 33% (See Table 10).

Table 10 Summary of Pharmacokinetics of PF-04449913 in Rats

(Excerpted from Submission)

PK Parameters:	IV	PO
Clearance (mL/min/kg)	50.9	n/a
Volume of Distribution (L/kg)	4.78	n/a
Half-life (h)	1.4	n/a
Cmax (ng/mL)	n/a	38.6
tmax (h)	n/a	0.33
$AUC_{(0-\infty)}$ (ng·h/mL)	329	109
Bioavailability (%)	n/a	33
Estimated Blood Clearance[a] (mL/min/kg)	30.3	n/a

[a]Blood clearance = Plasma clearance/blood to plasma ratio (PF-04449913/12Nov08/104333)
PK = Pharmacokinetics; IV = Intravenous; PO = Oral; n/a = Not applicable; C_{max} = Maximum observed plasma concentration; t_{max} = Time to reach Cmax; $AUC_{(0-\infty)}$ = Area under plasma concentration-time curve from time zero to infinity

Study PF-04449913/14Nov08/143627. Single Dose Pharmacokinetics and Oral Bioavailability of PF-04449913 [redacted] (b) (4) in Beagle Dogs Following Intravenous and Oral Administration.

The Applicant evaluated the PK and oral bioavailability of glasdegib in male Beagle dogs after administration of a 0.5 mg/kg single IV bolus or 3 mg/kg oral dose of glasdegib [redacted] (b) (4) salt. Following oral administration, glasdegib had an oral bioavailability of 68% (See Table 11).

Table 11 Summary of Pharmacokinetics of PF-04449913 in Dogs

(Excerpted from Submission)

PK Parameters	IV	PO
Clearance (mL/min/kg)	22.9	n/a
Volume of Distribution (L/kg)	4.21	n/a
Half-life (h)	2.3	n/a
C_{max} (ng/mL)	n/a	1070
t_{max} (h)	n/a	0.50
$AUC_{(0-\infty)}$ (ng·h/mL)	368	3860
Estimated Bioavailability (%)[a]	n/a	68
Estimated Blood Clearance (mL/min/kg)	1.99	n/a

[a]Bioavailability was estimated by converting plasma concentrations to blood concentrations to obtain blood AUCs
PK = Pharmacokinetics; IV = Intravenous; PO = Oral; n/a = Not applicable; C_{max} = Maximum observed plasma concentration; t_{max} = Time to reach C_{max}; $AUC_{(0-\infty)}$ = Area under plasma concentration-time curve from time zero to infinity

Distribution

Study PF-04449913/17Nov08/142017. Protein Binding of PF-04449913 in Rat, Dog, Mouse, and Human Plasma.
Study PF-04449913_08Dec15_022737. PF-04449913 Protein Binding in Rabbit Plasma.
Study PF-04449913_23Feb16_104614. PF-04449913 Protein Binding in Human Serum Albumin (HSA), and α1- Acid Glycoprotein (AAG).

The Applicant determined the extent of in vitro binding of glasdegib to CD-1 mice, SCID mice, Sprague Dawley rats, NZW rabbits, Beagle dogs, and human plasma proteins using an equilibrium dialysis method at concentrations ranging from 1 to 50 µM, depending on the species, and samples analyzed using LC/MS/MS. There was no concentration dependent change in free fraction of binding to plasma proteins in any of the species tested, therefore, the Applicant calculated the mean unbound fraction of drug (f_u) and used to determine unbound concentrations and exposure margins in the toxicity studies (See Table 12).

The Applicant determined the in vitro binding of glasdegib at 2 µM to human serum albumin (HSA) and alpha-1 glycoprotein (AAG) at physiological concentrations for these proteins using the same methodology as indicated above and the f_u calculated (See Table 12). Results suggests that glasdegib likely binds to both HSA and AAG in human plasma.

Table 12 Calculated Unbound Fraction of Glasdegib in Mouse, Rat, Rabbit, Dog and Human Plasma

Species / Matrix	F_u
CD-1 Mice	0.047
SCID mice	0.0932
Sprague Dawley rats	0.110
NZW rabbit	0.0203
Beagle dog	0.141
Human	0.0899
Human Serum Albumin	0.204
Alpha-1-Acid Glycoprotein	0.478

Study PF-04449913/12Nov08/104333. PF-04449913 Human, Rat, and Dog RBC Distribution.
Study PF-04449913_08Dec15_031858. PF-04449913 Rabbit RBC Distribution.
The Applicant determined the extent of glasdegib partitioning into blood cells in rat, dog, rabbit, and human whole blood over a concentration range of 0.2 to 50 µM (See Table 13). The in vitro results with human blood/plasma (Cb/Cp range 1.59 to 2.32) were consistent with those observed in vivo after oral administration of [^{14}C]glasdegib, with Cb/Cp values for glasdegib radioactivity ranging from 1.36 to 1.49 (Phase 1 open-label ADME of [^{14}C]glasdegib in healthy male volunteers Protocol B1371009). The in vitro and in vivo data suggest that glasdegib demonstrated species dependent blood partitioning with a modest preferential distribution into the blood cells of rats and humans, a limited distribution into the blood cells in rabbits, and a marked, concentration-dependent and saturable preferential distribution into the blood cells of dogs.

Table 13 Partition Coefficients and Blood to Plasma Concentration Ratios of Glasdegib in Human, Rat, Dog and Rabbit

(Excerpted from Submission)

	Human		Rat		Dog	
Substrate Conc. (µM)	Whole Blood (Cb)/Plasma Conc. (Cp)Ratio (Cb/CP)	RBC/Plasma Ratio or Partition Coefficient (Kp)	Whole Blood (Cb)/Plasma Conc. (Cp)Ratio (Cb/CP)	RBC/Plasma Ratio or Partition Coefficient (Kp)	Whole blood (Cb)/Plasma Conc. (Cp)Ratio (Cb/CP)	RBC/Plasma Ratio or Partition Coefficient (Kp)
0.2	1.25 ± 0.04	1.59 ± 0.10	1.28 ± 0.04	1.59 ± 0.08	6.90 (n=2)	11.6 (n=2)
0.5	1.36 ± 0.04	1.84 ± 0.08	1.33 ± 0.30	1.69 ± 0.62	3.68 ± 0.80	5.82 ± 1.43
2	1.27 ± 0.07	1.62 ± 0.16	1.79 ± 0.23	2.65 ± 0.48	2.24 ± 0.49	3.23 ± 0.87
5	1.36 ± 0.09	1.84 ± 0.21	1.80 ± 0.16	2.66 ± 0.34	2.33 ± 0.24	3.39 ± 0.43
20	1.46 ± 0.04	2.08 ± 0.08	1.86 ± 0.26	2.80 ± 0.53	1.09 ± 0.04	1.16 ± 0.07
50	1.56 ± 0.06	2.32 ± 0.15	2.01 ± 0.15	3.11 ± 0.31	1.27 ± 0.09	1.48 ± 0.16

Values are mean ± standard deviation (n =3 except where noted).

Species	Substrate Concentration (µM)	N	Time (hours)	BPR (Cb/Cp) (Mean ± SD)	RBC/Plasma Ratio or Partition coefficient (Kp) (Mean ± SD)
Rabbit	0.2	4	1	0.672±0.066	0.284±0.144
Rabbit	0.5	4	1	0.648±0.139	0.231±0.304
Rabbit	2	4	1	0.745±0.094	0.442±0.206
Rabbit	5	4	1	0.745±0.114	0.443±0.249
Rabbit	20	4	1	0.703±0.080	0.352±0.175
Rabbit	50	4	1	0.809±0.064	0.582±0.140
Mean				0.720±0.101	0.389±0.221

BPR = blood to plasma ratio; Cb = Concentration in blood; Cp = Concentration in plasma; n = Number of replicates; RBC = Red blood cell; SD = Standard Deviation.

Metabolism

(Adapted from Submission)

The Applicant evaluated the metabolism of glasdegib in vivo after oral administration of a single dose of [^{14}C]glasdegib to rats, dogs, and humans, and in vitro in liver microsomes from nonclinical species and humans and in human hepatocytes. In humans, oxidative metabolism was the major route of elimination, with minor contributions from glucuronidation, and approximately 20% of glasdegib was excreted unchanged in urine. After oral administration, the primary metabolic pathways of [^{14}C]glasdegib in humans were N-demethylation of the N-methylpiperidine group, glucuronidation, oxidation of the benzimidazole and N-methylpiperidine rings, and dehydrogenation of the piperidine ring. Oxidative metabolite profiles were qualitatively similar across all species. One primary and two secondary N-glucuronide metabolites (each <10%) were identified in the plasma and urine of humans only; however, additional safety testing of these metabolites was not considered necessary. Glasdegib was the most abundant circulating component in plasma, constituting 69% of plasma radioactivity, with minor circulating metabolites each being <8% of radioactivity. Chiral inversion to glasdegib epimers was considered minimal. (See Figure 3).

Excretion

(Adapted from Submission)

Following oral administration of [^{14}C]glasdegib to rats, dogs, and humans, recovery of radioactivity was essentially complete. The major route of excretion of drug-derived radioactivity was via feces and/or bile in rats and dogs; whereas, in humans there was

relatively equal excretion of drug-derived radioactivity in urine and feces with unchanged glasdegib being the major drug-related components in each matrix.

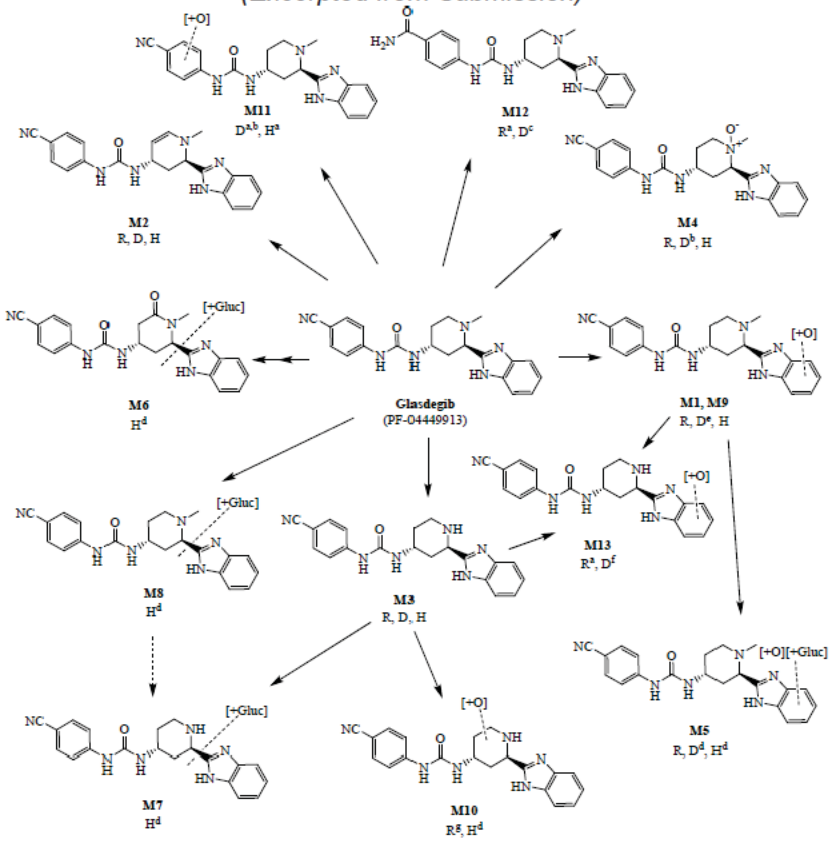
Table 14 Excretion of Radiolabeled Glasdegib

Species	Dose [^{14}C]glasdegib	Fecal (%)	Renal (%)	Total Radioactivity Recovery over 168h (%)
Rat	10 mg/kg PO	M: 93.7	<6	100
		F: 89.2	<6	96.7
Dogs	5 mg/kg PO	M: 82.0	7.2	~93
		F: 83.0	7.5	
Human	100 mg (100 µCi)	42	49	~91

The potential for glasdegib or its metabolites to be excreted in the breast milk of nonclinical species has not been evaluated.

Figure 3 Proposed Glasdegib In Vivo Metabolites Present in Rat, Dog, and Human Plasma and/or Excreta

(Excerpted from Submission)



Note: Excretory metabolites detected in urine and feces from rat, dog, or human. Metabolites M1, M2, M3, M4, M5, and M13 were detected in rat bile. Metabolite to metabolite pathways were not confirmed experimentally.
Abbreviations: D = Dog; H = Human; M = Metabolite; R = Rat.
a. Detected in feces only.
b. M4 and M11 co-elute in feces.
c. Detected in plasma and feces only.
d. Detected in plasma and urine only.
e. M9 detected in plasma and feces only.
f. Detected in urine and feces only.
g. Detected in plasma only.

5.2 Toxicokinetics

The Applicant evaluated the TK of glasdegib after once daily oral administration as part of oral repeat-dose toxicity studies in rats and dogs, embryo-fetal development (EFD) toxicity studies in rats and rabbits, and in support of a phototoxicity study in pigmented rats. The summary of all TK evaluations in animals is presented in the following tables as reference.

Table 15 Mean Toxicokinetics of Glasdegib in Sprague Dawley Rats (Male and Female Combined) Following Oral Administration in Repeat-Dose Toxicity Studies

(Excerpted from Submission)

Dose (mg/kg/day)	N/Sex	Study Day	C_{max} (ng/mL)	Unbound[a] C_{max} (ng/mL)	T_{max} (hour)	AUC_{24} (ng·hour/mL)	Unbound[a] AUC_{24} (ng·hour/mL)
1-Month Study (6348-650; 08LJ094)[b]							
1	4/M; 4/F	1	31.5	3.47	2.9	306	33.7
10	4/M; 4/F	1	743	81.7	3.4	7030	773
50	4/M; 4/F	1	4280	471	3.6	53400	5870
1	4/M; 4/F	29	36.0	3.96	2.4	288	31.7
10	4/M; 4/F	29	884	97.2	1.5	8700	957
50	4/M; 4/F	29	5610	617	2.3	66900	7360
1-Month Impurity Study (17GR041)							
10	4/M	1	286	31.5	0.5	1830	201
10	4/F	1	1060	117	1.4	5440	598
10	4/M	29	333	36.6	1.4	2500	275
10	4/F	29	2810	309	0.5	10200	1120
10[c]	4/M	1	380	41.8	0.5	2680	295
10[c]	4/F	1	847	93.2	1.5	5170	569
10[c]	4/M	29	614	67.5	0.6	3510	386
10[c]	4/F	29	1570	173	0.5	6970	767
13-Week Study (8299121; 14LJ052)[b]							
10	4/M; 4/F	87	1060	117	2.0	10000	1100
50	4/M; 4/F	87	5790	637	2.0	84200	9260
100	4/M; 4/F	87	14400	1580	6.0	215000	23700
26-Week Study (8303065; 14LJ108)							
10	4/M; 4/F	1	170	18.7	3.3	1740	191
50	4/M; 4/F	1	3560	392	4.8	46600	5130
100	4/M; 4/F	1	6320	695	4.8	81900	9010
10	4/M; 4/F	Week 13	813	89.4	2.1	7080	779
50	4/M; 4/F	Week 13	7460	821	3.5	92400	10200
100	4/M; 4/F	Week 13	13600	1500	4.4	178000	19600
10	4/M; 4/F	Week 26[d]	1050	116	1.4	7800	858
50	4/M; 4/F	Week 26[d]	8530	938	4.1	110000	12100
Embryo-Fetal Development Study (20069225; 15GR056)							
10	5/F	17[e]	726[f]	79.9[f]	4.0[f]	ND	ND
50	5/F	17[e]	4500[f]	495[f]	4.0[f]	ND	ND
100	5/F	17[e]	7140[f]	785[f]	4.0[f]	ND	ND
Phototoxicity Study (20111045; 16LJ129)							
10	3/F	3	976	107	0.50	5110	562
30	3/F	3	4460	491	0.50	22900	2520
100	3/F	3	14200	1560	0.67	137000	15100

AUC_{24} = Area under the concentration-time curve from zero to 24 hours postdose; C_{max} = Maximal plasma concentration; F = Female; f_u = Unbound fraction of drug; GD = Gestation day; M = Male; N = Number; ND = Not determined; T_{max} = Time to reach C_{max}.

a. Rat f_u = 0.110

b. Mean pharmacokinetic parameters were calculated from N=4/sex/timepoint.

c. Glasdegib blended with 0.65% PF-06658120 and 0.70% PF-06772998 impurities.

d. No data for animals in the 100 mg/kg/day dose group for Week 26, dosing suspended on Day 126.

e. Repeat oral dosages were administered to gravid rats from GD6 to GD17.

f. Results are from a single time point at 4 hours post dosing on GD17.

Plasma concentrations of glasdegib in these toxicology species are reported as total drug and unbound drug, and were used to calculate exposure ratios for various safety pharmacology and toxicity studies to assist in the assessment of safety margins relative to human exposure.

Table 16 Mean Toxicokinetics of Glasdegib in Beagle Dogs (Male and Female Combined) Following Oral Administration in Repeat-Dose Toxicity Studies

(Excerpted from Submission)

Dose (mg/kg/day)	N/Sex	Study Day	C_{max} (ng/mL)	Unbound[a] C_{max} (ng/mL)	T_{max} (hour)	AUC_{24} (ng•hour/mL)	Unbound[a] AUC_{24} (ng•hour/mL)
1-Month Study (6348-651; 08LJ093)							
1	3/M; 3/F	1	111[b]	15.7[c]	1.0	510[b]	71.9[c]
5	3/M; 3/F	1	2860	403	0.50	8300	1170
30	5/M; 5/F	1	14400	2030	0.75	115000	16200
1	3/M; 3/F	29	151	21.3	0.92	723	102
5	3/M; 3/F	29	3350	472	0.75	13500	1900
13-Week Study (8299265; 14LJ055)							
1	3/M; 3/F	89	65.5	9.24	2.3	487	68.7
5	3/M; 3/F	89	1100	155	2.0	8010	1130
10	3/M; 3/F	89	3060	431	2.2	25800	3640
39-Week Study (8303066; 14LJ109)							
1	4/M; 4/F	1	137	19.3	0.94	692	97.6
5	6/M; 6/F	1	1830	258	0.92	9060	1280
10	6/M; 6/F	1	5050	712	1.0	35200	4960
1	4/M; 4/F	Week 13	139	19.6	1.1	834	118
5	6/M; 6/F	Week 13	2340	330	1.2	13000	1830
10	4/M; 6/F	Week 13	4560	643	1.2	20100	2830
1	3/M; 3/F	160	112	15.8	0.92	381	53.7
5	2/M; 4/F	160	1400	197	1.3	6300	888
10	3/M; 6/F	160	4070	574	1.2	20000	2820
1	3/M; 3/F	Week 39[d]	95.9	13.5	0.67	372	52.5

AUC_{24} = Area under the concentration-time curve from zero to 24 hours postdose; C_{max} = Maximal plasma concentration; F = Female; f_u = Unbound fraction; h = Hour; M = Male; N = Number; NC = Not calculated due to insufficient quantifiable concentration data; ND = Not determined; T_{max} = Time to reach C_{max}.
a. Dog f_u = 0.141
b. C_{max} in males and females was 145 and 77.3 ng/mL on Day 1 and 201 and 100 ng/mL on Day 29, respectively while the AUC_{24} in males and females was 701 and 318 ng*hr/mL on Day 1 and 1020 and 427 ng*hr/mL on Day 29, respectively.
c. The unbound C_{max} in males and females was 20.5 and 10.9 ng/mL on Day 1 and 28.3 and 14.1 ng/mL on Day 29, respectively while the unbound AUC_{24} in males and females was 98.8 and 44.8 ng*hr/mL on Day 1 and 144 and 60.2 ng*hr/mL on Day 29, respectively.
d. No data for animals in the 5 or 10 mg/kg/day dose groups due to mortality prior to Week 39.

Table 17 Mean Toxicokinetics in Pregnant Female Rabbits Following Repeat Oral Doses of Glasdegib

(Excerpted from Submission)

Study	GD Day[a]	Dosage (mg/kg/day)	C_{max} (ng/mL)	Unbound[b] C_{max} (ng/mL)	T_{max} (hour)	AUC_{24} (ng•hour/mL)	Unbound[b] AUC_{24} (ng•hour/mL)
20069230 (15GR057)	19	5	3120	63.3	2.0	43200	877
		10	22700	461	1.0	262000	5320
		50	79800	1620	5.0	1790000	36300

AUC_{24} = Area under the concentration-versus-time curve from time 0 to 24 hours after dosing; C_{max} = Maximal plasma concentration; GD = Gestation day; f_u = Unbound fraction.
a. Once daily oral doses were administered to gravid rabbits from GD 7 to 19; results are from GD19.
b. Rabbit f_u = 0.0203

6 General Toxicology

In both rats and dogs, body weight was negatively affected by glasdegib, and the major target organs were the kidney, liver, skin, bone, and general. Rat specific target organs of toxicity included testis and peripheral nerve; dog specific target organs of toxicity included the adrenal, gastrointestinal track, lymph nodes, and lung. Corresponding clinical pathology effects occurred in both species. The primary toxicity was azotemia with subsequent damage to the kidneys, leading to the moribund sacrifice of dogs.

6.1 Single-Dose Toxicity

No studies were conducted.

6.2 Repeat-Dose Toxicity

Study title: 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with PF-04449913 in Rats with a 13-Week Recovery Phase

Study no.:	8303065 (14LJ108)
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 18, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; GR08041; 99.4%

Key Study Findings

- Glasdegib-related mortality and early termination of the 100 mg/kg/day dose group during Week 19 and subsequent mortality in 50 mg/kg/day females during Week 26.
- Glasdegib-related and adverse clinical signs at ≥50 mg/kg/day included tremors; malocclusions; missing teeth; thin appearance; clear oral discharge; labored respiration; hypoactivity, and hunched posture.
- Glasdegib-related and adverse effects at ≥50 mg/kg/day included lower grip strength, reductions in locomotor activity, lower mean body weights, body weight gains, food consumption, and microscopic findings in the kidney with corresponding clinical pathology findings, physis of the femur and sternum, loss of one or more incisor teeth.
- Target organs of toxicity included kidney, bone/cartilage, peripheral nerve, general body weight and the testis.

Methods

Doses:	0, 10, 50, or 100 mg/kg/day Doses selected based on the 13-W rat study (8299121 (14LJ052))
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% (w/v) methylcellulose (Methocel A4M, 4000 cps) in reverse osmosis water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	20/sex/group; 5-6 rats/sex in control, mid dose (MD) and high dose (HD) were allocated for the 13-w recovery phase
Age:	7 weeks
Weight:	M: 212 to 285 g; F: 163 to 208 g
Satellite groups:	4/sex/group for TK blood collection
Unique study design:	FOB elicited behavior and open field observations were evaluated
Deviation from study protocol:	None that affected the overall interpretation of study findings or compromised the integrity of the study

Observations

Table 18 Experimental Design for the 26-W Rat Study

(Excerpted from Submission)

Group	Subgroup	No. of Animals[a,d] Male	No. of Animals[a,d] Female	Dose (mg/kg/day)	Dose Concentration[b] (mg/mL)
1 (Control)[c]	1 (Toxicity)	20	20	0	0
	2 (Toxicokinetic)	4	4	0	0
2 (Low)	1 (Toxicity)	16	16	10	1
	2 (Toxicokinetic)	4	4	10	1
3 (Mid)	1 (Toxicity)	20	20	50	5
	2 (Toxicokinetic)	4	4	50	5
4 (High)	1 (Toxicity)	20	20	100	10
	2 (Toxicokinetic)	4[d]	4[d]	100	10

a All surviving toxicity animals in Group 4 (except the last 5 animals/sex in Group 4) were euthanized at Terminal Euthanasia 1 (Day 127 of the dosing phase). On Day 127 of the dosing phase, the last 5 toxicity animal/sex in Group 4 began the recovery phase (considered Day 1 of the recovery phase for these animals). The first 14 toxicity animals in Groups 1 and 3 (dependent on survival), and all surviving toxicity animals in Group 2 were euthanized at the Terminal Euthanasia 2 (Day 183 of the dosing phase). All surviving toxicity animals (Groups 1, 3, and 4) were euthanized at the Recovery Euthanasia on Study Day 277. All surviving toxicokinetic animals were euthanized after respective final blood collections.

b Concentrations were corrected for salt content and lot-specific purity. A correction factor of 1.243 was used.

c Group 1 received vehicle control article only.

d The final blood collection interval for the Group 4 toxicokinetic animals was considered Week 13 of the dosing phase and animals were euthanized and discarded at Terminal Euthanasia 1 (Day 127 of the dosing phase).

Results

Mortality

Glasdegib-related mortality occurred at ≥50 mg/kg/day. The extent of mortality at 100 mg/kg/day by Week 19 prompted euthanasia of all surviving toxicity rats at 100 mg/kg/day (except the last 5 rats/sex) on Day 127 (Week 19) of the dosing phase. The last 5 rats/sex administered 100 mg/kg/day went on an extended recovery phase of 21 weeks (+ 4 days). A total of nineteen (19) unscheduled deaths of toxicity animals and 2 toxicokinetic animals occurred between Days 22 and 176 of the dosing phase or Day 68 of the recovery phase, and 11 of the toxicity animal deaths were considered glasdegib-related due to dental abnormalities and/or degeneration/necrosis of the renal tubule cells noted histologically (See Table 19).

Table 19 Incidence of GLASDEGIB-related Mortality in the 26-W Rat Study

Excerpted from Submission)

	Group 1	Group 2	Group 3	Group 4
Dose (mg/kg/day)	0	10	50	100
Males	1/20	0/16	1/20[a]	7/20 Tox 2/4 TK
Females	1/20[b]	2/16[c]	4/20[d]	3/20[e]

a Attributed to blood collection procedure (Animal B13913 on Day 127)
b Attributed to blood collection procedure (Animal B14078 on Day 27)
c Both deaths attributed to blood collection procedure (Animal B14012 on Day 27; Animal B14045 on Day 90)
d One death attributed to blood collection procedure (Animal B14079 on Day 27). One moribund euthanasia occurred during the recovery phase (Animal B14091 on Day 68) and was not TA-related.
e One death attributed to the blood collection procedure (Animal B14015 on Day 54)

Clinical Signs

Clinical signs of early decedents included continuous or intermittent body tremors, labored respiration, piloerection, hypoactivity, hunched posture, clear oral discharge, thinning hair coat, malocclusion, missing teeth, thin and/or body weight loss.

Clinical signs in surviving rats at ≥50 mg/kg/day included continuous or intermittent tremors (various body regions or whole body); malocclusions; missing teeth; thin appearance; clear oral discharge; squinting eyes; rough haircoat and piloerection; rough, thinning, or discolored (red, yellow, black, or brown) haircoat in various body regions; and alopecia. Onset of observations was typically noted earlier in males than in females at the same respective dose levels and with an earlier onset with increasing dose level.

Reviewer Note: Exposure to glasdegib tended to be higher in rat females in all toxicology studies, and males were more sensitive to glasdegib toxicity.

Body Weights

Glasdegib-related and dose-dependent lower mean body weights and body weight gains relative to controls were noted at ≥50 mg/kg/day (See Table 20).

Table 20 Glasdegib-related Effects on Body Weight in the 26-W Rat Study

Glasdegib (mg/kg/day)	Percent Difference vs Control in Mean Body Weight (Day 182)	Percent Difference vs Control in Mean BW Gain (Day 1 to 182)
MALES		
10	↑4	↑5
50	↓27*	↓45*
100	↓38[A]*	Early termination
FEMALES		
10	↑4	↑3
50	↓22*	↓48*
100	↓25[A]*	Early termination

[A] Differences compared to control on Day 120 before early termination
* Significant different compared to control P≥0.05

Feed Consumption

Table 21 Glasdegib-related Effects on Food Consumption in the 26-W Rat Study

Glasdegib (mg/kg/day)	Percent Difference vs Control in Mean Food Consumption
MALES	
10	↑4
50	↓6*
100	↓11[A]*
FEMALES	
10	↑5
50	↓7*
100	↓10[A]*

[A] Differences compared to control on Day 120 before early termination
* Significant different compared to control P≥0.05

Ophthalmoscopy

No Glasdegib-related findings.

ECG

Not conducted

Hematology

Table 22 Percent Change in Hematology Parameters on Day 183 in the 26-W Rat Toxicology Study

Parameter	Glasdegib (mg/kg/day)			
	50		100[A]	
	Males	Females	Males	Females
RBC	↓7[B]*	↓4*	↓14[B]*	↓11[B]*
Hemoglobin	↓7[B]*	↓3	↓12[B]*	↓9[B]*
HCT	↓7[B]*	↓4*	↓13[B]*	↓9[B]*
RDW	↑11[B]*		↑24[B]*	↑9[B]*
Reticulocytes	↑50[B]*	↑32*	↑73[B]*	↑74[B]*
WBC		↑3*	↑55[B]*	↑64[B]*
Neutrophils			↑85[B]*	↑32[B]*
Lymphocytes		↑31[B]*	↑48[B]*	↑48[B]*
Monocytes			↑34[B]*	↑95[B]*
LUC			↑86[B]*	↑100[B]*

Platelet	↑4	↑3	↑26[B*]	↑11[B*]
MPV	↑6*	↑5	↑8*	↓5
Fibrinogen			↑27[B*]	↑24

Empty spaces denote no changes occurred compared to control
[A] Differences compared to control on Day 127 before early termination
[B] Differences also occurred on other assessment days
* Significant different compared to control P≥0.05

Clinical Chemistry

Table 23 Percent Change in Clinical Chemistry Parameters on Day 183 in the 26-W Rat Toxicology Study

Parameter	Glasdegib (mg/kg/day)			
	50		100[A]	
	Males	Females	Males	Females
Glucose	↓8*			
Urea nitrogen	↑25[B*]	↑39[B*]	↑31[B*]	↑27[B*]
Creatinine	↑29[B*]	↑25[B*]	↑67[B*]	↑29[B*]
Albumin			↓11[B*]	↓9[B]
Globulin			↑14[B*]	↑19[B*]
A:GR		↓9[B]	↓20[B*]	↓23[B*]
ALT		↑26	↑31[B*]	↓14[B*]
Alkaline Phosphatase	↓18[B*]	↓33[B*]	↓25[B*]	↓31[B*]
Phosphorus	↑16[B*]	↑20[B*]	↑32[B*]	↑53[B*]

Empty spaces denote no changes occurred compared to control
[A] Differences compared to control on Day 127 before early termination
[B] Differences also occurred on other assessment days
* Significant different compared to control P≥0.05

Urinalysis

Glasdegib-related effects in urinalysis parameters were consistent with microscopic evidence of adverse changes in the kidney.

Gross Pathology

Glasdegib-related macroscopic observations in ≥50 mg/kg/day consisted of 1 or more missing incisors, which corresponded histologically to fibrosis/new bone formation, and bilaterally discolored kidneys, which were associated with degeneration/necrosis of the tubular epithelial cells.

Organ Weights

No Glasdegib-related organ weight differences occurred.

Histopathology

Adequate Battery: Yes
Peer Review: No
Histological Findings
Glasdegib-related microscopic observations for were present in the kidney, bone and marrow of femur and sternum, 1 or more incisor teeth, oral mucosa, testis (males), and peripheral nerve adjacent to the mesenteric lymph node (nerve-(other)). In the testis, Glasdegib-related minimal to severe hypospermatogenesis occurred in

50 mg/kg/day males and was characterized by partial to complete loss of spermatogonia, spermatocytes, and spermatids; some animals also exhibited degeneration of seminiferous tubules (See Table 24).

Table 24 Incidence and Severity of Glasdegib-related Microscopic Findings at Terminal Sacrifice of the 26-W Study in Rats

(Excerpted from Submission)

	PF-04449913							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	10	50	100	0	10	50	100
Kidney								
Number Examined	14	16	14	8	14	14	12	12
Cast, proteinaceous								
Not Present	14	14	14	4	14	14	10	8
Minimal	0	2	0	4	0	0	2	4
Degeneration/necrosis, tubule								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	14	0	0	0	12	0
Moderate	0	0	0	8	0	0	0	12
Karyomegaly, renal tubule epithelium								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	14	8	0	0	12	12
Regeneration								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	14	0	0	0	12	0
Moderate	0	0	0	8	0	0	0	12
Tooth, Left Lower Incisor								
Number Examined	14	16	14	8	14	14	12	12
Dental hard substance, altered								
Not Present	14	16	3	6	14	14	4	4
Present	0	0	11	2	0	0	8	8
Fibrosis/New bone formation								
Not Present	14	16	0	0	14	14	0	0
Mild	0	0	1	0	0	0	0	0
Moderate	0	0	0	0	0	0	2	0
Marked	0	0	13	8	0	0	10	12
Infiltrate, mixed cell								
Not Present	14	16	12	5	14	13	12	12
Minimal	0	0	1	1	0	1	0	0
Mild	0	0	1	1	0	0	0	0
Moderate	0	0	0	1	0	0	0	0
Tooth, Left Upper Incisor								
Number Examined	14	16	14	8	14	14	12	12
Dental hard substance, altered								
Not Present	13	16	4	2	14	14	4	6
Present	1	0	10	6	0	0	8	6
Fibrosis/New bone formation								
Not Present	13	16	0	1	14	14	0	0
Minimal	1	0	0	0	0	0	0	0
Moderate	0	0	0	0	0	0	4	0
Marked	0	0	14	7	0	0	8	12
Infiltrate, mixed cell								
Not Present	12	15	9	8	14	14	11	11
Minimal	2	1	5	0	0	0	1	0
Mild	0	0	0	0	0	0	0	1
Tooth, Right Lower Incisor								
Number Examined	14	16	14	8	14	14	12	12
Dental hard substance, altered								
Not Present	14	16	7	4	14	14	6	5
Present	0	0	7	4	0	0	6	7
Fibrosis/New bone formation								
Not Present	14	16	0	0	14	14	0	0
Moderate	0	0	1	0	0	0	3	0
Marked	0	0	13	8	0	0	9	12
Infiltrate, mixed cell								
Not Present	14	16	11	5	14	13	12	12
Minimal	0	0	2	1	0	1	0	0
Mild	0	0	1	2	0	0	0	0

Continued…

	PF-04449913							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	10	50	100	0	10	50	100
Tooth, Right Upper Incisor								
Number Examined	14	16	14	7	14	14	12	12
Degeneration/necrosis/absence, apical portion								
Not Present	13	16	14	7	14	14	11	12
Mild	1	0	0	0	0	0	1	0
Dental hard substance, altered								
Not Present	12	16	3	1	14	14	4	3
Present	2	0	11	6	0	0	8	9
Fibrosis/New bone formation								
Not Present	12	16	0	0	14	14	1	0
Mild	2	0	0	0	0	0	1	0
Moderate	0	0	2	0	0	0	3	0
Marked	0	0	12	7	0	0	7	12
Infiltrate, mixed cell								
Not Present	13	16	12	7	14	14	11	10
Minimal	1	0	2	0	0	0	1	2
Oral Mucosa								
Number Examined	14	16	14	8	14	14	12	12
Infiltrate, mixed cell								
Not Present	14	14	14	6	14	14	10	12
Minimal	0	2	0	2	0	0	0	0
Mild	0	0	0	0	0	0	1	0
Moderate	0	0	0	0	0	0	1	0
Ulcer								
Not Present	14	16	14	5	14	14	12	12
Mild	0	0	0	2	0	0	0	0
Marked	0	0	0	1	0	0	0	0
Femur								
Number Examined	14	16	14	8	14	14	12	12
Disorganization, chondrocytes, physis								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	4	0	0	0	8	0
Mild	0	0	5	0	0	0	2	0
Moderate	0	0	5	8	0	0	2	12
Sternum								
Number Examined	14	16	14	8	14	14	12	12
Disorganization, chondrocytes, physis								
Not Present	14	16	0	0	14	14	0	0
Minimal	0	0	3	0	0	0	9	0
Mild	0	0	7	8	0	0	2	12
Moderate	0	0	4	0	0	0	1	0
Marrow, Femur								
Number Examined	14	16	14	8	14	14	12	12
Hypercellular								
Not Present	14	16	10	0	14	14	12	0
Minimal	0	0	3	0	0	0	0	0
Mild	0	0	1	8	0	0	0	12
Marrow, Sternum								
Number Examined	14	16	14	8	14	14	12	12
Hypercellular								
Not Present	14	16	10	0	14	14	12	0
Minimal	0	0	3	0	0	0	0	0
Mild	0	0	1	8	0	0	0	12

Continued…

	PF-04449913							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	10	50	100	0	10	50	100
Testis								
Number Examined	14	16	14	8	NA	NA	NA	NA
Degeneration, seminiferous tubule								
Not Present	14	16	11	8	NA	NA	NA	NA
Minimal	0	0	3	0	NA	NA	NA	NA
Hypospermatogenesis								
Not Present	14	16	10	8	NA	NA	NA	NA
Minimal	0	0	2	0	NA	NA	NA	NA
Marked	0	0	1	0	NA	NA	NA	NA
Severe	0	0	1	0	NA	NA	NA	NA
Nerve-*(other)*								
Number Examined	14	15	14	8	14	14	12	12
Degeneration, axon								
Not Present	14	15	12	1	14	14	4	4
Minimal	0	0	2	3	0	0	7	6
Mild	0	0	0	0	0	0	1	2
Moderate	0	0	0	2	0	0	0	0
Marked	0	0	0	2	0	0	0	0

NA = Not applicable.

Table 25 Incidence and Severity of Glasdegib-related Microscopic Findings at Recovery Sacrifice of the 26-W Study in Rats

(Excerpted from Submission)

	PF-04449913					
Sex	Males			Females		
Dose Level (mg/kg/day)	0	50	100	0	50	100
Kidney						
Number Examined	5	5	5	5	4	5
Cast, proteinaceous						
Not Present	5	5	5	5	4	3
Minimal	0	0	0	0	0	2
Karyomegaly, renal tubule epithelium						
Not Present	5	0	0	5	1	5
Minimal	0	5	5	0	3	0
Regeneration						
Not Present	5	2	2	5	2	5
Minimal	0	3	3	0	2	0
Tooth, Left Lower Incisor						
Number Examined	5	5	5	5	4	5
Degeneration/necrosis/absence, apical portion						
Not Present	5	5	4	5	4	5
Minimal	0	0	1	0	0	0
Dental hard substance, altered						
Not Present	5	1	2	5	2	4
Present	0	4	3	0	2	1
Fibrosis/New bone formation						
Not Present	5	0	0	5	0	0
Mild	0	2	1	0	1	0
Moderate	0	0	0	0	1	0
Marked	0	3	4	0	2	5
Infiltrate, mixed cell						
Not Present	5	4	5	5	4	5
Mild	0	1	0	0	0	0
Tooth, Left Upper Incisor						
Number Examined	5	5	5	5	3	5
Dental hard substance, altered						
Not Present	5	2	3	5	2	4
Present	0	3	2	0	1	1
Fibrosis/New bone formation						
Not Present	5	0	0	5	0	0
Mild	0	1	1	0	0	0
Moderate	0	0	0	0	2	0
Marked	0	4	4	0	1	5
Infiltrate, mixed cell						
Not Present	5	4	4	5	2	4
Minimal	0	1	1	0	1	1

At recovery necropsies, glasdegib-related microscopic observations persisted in one or more incisor teeth, femur, and sternum of ≥50 mg/kg/day rats; karyomegaly, proteinaceous casts, and regeneration of renal tubule epithelium in the kidney, and hypospermatogenesis in the testis persisted indicating a lack of recovery (See Table 25). The kidney degeneration/necrosis, bone marrow hypercellularity, testicular degeneration of seminiferous tubules, and axonal degeneration nerve-(other) observed at the terminal euthanasia were not observed at the recovery euthanasia.

Special Evaluation

(Adapted from Submission)
FOB
Glasdegib-related effects on quantitative FOB parameters included reduced forelimb grip strength at ≥50 mg/kg/day beginning at Week 9; and hindlimb grip strength at ≥50 mg/kg/day in males and at 100 mg/kg/day in females. Some of the differences were statistically significant. The maximum magnitude of the reductions relative to controls in 100 mg/kg/day rats was 0.62x during Week 9, and in 50 mg/kg/day rats was 0.71x during Week 26. There was a lack of recovery in grip strength.

Locomotor Activity
Glasdegib-related effects on locomotor activity were noted at 100 mg/kg/day beginning at the Week 9 evaluations, and was related to poor animal health.

Toxicokinetics

Due to early termination, 100 mg/kg rats were unavailable for blood sample collections during Week 26. Toxicokinetic parameters were calculated for LD, MD and HD on Day 1 and during Week 13 and for LD and MD during Week 26. No marked (≤2-fold) sex-related differences were noted in exposure.

Systemic exposure, as assessed by C_{max} and AUC_{24} increased with increasing dose from 10 to 100 mg/kg/day on Day 1 and during Week 13, and from 10 to 50 mg/kg/day during Week 26 (See Table 26). The increases in exposure were generally greater than dose-proportional from 10 to 50 mg/kg/day on Day 1 and during Weeks 13 and 26. The increases in exposure were approximately dose-proportional from 50 to 100 mg/kg/day on Day 1 and during Week 13. Exposure was higher during Weeks 13 and 26 when compared with Day 1. During Week 13, mean C_{max} values were 4.8x, 2.1x, and 2.2x and mean AUC_{24} values were 4.1x, 2.0x, and 2.2x when compared with Day 1 for the 10, 50, and 100 mg/kg/day dose groups, respectively. During Week 26, mean C_{max} values were 6.2x and 2.4x and mean AUC_{24} values were 4.5x and 2.4x when compared with Day 1 for the 10 and 50 mg/kg/day dose groups, respectively.

Table 26 Mean Toxicokinetic Parameters for Glasdegib in Combined Sex on Day 1 and during Weeks 13 and 26 in the 26-W Rat Study

(Excerpted from Submission)

Interval	Dose Group	Dose Level (mg/kg/day)		C_{max} (ng/mL)	T_{max} (hr)	AUC_{24} (ng·hr/mL)	AUC_{24}/Dose [(ng·hr/mL)/(mg/kg/day)]
Day 1	2	10	Mean	170	3.3	1740	174
			SD	76.6	1.4	771	77.1
			N	8	8	8	8
	3	50	Mean	3560	4.8	46600	933
			SD	1280	1.0	18000	360
			N	8	8	8	8
	4	100	Mean	6320	4.8	81900	819
			SD	1700	1.0	19400	194
			N	8	8	8	8
Week 13	2	10	Mean	813	2.1	7080	708
			SD	448	1.6	3600	360
			N	8	8	8	8
	3	50	Mean	7460	3.5	92400	1850
			SD	2900	1.7	24500	490
			N	8	8	8	8
	4	100	Mean	13600	4.4	178000	1780
			SD	2500	1.7	32200	322
			N	8	8	8	8
Week 26	2	10	Mean	1050	1.4	7800	780
			SD	596	1.1	3860	386
			N	8	8	8	8
	3	50	Mean	8530	4.1	110000	2200
			SD	3450	1.6	53500	1070
			N	8	8	8	8

AUC_{24}	Area under the concentration-time curve from hour 0 to hour 24.
AUC_{24}/Dose	Area under the concentration-time curve from hour 0 to hour 24 divided by dose level.
C_{max}	Maximum concentration in plasma.
N	Number of values in calculation.
SD	Standard deviation.
T_{max}	Time to maximum plasma concentration.
Note:	Group 4 (100 mg/kg/day) animals were sacrificed prior to their Week 26 TK sample collection.

Dosing Solution Analysis

Stability of samples prepared at concentrations of 0.1 and 10 mg/mL was established under (b) (4) Study 8299121 (13-W rat study). Dose formulations from the Day 1 preparations (1 and 10 mg/mL) and Week 21 preparations (1 and 5 mg/mL) met specifications and were considered homogeneous. The percent relative standard deviation (RSD) ranged from 1.1 to 3.2. All dose formulations from the Days 1, 57, 120, and 176 preparations were within ±10% of the target concentrations (mean values ranging from 95.5% to 101.5% of theoretical). No glasdegib was detected in the control vehicle.

Study title: 39-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with PF-04449913 in Dogs with a 16-Week Recovery Phase

Study no.:	8303066 (14LJ109)
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	September 17, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; GR08041; 99.4%

Key Study Findings

- Glasdegib-related mortality at all dose levels occurred due to body weight loss, inappetence and adverse clinical observations of thin appearance, hypoactivity, periodic vomiting, evidence of dehydration, and being cold to the touch.
- Glasdegib adverse microscopic findings in the kidney (tubular degeneration/ necrosis, tubular regeneration, granular/cellular casts, and tubular dilatation) and associated clinical pathology findings of increased relative to baseline urea nitrogen and creatinine concentrations, positive urine glucose, and granular casts and adverse microscopic findings in the liver (centrilobular/midzonal mixed cell inflammation, individual hepatocyte necrosis, Kupffer cell/macrophage pigment, and centrilobular hepatocyte vacuolation).
- Findings in the kidney were not present at the end of the 16-week recovery period. Partial recovery observed for liver findings.
- Target organs for toxicity included the kidney, liver, skin, gastrointestinal trach, lung and general body weight.

Methods

Doses:	0, 1, 5, or 10 mg/kg/day Doses selected based on the 13-W dog study (8299265 (14LJ055))
Frequency of dosing:	Once daily
Route of administration:	Oral
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% (w/v) methylcellulose [Methocel A4M (4000 cps)] in reverse osmosis water
Species/Strain:	Beagle dogs
Number/Sex/Group:	6/sex/group except at LD 4/sex.
Age:	10 months
Weight:	M: 7.1-9.7 kg; F: 5.5-8.6 kg
Satellite groups:	None
Unique study design:	None

Deviation from study protocol: None that affected the overall interpretation of study findings or compromised the integrity of the study

Observations

Table 27 Experimental Design for the 39-W Dog Study

(Excerpted from Submission)

Group	No. of Animals[a] Male	Female	Dose Level (mg/kg/day)	Dose Concentration[b] (mg/mL)
1 (Control)[c]	6	6	0	0
2 (Low)	4	4	1	0.2
3 (Mid)	6	6	5	1.0
4 (High)	6	6	10	2.0

a All surviving animals, except 2 animals/sex in Group 1 and all surviving animals in Group 2 were euthanized on Day 274 of the dosing phase (Terminal Euthanasia 2). All surviving animals in Group 3 and all surviving animals, except 3 animals/sex in Group 4 were euthanized on Day 162 of the dosing phase (Terminal Euthanasia 1). The last 2 animals/sex in Group 1 and last 3 animals/sex in Group 4 underwent recovery beginning on Day 162 of the dosing phase (also considered Day 1 of recovery phase); these animals were assigned to the recovery euthanasia (Study Day 274; Day 113 of the recovery phase).

b Concentrations were corrected for salt content and lot-specific purity. A correction factor of 1.243 was used.

c Group 1 received vehicle control article only (0.5% (w/v) methylcellulose (4000 cps) in reverse osmosis water).

Results

Mortality

A total of 8 males and 3 females were euthanized in moribund condition during the dosing phase (See Table 28). Two HD males presented clinical signs of vomitus, abnormal feces, hypoactivity, dehydration, excessive salivation, and clenched teeth along with significant body weight loss and reduced food consumption during Week 2. Canned food supplementation was offered, and dosing holiday started on Day 15. These dogs did not recover and were euthanized on Day 19. Canned food supplementation was offered to the 10 mg/kg/day starting on Day 23.

Table 28 Incidence of Glasdegib-related Mortality in the 39-W Dog Study

Glasdegib (mg/kg/day)	Sex	Study Day 19	112	114	121	126	138	141	155	Total # of Early Decedents
1	Males			1						1
	Females					1				1
5	Males		1		1		1		1	4
	Females						1	1		2
10	Males	2							1	3
	Females									0

The remaining unscheduled moribund sacrifices occurred during Weeks 16-23 and were associated with significant body weight loss, reduced or no food consumption, poor body condition (thin appearance), and/or evidence of dehydration; hypoactivity;

periodic vomitus; abnormal feces (liquid, mucoid); thinning hair coat; discolored (red) skin around the head, feet, muzzle, or legs; and cold to touch. All early decedents went through a dosing holiday for one or more days.

Glasdegib target organs of toxicity in early decedents included the kidney and liver with corresponding clinical pathology findings of azotemia and glucosuria.

Clinical Signs

In addition of the glasdegib-related and adverse clinical signs described for early decedents, other clinical signs included:

- At ≥1 mg/kg/day, skin and pelage observations (discolored red or blue skin; dry skin; alopecia; thinning hair coat on the entire body, muzzle, ears, eyes, lips, feet, legs, ventral cervical or thorax, perioral, or periorbital areas; or oily hair coat), evidence of dehydration, clear or cloudy ocular discharge. Skin findings were less prominent over the course of the dosing phase and did not present microscopic correlates for the skin/subcutis except for atrophy of the hair shafts, which the Applicant claims is a pharmacological effect.
- At ≥5 mg/kg/day, hypoactivity, abnormal (liquid, mucoid, discolored orange) feces, and cold to touch (5 mg/kg/day females).
- At 10 mg/kg/day, excessive salivation and vomitus.

The incidence and severity of most of glasdegib-related clinical signs were reduced over the course of the 16-W recovery phase.

Body Weights

Glasdegib-related body weight loss occurred starting on Week 2 of the dosing phase for 10 mg/kg/day dogs and later for ≥1 mg/kg/day individual dogs that corresponded with reduced food consumption and often unscheduled euthanasia. By the end of the 16-W recovery phase, body weight of 10 mg/kg/day dogs remained stable while control dogs gained weight.

Feed Consumption

Glasdegib-related reduced food consumption occurred starting on Week 2 of the dosing phase for 10 mg/kg/day dogs and later for ≥1 mg/kg/day individual dogs that corresponded with body weight loss and often unscheduled euthanasia. Canned food supplementation was offered at different starting dates to all dogs including control dogs and quantitative food consumption was not conducted.

Ophthalmoscopy

No glasdegib-related ophthalmic findings occurred during the dosing phase and were not conducted during the recovery phase.

ECG

Changes of small magnitude occurred in 1 mg/kg/day dogs:

- statistically decreased mean PR in females predose and 1-2 hours postdose on Day 135 and predose on Day 268 of the dosing phase compared with controls,

- statistically decreased mean QTc in males predose on Day 268 of the dosing phase compared with controls.

All the electrocardiograms evaluated in this study were qualitatively and quantitatively considered normal for the canine. No arrhythmias were found. No glasdegib-related abnormal electrocardiographic findings occurred.

Hematology

The only consistent glasdegib-related hematology effect in the 11 dogs euthanized at an unscheduled interval was increased red cell mass that corresponded with dehydration. Hematocrit values were ≥1.6X in early decedents. Increased fibrinogen concentrations (2.8X and 2.3X baseline, respectively) indicative of inflammation occurred only in the two 10 mg/kg/day males euthanized on Day 19 (See Table 28).

Clinical Chemistry

Glasdegib-related serum chemistry effects in the 11 animals euthanized at an unscheduled interval included increased urea nitrogen (≥1.31X) and creatinine concentrations (≥1.7X) in 10 mg/kg/day dogs, and increased ALT (up to 3.7X baseline on Days 90 and/or 160 in 1 or 5 mg/kg/day males), AST (up to 3.7X baseline), ALP, and GGT activities as well as increased cholesterol (up to 1.7X baseline on Days 21 and 90 in 5 mg/kg/day males and 10 mg/kg/day females). One male early decedent at 10 mg/kg/day had ≥10X increased urea nitrogen and creatinine concentration that was accompanied with the highest inorganic phosphorus concentration and lowest potassium and chloride concentrations.

Urinalysis

Glasdegib-related urinalysis effect in early decedents included urine glucose (which is a rare event in dogs) in 7/11 dogs and the presence granular casts in the urine sediment of 2/11 dogs. These findings indicated effects on kidney function and integrity. Glasdegib-related urinalysis effect in surviving dogs included the presence of granular casts in urine sediment in one 5 mg/kg/day male and one 10 mg/kg/day female. In all cases, granular cases, corresponded with the kidney microscopic findings of tubular degeneration/necrosis and granular/cellular casts. Granular casts were not present in the urine sediment of 10 mg/kg/day dogs at the end of the recovery sacrificed.

Gross Pathology

Glasdegib-related macroscopic observations in early decedents and surviving dogs at terminal sacrifice included discolored kidney in ≥5 mg/kg/day dogs with microscopic correlates of tubular degeneration/necrosis and regeneration; alopecia that corresponded with microscopic findings of hair shaft atrophy; discoloration (red, dark red, black, or brown) of 1 or more segments of the gastrointestinal tract (stomach, duodenum, jejunum, ileum, and colon) at ≥1 mg/kg/day. All macroscopic findings were resolved at the end of the recovery sacrificed.

Organ Weights

Organ weights were collected at the terminal euthanasia on Day 274 of the dosing phase (Terminal Euthanasia 2) for designated control and surviving 1 mg/kg/day dogs (Group 2) and at the recovery euthanasia on Day 113 of the recovery phase for remaining control and surviving 10 mg/kg/day dogs (Group 4).

Adrenals to brain and body weight ratios were significantly increased 1.33X and 1.44X, respectively, in 1 mg/kg/day males at the terminal euthanasia. The brain weight/body weight ratio was significantly decreased, 0.84X, in 1 mg/kg/day males. The significance of the brain weight changes is uncertain in the absence of terminal euthanasia for the MD and HD data.

Histopathology

Adequate Battery: Yes
Peer Review: Yes
Histological Findings
Early mortality of 5 or 10 mg/kg/day dogs resulted in the cessation of dosing and the necropsy on Week 24. A subset of 10 mg/kg/day dogs underwent a 16-week recovery phase. Dosing continued for 39 weeks for surviving 1 mg/kg/day dogs.

Glasdegib-related microscopic findings in early decedents and at 5 or 10 mg/kg/day terminal sacrifice occurred in the kidney, liver, skin/subcutis, gut-associated lymphoid tissue (GALT)/Peyer's Patch, thymus, mesenteric lymph node, pancreas, and prostate (See Table 29). Additionally, vascular/perivascular inflammation in the stomach, uterus, and oviduct of one female administered 5 mg/kg/day and euthanized in moribund condition was considered most likely secondary to glasdegib-related kidney failure/uremia. Glasdegib-related microscopic findings in 1 mg/kg/day dogs and euthanized on Day 274 of the dosing phase occurred in the liver (See Table 30).

Table 29 Incidence and Severity of Glasdegib-related Microscopic Findings in the 39-W Dog Study - Terminal Necropsy 5 and 10 mg/kg/day

(Excerpted from Submission)

Tissue/ Observation	Group/Sex: Number of Animals:	3/M 2	4/M 0	3/F 4	4/F 3
Brain	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	1
Dilatation, ventricles					
	finding not present -	2	0	4	2
	slight 2	0	0	0	1
	Total Incidence:	0	0	0	1
Infiltrate, mononuclear cell, perivascular					
	finding not present -	2	0	3	2
	minimal 1	0	0	1	1
	Total Incidence:	0	0	1	1
Cecum	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	3
Hemorrhage, mucosa					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
Cervix	Number Examined:	0	0	4	3
	Unremarkable:	0	0	4	3
Colon	Number Examined:	2	0	4	3
	Unremarkable:	2	0	4	3
Duodenum	Number Examined:	2	0	4	3
	Unremarkable:	1	0	2	1
Congestion					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Ectasia, crypt/gland					
	finding not present -	1	0	2	1
	minimal 1	1	0	2	2
	Total Incidence:	1	0	2	2
Gall Bladder	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	3
Infiltrate, mononuclear cell					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
GALT/Peyer's Patch	Number Examined:	2	0	4	3
	Unremarkable:	1	0	2	2
Hemorrhage					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Lymphoglandular complex, increased					
	finding not present -	1	0	2	2
	minimal 1	1	0	2	1
	Total Incidence:	1	0	2	1
Heart	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	3
Degeneration, myofiber					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Hemorrhage					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
Infiltrate, mononuclear cell					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
Ileum	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	3
Ectasia, crypt/gland					
	finding not present -	2	0	3	3
	slight 2	0	0	1	0
	Total Incidence:	0	0	1	0
Jejunum	Number Examined:	2	0	4	3
	Unremarkable:	1	0	4	3
Congestion					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Kidney	Number Examined:	2	0	4	3
	Unremarkable:	0	0	0	0
Cast, granular/cellular					
	finding not present -	2	0	4	2
	moderate 3	0	0	0	1
	Total Incidence:	0	0	0	1
Cast, proteinaceous					
	finding not present -	2	0	4	2
	minimal 1	0	0	0	1
	Total Incidence:	0	0	0	1
Degeneration/necrosis, tubule					
	finding not present -	0	0	4	1
	minimal 1	2	0	0	1
	moderate 3	0	0	0	1
	Total Incidence:	2	0	0	2
Dilatation, tubule(s)					
	finding not present -	2	0	4	2
	slight 2	0	0	0	1
	Total Incidence:	0	0	0	1
Infiltrate, mononuclear cell					
	finding not present -	2	0	3	2
	minimal 1	0	0	1	0
	slight 2	0	0	0	1
	Total Incidence:	0	0	1	1
Lipid, glomerulus					
	finding not present -	2	0	4	2
	Present	0	0	0	1
Mineralization, tubule					
	finding not present -	0	0	1	1
	minimal 1	2	0	3	2
	Total Incidence:	2	0	3	2

Continued…

Tissue/ Observation	Group/Sex: / Number of Animals:	3/M 2	4/M 0	3/F 4	4/F 3
Kidney	Number Examined:	2	0	4	3
	Unremarkable:	0	0	0	0
Pigment, tubule cell					
	finding not present -	1	0	2	2
	minimal 1	1	0	2	1
	Total Incidence:	1	0	2	1
Regeneration, tubule cell					
	finding not present -	0	0	4	0
	minimal 1	1	0	0	2
	slight 2	1	0	0	0
	moderate 3	0	0	0	1
	Total Incidence:	2	0	0	3
Vacuolation, tubule cell					
	finding not present -	2	0	4	1
	minimal 1	0	0	0	2
	Total Incidence:	0	0	0	2
Liver	Number Examined:	2	0	4	3
	Unremarkable:	0	0	0	0
Hypertrophy, Ito cell					
	finding not present -	2	0	4	1
	minimal 1	0	0	0	2
	Total Incidence:	0	0	0	2
Infiltrate, mononuclear cell					
	finding not present -	2	0	0	0
	minimal 1	0	0	4	3
	Total Incidence:	0	0	4	3
Inflammation, mixed cell, centrilobular/midzonal					
	finding not present -	0	0	3	1
	minimal 1	0	0	1	0
	slight 2	0	0	0	1
	moderate 3	2	0	0	1
	Total Incidence:	2	0	1	2
Pigment, Kupffer cell/macrophage					
	finding not present -	0	0	2	1
	minimal 1	0	0	2	1
	slight 2	2	0	0	1
	Total Incidence:	2	0	2	2
Single cell necrosis, hepatocyte					
	finding not present -	0	0	4	1
	minimal 1	2	0	0	1
	slight 2	0	0	0	1
	Total Incidence:	2	0	0	2
Vacuolation, hepatocyte					
	finding not present -	2	0	3	3
	minimal 1	0	0	1	0
	Total Incidence:	0	0	1	0
Vacuolation, hepatocyte, centrilobular					
	finding not present -	1	0	4	2
	minimal 1	0	0	0	1
	slight 2	1	0	0	0
	Total Incidence:	1	0	0	1

Tissue/ Observation	Group/Sex: / Number of Animals:	3/M 2	4/M 0	3/F 4	4/F 3
Lung	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	1
Hemorrhage					
	finding not present -	2	0	3	3
	moderate 3	0	0	1	0
	Total Incidence:	0	0	1	0
Hyperplasia, epithelium, terminal bronchial					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Infiltrate, macrophages, alveolus					
	finding not present -	2	0	3	2
	minimal 1	0	0	0	1
	slight 2	0	0	1	0
	Total Incidence:	0	0	1	1
Infiltrate, mixed cell					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Inflammation					
	finding not present -	2	0	3	2
	slight 2	0	0	0	1
	marked 4	0	0	1	0
	Total Incidence:	0	0	1	1
Lymph Node, Mesenteric	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	1
Erythrocytes, sinus					
	finding not present -	1	0	3	1
	minimal 1	1	0	0	2
	slight 2	0	0	1	0
	Total Incidence:	1	0	1	2
Lymph Node, Popliteal	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	2
Erythrocytes, sinus					
	finding not present -	2	0	3	2
	minimal 1	0	0	0	1
	slight 2	0	0	1	0
	Total Incidence:	0	0	1	1
Mammary Gland	Number Examined:	2	0	4	3
	Unremarkable:	2	0	3	2
Ectasia, duct					
	finding not present -	2	0	3	2
	minimal 1	0	0	1	0
	slight 2	0	0	0	1
	Total Incidence:	0	0	1	1

Continued…

Tissue/ Observation	Group/Sex: / Number of Animals:	3/M 2	4/M 0	3/F 4	4/F 3
Mandibular Salivary Gland	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	2
Infiltrate, mononuclear cell					
	finding not present -	1	0	3	2
	minimal 1	0	0	1	1
	slight 2	1	0	0	0
	Total Incidence:	1	0	1	1
Skin/Subcutis	Number Examined:	2	0	4	3
	Unremarkable:	0	0	1	1
Acanthosis					
	finding not present -	1	0	4	2
	minimal 1	1	0	0	0
	slight 2	0	0	0	1
	Total Incidence:	1	0	0	1
Atrophy, hair shaft					
	finding not present -	0	0	1	1
	slight 2	2	0	3	2
	Total Incidence:	2	0	3	2
Ectasia, follicular					
	finding not present -	1	0	2	2
	minimal 1	1	0	1	1
	slight 2	0	0	1	0
	Total Incidence:	1	0	2	1
Erosion/ulcer					
	finding not present -	2	0	4	2
	slight 2	0	0	0	1
	Total Incidence:	0	0	0	1
Granuloma, hair shaft					
	finding not present -	1	0	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Infiltrate, mononuclear cell					
	finding not present -	0	0	3	2
	minimal 1	2	0	1	1
	Total Incidence:	2	0	1	1
Inflammation					
	finding not present -	2	0	4	2
	slight 2	0	0	0	1
	Total Incidence:	0	0	0	1
Spinal Cord	Number Examined:	2	0	4	3
	Unremarkable:	2	0	4	3
Spleen	Number Examined:	2	0	4	3
	Unremarkable:	1	0	1	3
Pigment					
	finding not present -	1	0	1	3
	minimal 1	1	0	3	0
	Total Incidence:	1	0	3	0
Sternum	Number Examined:	2	0	4	3
	Unremarkable:	2	0	4	3
Stomach	Number Examined:	2	0	4	3
	Unremarkable:	2	0	4	3
Thymus	Number Examined:	2	0	4	3
	Unremarkable:	0	0	1	0
Cyst					
	finding not present -	1	0	3	2
	minimal 1	1	0	0	1
	slight 2	0	0	1	0
	Total Incidence:	1	0	1	1
Decreased cellularity, lymphocytes					
	finding not present -	1	0	1	0
	minimal 1	0	0	1	1
	slight 2	1	0	2	1
	moderate 3	0	0	0	1
	Total Incidence:	1	0	3	3
Hemorrhage					
	finding not present -	0	0	2	3
	minimal 1	1	0	2	0
	slight 2	1	0	0	0
	Total Incidence:	2	0	2	0
Thyroid	Number Examined:	2	0	4	3
	Unremarkable:	1	0	3	0
Cyst, follicle					
	finding not present -	2	0	3	2
	minimal 1	0	0	1	1
	Total Incidence:	0	0	1	1
Decreased, colloid					
	finding not present -	2	0	4	1
	minimal 1	0	0	0	2
	Total Incidence:	0	0	0	2
Hyperplasia, C-cell					
	finding not present -	1	0	4	1
	minimal 1	0	0	0	2
	slight 2	1	0	0	0
	Total Incidence:	1	0	0	2

Table 30 Incidence and Severity of Glasdegib-related Microscopic Findings in the 39-W Dog Study - Terminal Necropsy 0 and 1 mg/kg/day

(Excerpted from Submission)

Tissue/ Observation	Group/Sex: Number of Animals:	1/M 4	2/M 3	1/F 4	2/F 3
Adrenal, Cortex	Number Examined:	4	3	4	3
	Unremarkable:	2	3	3	0
Vacuolation, zona fasciculata					
	finding not present -	4	3	3	0
	minimal 1	0	0	1	3
	Total Incidence:	0	0	1	3
Liver	Number Examined:	4	3	4	3
Inflammation, mixed cell, centrilobular/midzonal					
	finding not present -	4	1	4	3
	minimal 1	0	2	0	0
	Total Incidence:	0	2	0	0
Pigment, Kupffer cell/macrophage					
	finding not present -	4	0	4	3
	minimal 1	0	3	0	0
	Total Incidence:	0	3	0	0
Vacuolation, hepatocyte					
	finding not present -	3	3	4	3
	minimal 1	1	0	0	0
	Total Incidence:	1	0	0	0
Vacuolation, hepatocyte, centrilobular					
	finding not present -	4	1	4	3
	minimal 1	0	2	0	0
	Total Incidence:	0	2	0	0
Lung	Number Examined:	4	3	4	3
	Unremarkable:	3	2	3	2
Foreign material					
	finding not present -	4	2	4	3
	Present	0	1	0	0
Infiltrate, macrophages, alveolus					
	finding not present -	4	3	3	2
	minimal 1	0	0	1	1
	Total Incidence:	0	0	1	1
Inflammation, granulomatous					
	finding not present -	4	2	4	3
	minimal 1	0	1	0	0
	Total Incidence:	0	1	0	0
Skin/Subcutis	Number Examined:	4	3	4	3
	Unremarkable:	4	2	3	3
Acanthosis					
	finding not present -	4	2	4	3
	slight 2	0	1	0	0
	Total Incidence:	0	1	0	0
Crust, serocellular					
	finding not present -	4	2	4	3
	minimal 1	0	1	0	0
	Total Incidence:	0	1	0	0
Erosion/ulcer					
	finding not present -	4	2	4	3
	moderate 3	0	1	0	0
	Total Incidence:	0	1	0	0

Special Evaluation

None

Toxicokinetics

Due to early termination, blood samples were collected and toxicokinetic parameters calculated for LD, MD and HD on Day 1, during Week 13 and on Day 160 and for the LD during Week 39. Plasma preparations were analyzed with a validated bioanalytical method under Study 6348-660 "Method Validation for Glasdegib Dog Plasma using LC/MS/MS." The calibration standard data, quality control (QC) sample data, incurred sample reanalysis (ISR) data, and chromatograms indicate the method performed acceptably during the sample analysis phase. Systemic exposure, as assessed by C_{max} and AUC_{0-24} increased with increasing dose although the increase was generally greater than dose proportional at all intervals. There were no apparent gender-related differences in exposure in this study. Mean T_{max} ranged from 0.92 to 1.3 hours on Day 1, during Week 13, and on Day 160.

Table 31 Mean Toxicokinetic Parameters for Glasdegib in Combined Sex in the 39-W Dog Study: Day 1 and Week 13

(Excerpted from Submission)

Interval	Dose Group	Dose Level (mg/kg/day)		C_{max} (ng/mL)	T_{max} (hr)	AUC_{24} (ng·hr/mL)	AUC_{24}/Dose [(ng·hr/mL)/(mg/kg/day)]
Day 1	2	1	Mean	137	0.94	692	692
			SD	18.7	0.50	236	236
			N	8	8	8	8
	3	5	Mean	1830	0.92	9060	1810
			SD	273	0.42	2740	548
			N	12	12	12	12
	4	10	Mean	5050	1.0	35200	3520
			SD	892	0.0	10900	1090
			N	12	12	12	12
Week 13	2	1	Mean	139	1.1	834	834
			SD	29.9	0.78	327	327
			N	8	8	8	8
	3	5	Mean	2340	1.2	13000	2600
			SD	892	0.39	6020	1200
			N	12	12	12	12
	4	10	Mean	4560	1.2	20100	2010
			SD	1430	0.63	15300	1530
			N	10	10	10	10

AUC_{24}	Area under the concentration-time curve from hour 0 to hour 24.
AUC_{24}/Dose	Area under the concentration-time curve from hour 0 to hour 24 divided by dose level.
C_{max}	Maximum concentration in plasma.
N	Number of values in calculation.
SD	Standard deviation.
T_{max}	Time to maximum plasma concentration.

Table 32 Mean Toxicokinetic Parameters for Glasdegib in Combined Sex in the 39-W Dog Study: Day 160 and Week 39

(Excerpted from Submission)

Interval	Dose Group	Dose Level (mg/kg/day)		C_{max} (ng/mL)	T_{max} (hr)	AUC_{24} (ng·hr/mL)	AUC_{24}/Dose [(ng·hr/mL)/ (mg/kg/day)]
Day 160	2	1	Mean	112	0.92	381	381
			SD	22.7	0.59	65.3	65.3
			N	6	6	6	6
	3	5	Mean	1400	1.3	6300	1260
			SD	498	0.52	3390	678
			N	6	6	6	6
	4	10	Mean	4070	1.2	20000	2000
			SD	606	0.62	13200	1320
			N	9	9	9	9
Week 39	2	1	Mean	95.9	0.67	372	372
			SD	39.0	0.26	110	110
			N	6	6	6	6

AUC_{24}	Area under the concentration-time curve from hour 0 to hour 24.
AUC_{24}/Dose	Area under the concentration-time curve from hour 0 to hour 24 divided by dose level.
C_{max}	Maximum concentration in plasma.
N	Number of values in calculation.
SD	Standard deviation.
T_{max}	Time to maximum plasma concentration.
Note:	Groups 3 and 4 were sacrificed prior to the Week 39 TK collection.

Dosing Solution Analysis

All dose formulations from the Day 1 (0.2 and 2 mg/mL) and Day 267 (0.2 mg/mL) met specifications and were considered homogeneous. The RSD ranged from 1.1%-4.7%. All dose formulations from the Day 1 and Weeks 10, 20, 30, and 39 preparations (Days 1, 64, 134, 204 and 267, respectively) were within +10% of the target concentrations (mean values ranging from 97.7%-106.5% of theoretical). No glasdegib was detected in the control vehicle.

7 Genetic Toxicology

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

Study title: Bacterial Reverse Mutation Assay with a Confirmatory Assay

Study no.:	6348-654 (08GR352)
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 5, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; GR01590 121657-119-8; 99.8%

Key Study Findings

- Using a plate incorporation method with *Salmonella* strains (TA98, TA100, TA1535, and TA1537) and *E. coli* tester strain WP2uvrA(pKM101), glasdegib did not induce genotoxic responses with or without S9 metabolic activation. This study used the highest level recommended by ICH S2(R1) (5.0 mg/plate). The results are considered valid and adequate.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Genetic Toxicology Human Lymphocyte Assay of PF-04449913-01 (Clastogenicity Assay)

Study no.:	09GR016
Study report location:	eCTD 4.2.3.3.1
Conducting laboratory and location:	Pfizer Global Research & Development Safety Sciences Genetic Toxicology Groton, CT
Date of study initiation:	February 2, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913-01; GLASDEGIB-01-0014; 99.04% (Potency 77.6%)

Key Study Findings

- Using cultured primary human lymphocytes, glasdegib did not induce chromosomal aberrations or increases of numerical chromosome changes with or without S9 metabolic activation when tested up to concentrations that

produced marked mitotic suppression (≥58%). The results are considered valid and adequate.

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

Study title: 1-Month Oral Toxicity Study of PF-04449913 in Rats with 1-Month Recovery with Micronucleus Assessment

(Parts were adapted from IND 105453 Dr. T. Kropp review)

Study no.:	6348-650 (08LJ094)
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 26, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; 121657-119-8; 98% (Potency factor of 0.776)

Key Study Findings

Glasdegib was negative for inducing micronuclei in the rat bone marrow.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Not conducted

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not conducted

9.2 Embryonic Fetal Development

Study title: A Preliminary Embryo-Fetal Development Study of PF-04449913 by Oral Gavage in Rats

Study no.:	20069225 (15GR056)
Study report location:	eCTD 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 10, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PF-04449913; E010015565; Chiral potency factor 0.761.

Key Study Findings

- Glasdegib produced fetal toxicity in rats based on lower embryo-fetal survival and increased post-implantation loss at ≥50 mg/kg/day with maternal exposures approximately 4-times the human exposure at the recommended dose based on unbound C_{max}.
- Glasdegib is a teratogen in rats based on a higher incidence of skeletal anomalies at ≥10 mg/kg/day, and higher incidences of external, visceral, and skeletal malformations and variations at ≥50 mg/kg/day.
- Teratogenicity in rats occurred in the absence of maternal toxicity at maternal exposures of approximately 0.7-times the human exposure at the recommended dose based on unbound C_{max} at 10 mg/kg/day of 79.9 ng/mL.

Methods

Doses:	10, 50, or 100 mg/kg/day on GD 7 though GD 17. Doses based on 13-w repeat-dose toxicology study 8299265 (14LJ055) in rats.
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) methylcellulose (4000 cps) in reverse osmosis water
Species/Strain:	Crl:CD(SD) Sprague Dawley rat
Number/Sex/Group:	8 time-mated females/group
Satellite groups:	None
Study design:	Standard. Cesarean section conducted on GD 21. Plasma concentration analysis on GD 17 4 hours after dosing.
Deviation from study protocol:	None that had an impact on study data or interpretation of results.

Observations

Table 33 Experimental Design of EFD in Rats

(Excerpted from Submission)

Group No.	Test Article	PF-04449913 Doses (mg/kg/day)[a]	PF-04449913 Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Rats (Assigned Female Rat Numbers)
1	Vehicle Control Article	0	0	10	8 (161-168)
2	PF-04449913	10	1	10	8 (169-176)
3	PF-04449913	50	5	10	8 (177-184)
4	PF-04449913	100	10	10	8 (185-192)

[a] The potency of PF-04449913 was 0.761. Dose calculations were corrected for potency.

Results

Mortality

All rats survived to scheduled euthanasia on GD 21.

Clinical Signs

Single 100 mg/kg dams presented an increase in activity and abnormal breathing sounds on GD 9. Vaginal discharge occurred in two 100 mg/kg dams on GD 19 and 20.

Body Weight

Glasdegib dose-related decrease in body weight gain occurred at 50 mg/kg (↓0.48x) and 100 mg/kg (↓0.65x) for the interval GD 7 through GD 21 that was attributed to significant lower gravid uterus weight (↓0.82x and 0.95x, respectively).

Table 34 Maternal Body Weight Gains in Pregnant Rats

(Excerpted from Submission)

Bodyweight Gain (Interval)

Sex: Female		Day(s) Relative to Mating (L)						
		7 → 10	10 → 12	12 → 15	15 → 18	7 → 18	18 → 21	7 → 21
0 mg/kg/day	Mean	10.6	13.1	19.3	36.3	79.3	52.4	131.7
	SD	3.3	4.6	6.0	6.0	10.3	10.2	19.3
	N	7	7	7	7	7	7	7
10 mg/kg/day	Mean	15.0	13.0	21.3	39.0	88.3	53.1	141.4
	SD	2.8	2.7	2.5	4.4	7.0	7.7	8.2
	N	8	8	8	8	8	8	8
50 mg/kg/day	Mean	14.8	14.0	15.4	16.6	60.8	7.8	68.5
	SD	6.0	3.8	5.2	6.5	10.6	4.1	11.4
	N	8	8	8	8	8	8	8
100 mg/kg/day	Mean	8.5	16.4	10.9	13.3	49.0	-2.8	46.3
	SD	5.3	4.4	4.1	2.6	8.1	8.0	9.2
	N	8	8	8	8	8	8	8

Feed Consumption

Mean maternal absolute food consumption in the 100 mg/kg/day dose group was lower than the control group mean for the interval of GD 7 to 8 (↓0.46x), GD 7 to 10 (↓0.12x) and GD 20 to 21 (↓0.24x). The lower mean food consumption was not considered an adverse effect of glasdegib because food consumption values were similar to the control group mean for the remainder of the dose period and there was no overall effect on mean food consumption for the interval of GD 7 to 21.

Toxicokinetics

A single blood sample was collected from the jugular vein at 4 hours postdose on GD 17, from the first three available females in the control group and the first five available females in glasdegib-groups. Plasma samples were analyzed for concentration of glasdegib using a validated analytical procedure (b) (4) Method No. P13RPP (See Table 35).

Table 35 Summary of Glasdegib Plasma Concentrations (ng/mL) on GD 17 in Pregnant Rats

(Excerpted from Submission)

Time Point: 4 hours postdose	Dose (mg/kg/day)		
	10	50	100
Mean	3631	22510	35680
SD	359	1371	2224
n	5	5	5

The Applicant calculated the unbound C_{max} of 79.9 ng/mL and 495 ng/mL in rats at 10 mg/kg/day and 50 mg/kg/day, respectively. In previously untreated patients with AML or high-risk MDS, exposure of glasdegib at 100 mg QD in combination with LDAC, expressed as unbound C_{max}, was 113 ng/mL. Fetal toxicity occurred at maternal exposure of approximately 4-times the human exposure based on unbound C_{max} in rats at 50 mg/kg/day of 495 ng/mL. Teratogenicity occurred at maternal exposures of approximately 0.7-times the human exposure at the recommended dose based on unbound Cmax at 10 mg/kg/day of 79.9 ng/mL.

Dosing Solution Analysis

The relative standard deviation of each concentration was ≤ 10% and the mean sample concentration results were within ± 15% of the theoretical concentration, meeting specifications for homogeneity and concentration verification. No significant detection of glasdegib occurred in the vehicle control samples.

Necropsy

No glasdegib-related maternal macroscopic observations. Placenta exams were normal.

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

The primary glasdegib-related effects were lower embryo-fetal viability and mean fetal body weights and higher incidences of fetal external, visceral, and/or skeletal malformations and/or variations. Glasdegib-related and significant effects occurred in all ovary and uterus parameters examined at 50 and 100 mg/kg/day (See Table 36). Overall, the higher number of resorptions resulted in higher mean postimplantation loss at 50 and 100 mg/kg/day (88.36% and 100%, respectively, compared with 4.72% in controls). No live fetuses were available for evaluations at 100 mg/kg/day due to total litter loss in all dams. Mean fetal body weights (male, female, and sexes combined) in the 50 mg/kg/day dose group were lower (0.62x) than control group means (See Table 37).

Table 36 Summary of Laparohysterectomy Evaluations in Rats

Glasdegib Dose (mg/kg/day)	0	10	50	100
Number of females tested	8	8	8	8
Number of females pregnant	7	8	8	8
Unscheduled euthanasia	0	0	0	0
Early delivery	0	0	0	0
Number of litters examined	7	8	5	0
Total number of resorptions N	0.6	0.9	8.0	12.5
Mean number Corpora lutea per liter	13.1	14.4	12.4	13.3
Mean number of Implantations	13.0	13.5	11.0	12.5
Mean Percent pre-implantation loss	1.0	4.8	11.9	3.7

Glasdegib Dose (mg/kg/day)	0	10	50	100
% Live fetuses	12.4	12.6	1.1	0
Post-implantation loss				
Mean Early resorptions/litter	0.6	0.9	8.0	12.5
Mean Late resorptions/litter	0.0	0.0	1.9	0.0
Mean Percent post-implantation loss	4.7	6.4	88.4	100.0

Percent Post-implantation loss = [(# implantations - # live fetuses) / # implantations] x 100

Table 37 Summary of Litter Observations in Rats

Glasdegib Dose (mg/kg/day)	0	10	50	100
Number of females pregnant	7	8	8	8
% Live fetuses	12.4	12.6	1.1	0
Sex distribution				
Mean # of Males (%)	5.7(46.8)	5.6(43.6)	0.2(6.7)	
Mean # of Females	6.7	6.9	1.6	
Mean fetal body weight (g) per litter	5.784	5.955	3.611	
Male fetuses	5.987	6.148	3.910	
Female fetuses	5.609	5.809	3.567	

Fetal Evaluations

Fetal observations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development).

Fetal evaluations were based on 87, 101, and 9 viable GD 21 cesarean-delivered fetuses in 7, 8, and 5 litters in the 0, 10, and 50 mg/kg/day groups, respectively. Each of these fetuses was examined for external, visceral (fixed head and/or fresh Staples), and skeletal (intact or body only) abnormalities. Total glasdegib-related litter loss at 100 mg/kg/day precluded fetal external, visceral, and skeletal examinations at this dose.

Fetal Examinations (External, Visceral and Skeletal)

No glasdegib-related external malformations or variations at 10 mg/kg/day, but at skeletal examinations there were delays in skeletal ossification, structural changes in the ribs, and one fetus had a few skeletal malformations (absent ribs, a fused lumbar vertebra and a fused thoracic vertebra) that were similar to findings noted at 50 mg/kg/day. The skeletal malformations noted at 10 mg/kg/day were considered glasdegib-related since these findings were considered part of the larger spectrum of anomalies at 50 mg/kg/day.

All 9 viable fetuses from 5 litters in the 50 mg/kg/day group were available for evaluation, and each fetus had multiple external malformations and/or variations (See Table 37).

- The most frequently observed glasdegib-related external malformations, which occurred in 89% to 100% of the fetuses available for examination in the 50 mg/kg/day group, consisted of small mouth, misshapen snout, short fore- and/or hindlimbs, absent fore and/or hindpaw digits, short fore and/or hindpaw digits, and a short tail. Additional malformations consisted of malformed eyes (malpositioned, open, depressed eye bulge), face (small mouth, snout misshapen), trunk (short trunk), and tail (narrow, thread-like). Most of the external malformations were confirmed to be the result of underlying skeletal dysmorphogenesis.
- The most frequently observed glasdegib-related visceral malformations consisted of severe dilation of the lateral ventricles of the brain, a malpositioned eye, a misshapen head (rhinocephaly), absent palatal rugae, a small tongue, an absent tooth (upper incisors), an interrupted nasopharynx, malformed adrenals (absent, large, small, and/or malpositioned), a malpositioned aorta, a malformed esophagus (atretic or narrow), large ovaries, persistent truncus arteriosus, a ventricular septum defect, malformed kidneys (absent, malpositioned, small), a misshapen liver, a malformed liver (absent, small), a narrow pulmonary trunk, a retroesophageal subclavian artery, an absent trachea, an absent ureter, and an absent urinary bladder.
- The most frequently observed glasdegib-related skeletal malformations, which occurred in 78% to 100% of the fetuses available for examination in the 50 mg/kg/day dose group, consisted of multiple rib or vertebral abnormalities, absent fore- and/or hindpaw phalanges, short long bones (humerus, radius, ulna, femur, tibia), an unossified fibula, a small scapula body, and absent metatarsals. Additional malformations consisted of fused skull bones (mandible and maxilla), a palatine cleft, an absent premaxilla, and a bent clavicle.

Table 38 Summary of Glasdegib-Related Abnormalities in Rats

Glasdegib Dose (mg/kg)		0	10	50
Fixed Head				
Total number of litters examined		7	8	5
Total number of fetuses examined		42	48	3
M- Brain: Severe dilated lateral ventricle	Litter incidence (%)	-	-	2 (66.7)
	Fetal incidence (%)	-	-	2 (66.7)
V- Brain: Moderate dilated lateral ventricle	Litter incidence (%)	-	-	1(33.3)
	Fetal incidence (%)	-	-	1(33.3)
M- Eye: Malpositioned	Litter incidence (%)	-	-	1(33.3)
	Fetal incidence (%)	-	-	1(33.3)
M- Head/neck: Head, misshapen	Litter incidence (%)	-	-	3(100.0)
	Fetal incidence (%)	-	-	3(100.0)
	Litter incidence (%)	-	-	3(33.3)

M- Mouth: Palatal rugae, Tongue absent, small	Fetal incidence (%)	-	-	3(33.3)
M- Mouth: Tooth, absent	Litter incidence (%)	-	-	3(100.0)
	Fetal incidence (%)	-	-	3(100.0)
M- Nasopharynx, interrupted	Litter incidence (%)	-	-	3(100.0)
	Fetal incidence (%)	-	-	3(100.0)
Fresh Visceral Body				
Total number of litters examined		7	8	5
Total number of fetuses examined		87	101	9
M- Adrenal Gland: absent, large, malpositioned	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	1(11.1)
M- Adrenal Gland: small	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	5(55.6)
M- Carotid artery: origin, malpositioned	Litter incidence (%)	-	-	2(40.0)
	Fetal incidence (%)	-	-	3(33.3)
M- Vessels : Truncus Arteriosus, persistent	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	1(11.1)
M- Esophagus: narrow	Litter incidence (%)	-	-	2(40.0)
	Fetal incidence (%)	-	-	2(22.2)
M- Gonad: Ovary large	Litter incidence (%)	-	-	2(40.0)
	Fetal incidence (%)	-	-	(2(22.2)
M- Heart: Ventricular Septum, defect	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	7(77.8)
V- Innominate Artery: absent	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	5(55.5)
M- Kidneys: absent	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	3(33.3)
M- Kidneys: small	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	2(22.0)
V- Kidneys: malpositioned	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	3(33.3)
M- Liver: misshapen	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	1(11.1)
M- Lung: absent	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	6(66.7)
M- Lung: small	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	5(55.6)
M- Pulmonary trunk: narrow	Litter incidence (%)	-	-	4(80.0)
	Fetal incidence (%)	-	-	6(66.7)
V- Spleen: large	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	4(44.4)
M- Subclavian Artery: retroesophageal	Litter incidence (%)	-	-	2(40.0)
	Fetal incidence (%)	-	-	3(33.3)
V- Subclavian Artery: malpositioned	Litter incidence (%)	-	-	5(100.0)
	Fetal incidence (%)	-	-	7(77.8)
M-Trachea: absent	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	5(55.6)
M- Ureter: absent	Litter incidence (%)	-	-	3(60.0)
	Fetal incidence (%)	-	-	3(33.3)

M- Urinary Bladder: absent	Litter incidence (%)	-	-	1(20.0)
	Fetal incidence (%)	-	-	3(33.3)
Skeletal[1]				
Total number of litters examined		7	8	5
Total number of fetuses examined		87	101	9
M- Mandible: fused	Litter incidence (%)			4(80.0)
	Fetal incidence (%)			5(83.3)
M- Maxilla: fused	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			6(100.0)
M- Palatine: cleft	Litter incidence (%)			1(20.0)
	Fetal incidence (%)			1(16.7)
M- Premaxilla: absent	Litter incidence (%)			4(80.0)
	Fetal incidence (%)			5(83.3)
M- Forepaw phalanges: absent	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Humerus: bent	Litter incidence (%)			1(20.0)
	Fetal incidence (%)			1(11.1)
M- Humerus: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Metacarpal: absent	Litter incidence (%)			3(60.0)
	Fetal incidence (%)			4(44.4)
V- Metacarpal: unossified	Litter incidence (%)		1(12.5)	5(100.0)
	Fetal incidence (%)		1(1.0)	8(88.9)
M- Radius: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Ulna: unossified	Litter incidence (%)			1(20.0)
	Fetal incidence (%)			1(11.1)
M- Ulna: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0
M- Femur: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			8(88.9)
M- Fibula: unossified	Litter incidence (%)			4(80.0)
	Fetal incidence (%)			7(77.8)
M- Fibula: short	Litter incidence (%)			2(40.0)
	Fetal incidence (%)			3(33.3)
M- Hindpaw phalanges: absent	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Metatarsal: absent	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
M- Tibia: short	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			8(88.9)
M- Tibia: unossified	Litter incidence (%)			1(20.0)
	Fetal incidence (%)			1(11.1)
V- Ischium: incomplete ossification	Litter incidence (%)		1(12.5)	4(80.0)
	Fetal incidence (%)		1(1.0)	7(77.8)
V- Pubis: incomplete ossification	Litter incidence (%)		1(12.5)	4(80.0)
	Fetal incidence (%)		1(1.0)	7(77.8)
M- Rib: multiple ribs	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			9(100.0)
V- Rib: nodulated	Litter incidence (%)		2(25.0)	
	Fetal incidence (%)		2(2.0)	
V- Rib: wavy	Litter incidence (%)		1(12.5)	
	Fetal incidence (%)		1(1.0)	

V- Rib: incomplete ossification	Litter incidence (%)		4(50.0)	
	Fetal incidence (%)		5(5.0)	
V- Sternebra: asymmetric	Litter incidence (%)		1(12.5)	
	Fetal incidence (%)		1(1.0)	
M- Scapula body: small	Litter incidence (%)			5(100.0)
	Fetal incidence (%)			8(88.9)

[1] Numerous skeletal variations occurred. Only skeletal malformations are listed except for few cases to document similar variations occurrence at 10 mg/kg and 50 mg/kg

Study title: A Preliminary Embryo-Fetal Development Study of PF-04449913 by Oral Gavage in Rabbits

Study no.: 20069230 (15GR057)
Study report location: eCTD 4.2.3.5.2
Conducting laboratory and location: (b) (4)
Date of study initiation: September 29, 2015
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: PF-04449913; E010015565; Chiral potency factor 0.761.

Key Study Findings

- Glasdegib produced maternal toxicity in rabbits based on maternal body weight losses and lower food intake at ≥5 mg/kg/day and abortions and elective euthanasia at ≥10 mg/kg/day.
- Glasdegib produced fetal toxicity and teratogenicity in rabbits at 5 mg/kg/day based on lower embryo-fetal survival, increased post-implantation loss, lower mean fetal body weights, and higher incidences of external, visceral, and skeletal malformations and variations at maternal exposures of approximately 0.6-times the human exposure at the recommended dose based on unbound C_{max} at 5 mg/kg/day of 63.3 ng/mL.

Methods

Doses: 5, 10, 50, or 100 mg/kg/day on GD 7 though GD 19. Doses based on 13-w repeat-dose toxicology study 8299265 (14LJ055) in rats.
Frequency of dosing: Daily
Dose volume: 10 mL/kg
Route of administration: Oral gavage
Formulation/Vehicle: 0.5% (w/v) methylcellulose (4000 cps) in reverse osmosis water
Species/Strain: New Zealand White [Crl:KBL(NZW)] rabbits
Number/Sex/Group: 8 time-mated females/group
Satellite groups: None

Study design: Standard. Cesarean section conducted on GD 29.

Deviation from study protocol: None that had an impact on study data or interpretation of results.

Observations

Table 39 Experimental Design of EFD in Rabbits

(Excerpted from Submission)

Group No.	Test Article	PF-04449913 Dose (mg/kg/day)	PF-04449913 Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Rabbits (Assigned Rabbit Numbers)
1	Vehicle Control Article	0	0	10	8 (4901-4908)
2	PF-04449913	5	0.5	10	8 (4909-4916)
3	PF-04449913	10	1	10	8 (4917-4924)
4	PF-04449913	50	5	10	8 (4925-4932)
5	PF-04449913	100	10	10	8 (4933-4940)

Results

Mortality

None of the rabbits in the 10, 50, and 100 mg/kg/day dose groups survived to scheduled C-section.

Table 40 Summary of Glasdegib-Related Mortality in Rabbits

Glasdegib (mg/kg/day)	Number	GD	Reason
0	1	21	Aborted ↓BW/FC. Litter: 3 early/1 late resorptions, 2 live fetuses
5	1	12	Euthanized acute paralysis. ↓BW/FC. Litter: 6 live fetuses
	1	23	Aborted ↓BW/FC. Litter: 6 late resorptions, 2 live fetuses
10	1	19	Found dead. Red gelatinous material present in the thoracic cavity and a perforation in the right diaphragmatic lobe of the lung. Litter: 62.5% post-implantation loss.
	5	19-21	Aborted. ↓BW/FC. Litter: 100% early resorptions.
	2	23	Euthanized ↓BW and adverse clinical signs. One dam no pregnant. Litter: 83.3% post-implantation loss.
50	1	18	Euthanized. ↓BW and adverse clinical signs. Litter: 100% early resorptions.
	3	18-20	Aborted. Litter: 100% early resorptions.
	4	19-20	Euthanized ↓BW and adverse clinical signs. Litter: 100% early resorptions.
100	1	14	Euthanized ↓BW and adverse clinical signs. Litter: 100% early resorptions.
	7	16-17	Euthanized ↓BW and adverse clinical signs. Litter: 100% early resorptions.

Clinical Signs

Glasdegib (mg/kg/day)	Common Clinical Signs
0	None
5	Unknown etiology for the acute paralysis. Ungroomed fur and limited usage of the left and right hindlimbs
10	Soft feces, decreased fetal output, red discharge from the vagina (aborted tissue, liquid material), ungroomed fur, and fur loss
50	Absent feces/decreased fecal output, ungroomed coat, fur loss, and red aborted tissue and red liquid material.
100	Absent feces/decreased fecal output, ungroomed coat, fur loss, and red discharge from the vagina and red liquid material.

Body Weight

Glasdegib-related and adverse lower maternal body weight gain occurred at all doses. The mean maternal body weight gain in the 5 mg/kg/day surviving dams was 0.15x control for the interval of GD 7 to 29.

Table 41 Maternal Body Weight Gains in Pregnant Rabbits

(Excerpted from Submission)

Sex: Female		Day(s) Relative to Mating (L)			
		7 → 10	10 → 13	13 → 16	16 → 20
0 mg/kg/day	Mean	55.84	-5.95	22.19	-1.41
	SD	29.09	55.51	55.59	100.61
	N	8	8	8	8
5 mg/kg/day	Mean	-23.95	31.40	-26.57	-20.36
	SD	44.65	45.99	56.14	106.80
	N	8	7	7	7
10 mg/kg/day	Mean	68.69	27.23	-39.41	-207.04
	SD	59.44	67.68	14.48	158.98
	N	7	7	7	5
50 mg/kg/day	Mean	33.73	-38.95	-131.01	-108.03
	SD	85.84	100.27	51.88	175.01
	N	8	8	8	3
100 mg/kg/day	Mean	-37.36	-177.18	-200.87	-
	SD	64.07	93.57	46.82	-
	N	8	8	7	-

7 → 20	20 → 24	24 → 29	20 → 29	7 → 29
70.66	22.33	27.70	50.03	108.51
121.43	61.41	73.03	98.97	134.05
8	7	7	7	7
-28.56	-34.18	41.23	7.05	15.92
150.65	100.67	54.19	125.20	209.80
7	6	6	6	6
-157.54	-	-	-	-
109.75	-	-	-	-
5	-	-	-	-
-219.80	-	-	-	-
357.13	-	-	-	-
3	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-

Feed Consumption

Glasdegib-related and adverse lower maternal food consumption occurred at all doses. The mean maternal food consumption in the 5 mg/kg/day group was 0.71x control for the interval of GD 7 to 20. The mean food consumption improved during the postdose phase GD 20 to 29.

Table 42 Maternal Food Consumption in Pregnant Rabbits (g/animal/day)

(Excerpted from Submission)

Group 1 - Vehicle Control Article
Group 2 - PF-04449913 5 mg/kg/day
Group 3 - PF-04449913 10 mg/kg/day
Group 4 - PF-04449913 50 mg/kg/day
Group 5 - PF-04449913 100 mg/kg/day

Group / Sex		Day (From/To) 7/20	20/29	7/29
1F	Mean	117.1	83.1	101.0
	SD	29.3	33.0	25.5
	N	8	7	7
2F	Mean	82.8	92.1	91.7
	SD	35.8	40.1	28.3
	N	7	6	6
3F	Mean	109.2	-	-
	SD	42.0	-	-
	N	4	-	-
4F	Mean	43.9	-	-
	SD	31.6	-	-
	N	2	-	-
5F	Mean	-	-	-
	SD	-	-	-
	N	-	-	-

Toxicokinetics

Blood samples were collected at approximately 1, 4, 7, and 24 hours postdose on GD 19 except for 100 mg/kg/day rabbits due to early elective euthanasia. Plasma samples were analyzed for concentration of glasdegib using a validated analytical procedure ([(b) (4)] Method No. PF44BPP, validated under [(b) (4)] 8325312). Toxicokinetic parameters were estimated using WinNonlin and fitted to a non-compartmental approach consistent with the oral route of administration (See Table 43).

Table 43 Summary of Toxicokinetic Parameters of Glasdegib in Pregnant Rabbits

(Excerpted from Submission)

Dose (mg/kg/day)		C_{max} (ng/mL)	T_{max} (hr)	AUC_{24} (ng·hr/mL)
5	Mean	3120	2.0	43200
	SD	1030	1.7	40400
	N	3	3	3
10	Mean	22700	1.0	262000
	SD	NA	NA	NA
	N	2	2	1
50	Mean	79800	5.0	1790000
	SD	5460	3.5	NA
	N	3	3	2

AUC_{24} Area under the concentration-time curve from hour 0 to hour 24.
C_{max} Maximum concentration in plasma.
N Number of values in calculation.
NA Not applicable.
SD Standard deviation.
T_{max} Time to maximum plasma concentration.
Note: All animals in the 100 mg/kg/day group were euthanized prior to GD 19.

The Applicant calculated the unbound C_{max} of 63.3 ng/mL in rabbits at 5 mg/kg/day. In previously untreated patients with AML or high-risk MDS, exposure of glasdegib at 100 mg QD in combination with LDAC, expressed as unbound C_{max}, was 113 ng/mL. Fetal toxicity and teratogenicity occurred at maternal exposure of approximately 0.6-times the human exposure at the recommended dose based on unbound C_{max} at 5 mg/kg/day of 63.3 ng/mL.

Dosing Solution Analysis

The relative standard deviation of each concentration was ≤ 10% and the mean sample concentration results were within ± 15% of the theoretical concentration, meeting specifications for homogeneity and concentration verification. No significant detection of glasdegib occurred in the vehicle control samples.

Necropsy

Macroscopic observations in early decedents were summarized in Table 40 under mortality. No other glasdegib-related maternal macroscopic observations were detected at necropsy examination in the rabbits that survived to scheduled euthanasia.

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

All rabbits that survived to scheduled euthanasia in the 0 and 5 mg/kg/day dose groups were pregnant and were examined for ovarian and uterine contents on GD 29. Glasdegib-related and significant effects occurred at 5 mg/kg/day including reduced number of live fetuses, increased late resorptions and mean percent post-implantation loss (See Table 44).

Table 44 Summary of Laparohysterectomy Evaluations in Rabbits

Glasdegib Dose (mg/kg/day)	0	5	10	50	100
Number of females tested	8	8	8	8	8
Number of females pregnant	8	8	7	8	8
Females with live fetuses	8	8	0	0	0
Females with dead or resorbed	0	0	7	8	8
Unscheduled euthanasia	1	2	8	8	8
Females aborted	1	1	5	3	0
Number of litters	7	6			
Mean number Corpora lutea	11.9	11.5			
Mean number of Implantations	10.0	8.3			
Mean Percent pre-implantation loss	16.0	28.6			
Number of live fetuses (mean)	68(9.7)	22(3.7)			
Post-implantation loss Mean Early resorptions Mean Late resorptions Mean Percent post-implantation loss	 0.3 0.0 3.0	 0.3 4.3 47.2			

Percent Post-implantation loss = [(# implantations - # live fetuses) / # implantations] x 100

Table 45 Summary of Litter Observations in Rabbits

Glasdegib Dose (mg/kg/day)	0	5
Number of females pregnant	8	8
Number of live fetuses	9.7	3.7
Sex distribution Mean # of Males (%) Mean # of Females	 5.6(57.5) 4.1	 1.5(40.7) 2.0
Mean fetal body weight (g) Male fetuses Female fetuses	38.9 39.8 37.7	29.2 30.7 29.2

Fetal Evaluations

Fetal observations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); or 2) variations (common findings in this species and strain and reversible delays or accelerations in development).

Fetal evaluations were based on 68 and 22 viable GD 29 cesarean-delivered fetuses in 7 and 6 litters in the 0 and 5 mg/kg/day dose groups, respectively. Each of these fetuses was examined for external, visceral, and skeletal abnormalities.

Fetal Examinations (External, Visceral and Skeletal)

- The most frequently observed glasdegib-related external malformations, which occurred in 54.5% to 63.6% of the fetuses available for examination in the 5 mg/kg/day group, consisted of domed head, absent teeth, and hyperextension of the forepaws. Additional malformations included ears (malpositioned), eyes (malpositioned, open, absent eye bulge, depressed eye bulge), face (proboscis, small mouth, fused nares, malpositioned nares, absent naris, cleft palate, a misshapen palate, misshapen snout, and short snout), head (cranial meningocele), limbs (malrotated fore- and hindlimbs and short fore- and hindlimbs), mouth (a protruding tongue, absent teeth), paws (hyperflexion of the hindpaw), trunk (omphalocele, short trunk, a herniated umbilicus), and tail (bent and short). Most of the external malformations were confirmed to be the result of underlying skeletal dysmorphogenesis.
- The most frequently observed glasdegib-related visceral malformations, which occurred in 36.4% to 68.2% of the fetuses available for examination in the 5 mg/kg/day group, consisted of a diaphragmatic hernia, a malpositioned atrium, absent chordae tendinae, absent papillary muscles, and a retroesophageal subclavian artery. Additional malformations included a malpositioned aorta, malformed eyes (absent or small), persistent truncus arteriosus, an absent ventricular septum, a ventricular septum defect, malpositioned intestines, malpositioned kidneys, absent kidneys, small lungs, and an absent trachea.
- The most frequently observed glasdegib-related skeletal malformation, which occurred in 77.3% of the fetuses available for examination in the 5 mg/kg/day group consisted of short maxilla. Additional malformations consisted of bent clavicles, absent skull bones (exoccipital, palatine, premaxillae, squamosals), fused skull bones (mandibles, maxillae), absent vertebrae (caudal, cervical, lumbar), fused centra in one or more vertebrae (cervical, sacral), fused sacral vertebrae, and multiple interrelated vertebral abnormalities (including absent, fused, misshapen, small, unilaterally ossified, misaligned, bipartite ossification, unossified or incompletely ossified cervical, thoracic, lumbar, sacral, and/or caudal arches, centra and/or vertebrae).

Table 46 Summary of Glasdegib-Related Abnormalities in Rabbits

Glasdegib Dose (mg/kg)	0	5
External		
Total number of litters examined	7	6
Total number of fetuses examined	68	22

M- Ear: Pinna, malpositioned	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	2(9.1)
M- Eye: malpositioned	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	3(13.6)
M- Eye: open	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	2(9.1)
M- Eye: Bulge absent /depressed	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Face: probosis	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Mouth: small	Litter incidence (%)	-	2(9.1)
	Fetal incidence (%)	-	1(16.7)
M- Nares: fused	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	6(27.3)
M- Nares: malpositioned/absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Palate: cleft	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Palate: misshapen	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Snout: short/ misshapen	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Head: Domed	Litter incidence (%)	-	5(83.3)
	Fetal incidence (%)	-	12(54.5)
M- Neck: cranial meningocele	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Forelimb: malrotated, short	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Hindlimb: malrotated, short	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Tongue: protruding	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	2(9.1)
M- Tooth: absent	Litter incidence (%)	-	5(83.3)
	Fetal incidence (%)	-	13(59.1)
M- Forepaw: hyperextension	Litter incidence (%)	-	6(100.0)
	Fetal incidence (%)	-	14(63.6)
M- Hindpaw: hyperextension/ hyperflexion	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Trunk: omphalocele/ short/ Umbilicus	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Tail: bent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Tail: short	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	2(9.1)
Fresh Visceral			
Total number of litters examined		7	6
Total number of fetuses examined		68	22
M- Aorta: malpositioned	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Diaphragm: hernia	Litter incidence (%)	-	5(83.3)

	Fetal incidence (%)	-	15(68.2)
M- Truncus arteriosus: persistent	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	3(16.7)
M-Heart: Atrium malpositioned	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	8(36.4)
M-Heart: Chordae tendinae absent	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	8(36.4)
M-Heart: Papillary muscle absent	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	8(36.4)
M-Heart: Ventricular septum absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M-Heart: Ventricular septum defect	Litter incidence (%)	-	4(66.7)
	Fetal incidence (%)	-	4(18.2)
M- Innominate artery: absent	Litter incidence (%)	-	6(100.0)
	Fetal incidence (%)	-	10(45.5)
M- Intestine: malpositioned	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	2(9.1)
M- Kidney: malpositioned absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Subclavian artery: retroesophageal/ malpositioned	Litter incidence (%)	-	6(100.0)
	Fetal incidence (%)	-	9(40.9)
M- Trachea: absent	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	4(18.2)
Skeletal			
Total number of litters examined		7	6
Total number of fetuses examined		68	22
M- Clavicle: bent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Skull: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Mandible: fused	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Maxilla: fused	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Maxilla: short	Litter incidence (%)	-	6(100.0)
	Fetal incidence (%)	-	17(77.3)
M- Palatine: absent	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Premaxilla: absent	Litter incidence (%)	-	3(50.0)
	Fetal incidence (%)	-	4(18.2)
M- Squamosal: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M-Caudal vertebrae: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Cervical centrum: fused	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	4(18.2)
M- Cervical vertebrae: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Lumbar vertebrae: absent	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	2(9.1)
M- Sacral centrum: fused	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	2(9.1)

M- Sacral vertebrae: fused	Litter incidence (%)	-	1(16.7)
	Fetal incidence (%)	-	1(4.5)
M- Vertebrae general: multiple abnormalities	Litter incidence (%)	-	2(33.3)
	Fetal incidence (%)	-	3(13.6)

9.3 Prenatal and Postnatal Development

No studies were conducted.

10 Special Toxicology Studies

(The following studies were not completely review; Summary Adapted from Submission)

Study No. 16GR159: Single Dose Intravenous and Perivascular Study of PF-04449913 in Female New Zealand White Rabbits. Non-GLP.

Glasdegib (Lot No. 00706780-0036-001) and its associated vehicle were tolerated when administered as a single intravenous and perivascular dose at 1 and 0.05 mg, respectively, followed by a 3-day observation period. No glasdegib-, vehicle- or saline-related local irritation, macroscopic, or microscopic findings occurred in this study.

Study No. 16LJ129: A Multiple Dose Phototoxicity Study to Determine the Effects of Oral Gavage Administration of PF-04449913 on Eyes and Skin in Pigmented Rats. GLP-compliant.

Glasdegib (Lot No. GR10340 (also known as E010015971)) was administered once daily by oral gavage once daily for 3 consecutive days at doses of 0, 10, 30 or 100 mg/kg/day on the eyes and skin of Crl:LE (Long-Evans) pigmented rats, followed by exposure to ultraviolet B, ultraviolet A and visible light from a xenon lamp. In addition, the toxicokinetic characteristics of glasdegib were determined.

No glasdegib-related effects occurred on survival, clinical signs, skin reaction observations in pigmented and non-pigmented skin, body weights or ophthalmology observations. Glasdegib was not phototoxic based on the absence of effects in pigmented and non-pigmented skin and eyes under the conditions of the study. Mean exposure to glasdegib on Day 3 at 100 mg/kg/day was C_{max} of 14200 ng/mL and AUC_{24} of 137000 ng•hr/mL.

Study No. 17GR182: 1-Day In Vitro Hemolysis Compatibility Study of PF-04449913 with Human Blood. Non-GLP.

Glasdegib (Lot No. 00711282-0063-001) was combined with heparinized whole blood and plasma in a series of compound (0.01-1.0 mg/mL) and vehicle dilutions. The samples were then incubated and centrifuged. At predetermined time intervals, the tubes were observed and recorded for hemolysis and precipitation. The extent of hemolysis from each concentration of whole blood and vehicle was determined visually using the positive control and negative control (normal saline) as the baseline. The extent of precipitation from each concentration of plasma and vehicle was determined visually using the negative control (normal saline) as the baseline.

Glasdegib does not cause hemolysis in human whole blood under the study conditions and it is compatible with human donor plasma.

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/s/

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