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RESEARCH**

*APPLICATION NUMBER:*

**207103Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## MEMORANDUM

Ibrance (palbociclib)

**Date:** January 23, 2015

**To:** File for NDA 207103

**From:** John K. Leighton, PhD, DABT

Acting Director, Division of Hematology Oncology Toxicology  
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting and labeling reviews for Ibrance conducted by Dr. Chen, and secondary memorandum and labeling provided by Dr. Palmby. I concur with Dr. Palmby's conclusion that Ibrance may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/  
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JOHN K LEIGHTON  
01/23/2015

## MEMORANDUM

**Date:** January 22, 2015  
**From:** Todd R. Palmby, PhD  
Pharmacology/Toxicology Supervisor  
Division of Hematology Oncology Toxicology (DHOT)  
Office of Hematology and Oncology Products (OHOP)  
**To:** File for NDA 207103 Ibrance (palbociclib)  
**Re:** Approvability for Pharmacology and Toxicology  
**Indication:** in combination with letrozole for the treatment of postmenopausal women with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer as initial endocrine-based therapy for their metastatic disease

Non-clinical pharmacology and toxicology literature and original reports for studies to support NDA 207104 for Ibrance (palbociclib) indicated for use in combination with letrozole for the treatment of postmenopausal women with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer as initial endocrine-based therapy for their metastatic disease were reviewed by Wei Chen, PhD. Studies conducted with palbociclib for which reports were submitted to this NDA include pharmacology, pharmacokinetics and ADME, safety pharmacology, general toxicology, genetic toxicology and reproductive and developmental toxicology.

Palbociclib is a kinase inhibitor, which has inhibitory activity against cyclin dependent kinase (CDK) 4 and 6. Palbociclib would be the first product approved by the US FDA with a mechanism of action involving inhibition of a cyclin-dependent kinase leading to anti-tumor activity. Palbociclib inhibited CDK4/cyclinD1, CDK4/cyclinD3 and CDK6/cyclinD2 complexes with IC<sub>50</sub> values of 11, 9 and 15 nM, respectively. The IC<sub>50</sub> values for CDK2/cyclinA, CDK1/cyclinB and CDK5/p25 were greater than 5 μM. In vitro, palbociclib blocked progression of the cell cycle from G1 to S phase, thereby reducing proliferation in estrogen receptor (ER)-positive breast cancer cell lines. The combination treatment of palbociclib and antiestrogens in breast cancer cell lines lead to reduced retinoblastoma (Rb) protein phosphorylation and E2F expression, reduced signaling and increased growth arrest compared to treatment with each drug alone. In vivo, the combination treatment of palbociclib and letrozole in an ER-positive breast cancer mouse xenograft model lead to increased inhibition of Rb phosphorylation, downstream signaling and dose-dependent tumor growth inhibition compared to treatment with each drug alone. This data supported the enrollment of patients with ER-positive/HER2-negative advanced breast cancer into the clinical trials that were conducted to support the proposed indication. "Kinase inhibitor" was selected as the Established Pharmacologic Class for palbociclib. This is scientifically valid based on the submitted data and clinically meaningful, since adverse events observed in clinical trials were not unique to palbociclib and did not warrant a distinct

pharmacologic class to alert prescribers to potential safety concerns that are not associated with other kinase inhibitors.

General toxicology studies were conducted with oral palbociclib administration for up to 27 weeks in rats and 39 weeks in dogs. The major adverse effects noted at clinically relevant exposures were in the bone marrow/hematolymphoid system and male reproductive organs in these studies. Additional target organs of toxicity included the gastrointestinal tract, liver, kidney, endocrine/metabolic system, respiratory system and adrenal glands in rats or dogs. Studies of 3-months duration would generally support marketing of a pharmaceutical intended to treat patients with advanced cancer. The studies of 26 and 39 weeks were not required by the US FDA to support submission of an NDA for this indication. FDA requested that the Applicant submit the reports for the 27-week study in rats and the 39-week study in dogs to this NDA after reviewing the 27-week rat study report, submitted to the IND, and noting findings of altered glucose metabolism.

Altered glucose metabolism including glycosuria, hyperglycemia and decreased insulin were noted in the 27-week rat toxicology study with palbociclib. Findings in the pancreas (islet cells vacuolation), eye (cataracts, lens degeneration), teeth (degeneration/necrosis of ameloblasts in actively growing teeth), kidney (tubule vacuolation, chronic progressive nephropathy), and adipose tissue (atrophy) were associated with the altered glucose metabolism in these animals. These findings were most prevalent in males at doses  $\geq 30$  mg/kg/day (approximately 11 times the human exposure (AUC) at the recommended dose). Some findings (glycosuria/hyperglycemia, pancreatic islet cell vacuolation, and kidney tubule vacuolation) were present in the 15-week rat study, but at lower incidence and severity. Altered glucose metabolism or associated changes in pancreas, eye, teeth, kidney and adipose tissue were not observed in dogs in repeat-dose toxicology studies up to 39 weeks duration. Hyperglycemia was not observed as an adverse reaction associated with palbociclib administration in clinical trials.

There are multiple publications reporting findings from CDK4, CDK6 or CDK4/6 double knockout mice. Mice that do not express CDK4 are viable, but are small in size and infertile. These mice develop insulin deficient diabetes due to a decrease in the number of pancreatic beta islet cells (Rane, Dubus, et al. 1999). A subsequent publication reported that CDK4 is essential for the postnatal proliferation of pancreatic beta cells (Martin, Hunt, et al. 2003). This suggests that inhibition of pancreatic beta cell proliferation during a time when these cells are undergoing expansion may lead to decreased pancreatic beta cell numbers resulting in insulin deficiency. There are potential reasons why altered glucose metabolism occurred in rats administered palbociclib, but was not observed in dogs or in patients in clinical trials and may not be clinically relevant in the indicated patient population. Rodent pancreatic beta cells are dependent on CDK4 for proliferation, while human beta islet cells may have compensatory CDK expression and, therefore, may be able to proliferate normally in the absence of CDK4 or even CDK4/6. Also, the rats used in the repeat-dose toxicology studies

administered palbociclib were adolescent animals (~8 weeks old at the start of the 27-week study). Beta cell proliferation follows an age-related decline. In humans, beta cell proliferation diminishes shortly after infancy and these cells remain relatively quiescent through adulthood. In rats, this decline occurs slower than in humans, with ~20% of cells proliferating in young animals in the postnatal period, ~10% proliferating during adolescence, ~2% proliferating in early adulthood, 0.07% proliferating in 1 year-old animals and 0.04% proliferating in older animals, approaching the level seen in adult humans. Therefore, the pancreatic beta cells in adolescent rats may be much more sensitive to the effects of palbociclib than in adults, in which beta cells are primarily quiescent. Finally, the altered glucose metabolism and associated findings observed in rats, but not humans, may involve higher relative exposures (AUC) in rats. There is insufficient data at this time to determine if the potential effects of palbociclib on glucose metabolism are clinically relevant. The Applicant has stated that they incorporated appropriate monitoring for these effects in their ongoing and planned clinical trials with palbociclib, so no additional nonclinical studies are necessary at this time. These findings may be important if Ibrance is studied in pediatric patients.

There was an imbalance in pulmonary embolisms in patients receiving palbociclib and letrozole compared to patients receiving letrozole alone in clinical trials, with a higher incidence in the palbociclib-treated patients. There were limited findings of thrombosis in repeat-dose toxicology studies conducted with palbociclib. However, based on available literature, there is a mechanistic plausibility for an increased risk of thrombus in patients receiving palbociclib. Mice overexpressing p16(Ink4), a cell cycle inhibitor that promotes senescence by inhibiting CDK4/6 and is upregulated during normal aging, were studied with four different vascular injury models to assess the time to thrombus formation, time to resolution and effect on inflammation-induced vascular dysfunction. The conclusions of this study were that venous thrombosis is enhanced by overexpression of p16(Ink4) in these models (Cardenas, Owens, et al. 2011). The pharmacological effects of palbociclib in these models may be similar to p16(Ink4) overexpression, since both are inhibitors of CKD4/6. Since pulmonary embolism was identified as an adverse reaction in clinical trials and is included as a Warning in the Ibrance label, no nonclinical studies further assessing the potential for palbociclib to induce thrombosis are required at this time.

Palbociclib was clastogenic in an in vitro micronucleus assay in Chinese Hamster Ovary cells and in vivo in the bone marrow of male rats that received doses of palbociclib  $\geq 100$  mg/kg/day for 3 weeks. The clastogenicity occurred by an aneugenic mechanism. Palbociclib was not mutagenic in an in vitro bacterial reverse mutation (Ames) assay and did not induce structural chromosomal aberrations in vitro in human lymphocytes. Rodent carcinogenicity studies were not required to support this NDA submission for palbociclib.

The Applicant conducted a fertility and early embryonic development study in female rats, which was not required or requested by the US FDA to support an NDA for this indication. There were no adverse effects on mating and fertility rates or embryonic development in treated female rats at exposures above the human exposure at the recommended dose. Testicular degeneration was observed at exposures (AUC) higher than human exposure in rats and at exposures lower than human exposure in dogs, which was partially reversible.

Ibrance can cause fetal harm when administered to a pregnant woman based on findings in animals and mechanism of action. In embryo-fetal development studies, pregnant rats and rabbits were administered oral doses of palbociclib during the period of organogenesis. The maternally toxic dose of 300 mg/kg/day was fetotoxic in rats, resulting in reduced fetal body weights. At doses  $\geq 100$  mg/kg/day in rats, there was an increased incidence of a skeletal variation (increased incidence of a rib present at the seventh cervical vertebra). At the maternally toxic dose of 20 mg/kg/day in rabbits, there was an increased incidence of skeletal variations, including small phalanges in the forelimb. At 300 mg/kg/day in rats and 20 mg/kg/day in rabbits, the maternal systemic exposures were approximately 4 and 9 times the human exposure (AUC) at the recommended dose. A study reported in the literature suggests that inhibition of CDK4/6 during late stages of fetal development may be significantly toxic by causing severe anemia. Mouse fetuses that did not express CDK4 or 6 died starting on embryonic day 14.5 with an increased frequency until birth, shortly after which no CDK4/6 deficient pups remained alive (Malumbres, Sotillo, et al. 2004). It is not clear that this study of CDK4/6 knockout animals is predictive of effects in pregnant humans treated with palbociclib, but this study identified a risk of fetal death, which was not observed in the embryo-fetal developmental toxicity studies conducted with palbociclib. In addition, the death in the CDK4/6 deficient fetuses occurred during a period late in development that was not fully evaluated in the rat and rabbit embryo-fetal developmental toxicity studies. The package insert for Ibrance includes a recommendation that if contraceptive methods are being considered during treatment, advise females of reproductive potential to use them during treatment and for at least two weeks following the last dose. The recommendation of two weeks is based on a relatively short plasma half-life of palbociclib in humans (six plasma half-lives of palbociclib in humans is approximately 7 days).

**Recommendation:** I concur with Dr. Chen's conclusion that submitted pharmacology and toxicology data support the approval of NDA 207103 for Ibrance. There are no outstanding non-clinical issues that would preclude the approval of Ibrance for the proposed indication.

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TODD R PALMBY  
01/22/2015

**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: 207,103  
Supporting document/s: 1  
Applicant's letter date: August 13, 2014  
CDER stamp date: August 13, 2014  
Product: Ibrance (palbociclib)  
Indication: Postmenopausal women with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer who have not received previous systemic treatment for their advanced disease.  
Applicant: Pfizer Inc.  
Review Division: Division of Hematology Oncology Toxicology  
(for Division of Oncology Products 1)  
Reviewer: Wei Chen, Ph.D.  
Supervisor/Team Leader: Todd Palmby, Ph.D.  
Division Director: John Leighton, Ph.D., D.A.B.T. (acting)  
(Amna Ibrahim, M.D. (acting))  
Project Manager: Amy Tilley

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# 1 Executive Summary

## 1.1 Introduction

Pfizer seeks to market Ibrance (palbociclib), a kinase inhibitor with activity against cyclin-dependent kinase (CDK) 4 and 6, for the treatment of advanced breast cancer. Palbociclib would be the first kinase inhibitor approved in the United States with activity against CDK 4/6, which inhibits cell proliferation and cellular DNA synthesis by preventing cell-cycle progression. In this NDA, the Applicant submitted reports and literature for nonclinical pharmacology, pharmacokinetic, and toxicology studies to support the approval of palbociclib in combination with letrozole for postmenopausal women with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer.

## 1.2 Brief Discussion of Nonclinical Findings

The nonclinical repeat-dose general toxicity studies with palbociclib were conducted in rats and dogs, consistent with the clinical route of administration. Non-clinical studies also included safety pharmacology studies, genetic toxicity studies, and reproductive and developmental toxicity studies. The 15-week and 27-week repeat-dose toxicology studies were conducted in compliance with Good Laboratory Practice regulations (21 CFR part 58). The pharmacokinetics and toxicokinetics of palbociclib were also evaluated in rats and dogs.

### *Pharmacology*

Palbociclib is an inhibitor of CDK4/cyclinD1 and CDK6/cyclinD2. Palbociclib modulated downstream targets of CDK4 and CDK6 in vitro including inhibition of retinoblastoma (Rb) protein phosphorylation, induction of G1 phase cell cycle arrest and inhibition of DNA synthesis and cell proliferation. Combination of palbociclib with anti-estrogen agents demonstrated additive inhibition of cell proliferation in ER+ breast cancer cells. Palbociclib showed anti-tumor activity in animal tumor models. The anti-tumor activity of palbociclib was enhanced by the combination of palbociclib and ER antagonism in ER+ breast cancer models.

### *Safety pharmacology*

In safety pharmacology studies, palbociclib administration had adverse effects on the cardiovascular function and on the respiratory function in dogs at 4 times and 50-times, respectively, the human clinical exposure at 125 mg/day based on mean unbound  $C_{max}$  (17 ng/mL). Palbociclib had no adverse effects on the function of the central nervous system in rats.

### *General toxicology*

Palbociclib was assessed in single-dose toxicity studies and repeat-dose studies in rats and dogs. Administration of palbociclib to rats and dogs resulted in adverse effects in the bone marrow, lymphoid tissues, and male reproductive organs. The toxicities in these organs were observed at clinically relevant exposures. Partial to complete reversibility of toxicities to the hematolymphopoietic and male reproductive systems was

demonstrated following a recovery period (4-12 weeks), with the exception of the male reproductive organ findings in dogs. Additional toxicities included gastrointestinal, liver, kidney, endocrine/metabolic, respiratory, and adrenal effects in rats and/or dogs. Minimal or mild degeneration and regeneration of kidney tubule epithelial cells was observed with increased incidence and/or severity following 15-weeks, or longer, of dosing in rats and minimal degeneration was observed in a 2-week dog study at a severely toxic dose. Altered glucose metabolism associated with pancreatic islet cell vacuolation, and secondary effects in the eye, teeth, kidney, and adipose tissue were identified in the 27-week rat study with palbociclib, with findings most prevalent at doses of  $\geq 30$  mg/kg/day in males and 300 mg/kg/day in females. The exposures in male and female rats at these doses were approximately 11 and 7 times the human exposure (AUC) at the recommended dose, respectively. Some of these findings were present in the 15-week repeat-dose toxicology study in rats, but with decreased incidence and severity. The rats used in these studies were approximately 7 weeks old at the beginning of the studies. The findings of pancreatic islet cell vacuolation, eye lens degeneration, degeneration of tooth ameloblasts, and renal tubuloepithelial cell vacuolation correlated with increased serum glucose levels and glucosuria. This suggests these findings may be secondary effects of increased glucose levels, although there are potential primary effects of palbociclib on these organs in the pathogenesis of these findings. Observed mortalities in the 27-week rat study resulted from degeneration and/or inflammation of the feet and other adverse effects, which are considered to be associated with treatment induced hyperglycemia. The lens degeneration and chronic progressive nephropathy remained at the recovery euthanasia, suggesting a permanent and/or progressive nature of these effects.

#### *Genetic toxicology*

Palbociclib was not mutagenic in the Ames bacterial mutagenicity assay in the presence or absence of metabolic activation. Palbociclib did not induce structural or numerical chromosome aberrations in cultured human peripheral blood lymphocytes in the presence or absence of metabolic activation. Palbociclib caused micronuclei formation due to an aneugenic mechanism in CHO-WBL cells. In addition, palbociclib induced micronuclei formation in male rats at doses  $\geq 100$  mg/kg/day (greater than 10 times the human exposure at the therapeutic dose) in an in vivo rat micronucleus assay. Therefore, palbociclib is an aneugen.

#### *Reproductive toxicology*

Palbociclib did not cause adverse effects on estrous cycling, mating, fertility and early embryonic development when administered to female rats before and during the mating time frame, and continuing until gestation day (GD) 7. Repeat dosing of palbociclib caused toxicities in male reproductive organs in rats and dogs. The observed toxicities included testicular degeneration, decreased prostate weights and/or prostate atrophy, were generally dose-dependent and did not fully recover after a non-dosing period of up to 12-weeks. When administered to animals during the period of organogenesis, palbociclib resulted in reduced fetal body weights, and lead to increased fetal incidence of cervical ribs at the 7<sup>th</sup> cervical vertebra (skeletal variation) in rats, and a low incidence of small phalanges on the forepaws in rabbits. In addition, available literature on the

function of CDK4/CDK6 in development suggests that palbociclib can cause fetal harm based on its mechanism of action. As reported in the literature, CDK4/6 knockout mice die in late stages of fetal development (gestation day 14.5 until birth) due to severe anemia.

#### *Carcinogenicity studies*

No studies were conducted to support this NDA, as the indicated population has advanced cancer.

### **1.3 Recommendations**

#### **1.3.1 Approvability**

Recommending approval. The nonclinical studies adequately support the safety of oral palbociclib in postmenopausal women with estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer who have not received previous systemic treatment for their advanced disease.

#### **1.3.2 Additional Non Clinical Recommendations**

Additional nonclinical studies are not needed at this time.

#### **1.3.3 Labeling**

Information needed for nonclinical sections of the label are provided in this review.

Therefore, a separate labeling review is not deemed necessary.

Dose level	AUC in animal	AUC in human	AUC ratio
30 mg/kg, male rat (27-week toxicology study)	21300 ng•hr/mL	1863 ng•h/mL (125 mg/dose, daily)	11.4
300 mg/kg, female rat (27-week toxicology study)	12800 ng.hr/mL		6. (b) (4)
300 mg/kg, rat (Embryonic Fetal Development)	7310 ng•h/mL		3.9
20 mg/kg, rabbit (Embryonic Fetal Development)	17200		9.2

## 2 Drug Information

### 2.1 Drug

CAS Registry Number: 571190-30-2

Trade name: Ibrane

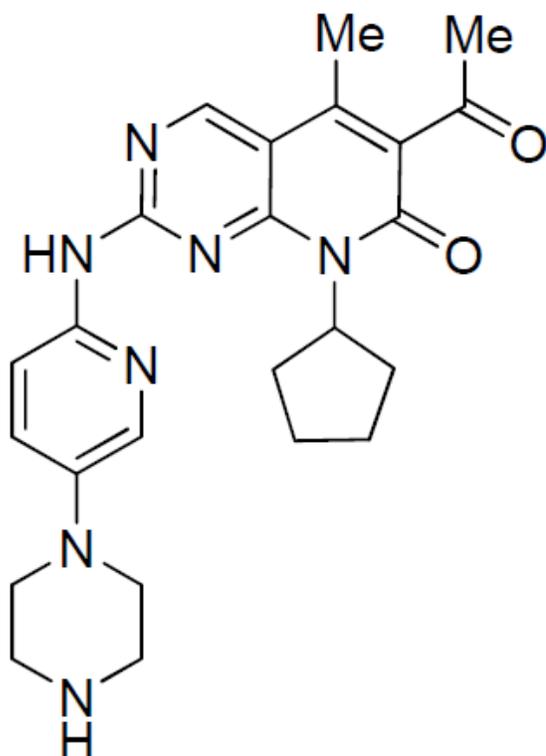
Generic Name: Palbociclib

Code Name: PD-0332991

Chemical Name: 6-Acetyl-8-cyclopentyl-5-methyl-2-[[5-(piperazin-1-yl)pyridin-2-yl]amino]pyrido[2,3-d]pyrimidin-7(8H)-one

Molecular Formula/Molecular Weight:  $C_{24}H_{29}N_7O_2$  / 447.54 g/mole

Structure or Biochemical Description:



Pharmacologic Class: kinase inhibitor

Mechanism of action: an inhibitor of CDK 4/6

Relevant INDs, NDAs, BLAs and DMFs: IND 69,324

### 2.2 Drug Formulation: capsule, 75 mg, 100 mg or 125 mg

The composition for all 3 strengths (b) (4); the following are the details for the composition of palbociclib 75 mg capsules  
(Copied from Applicant's submission)

Name of Ingredients	Reference to Standard	Function	Unit Formula	
			Unit (mg)	%
<b>Blend Composition</b>				
Palbociclib	Pfizer	Drug Substance	75.000 <sup>1</sup>	(b) (4)
Microcrystalline Cellulose (b) (4)	USP/NF, Ph Eur., JP	(b) (4)		
Lactose Monohydrate	USP/NF, Ph Eur., JP			
Sodium Starch Glycolate (Type A)	USP/NF, Ph Eur., JP			
Colloidal Silicon Dioxide	USP/NF, Ph Eur., JP			
Magnesium Stearate	USP/NF, Ph Eur., JP			
<b>Total Target Fill Weight</b>				
<b>Hard Gelatin Capsule Shell</b>				
Capsule Shells (Size #2, (b) (4) HG Capsules) <sup>3,4</sup>	Pfizer	Encapsulation	1 capsule	
Body (b) (4)				(b) (4)
Gelatin	USP/NF, Ph Eur., JP	(b) (4)		
Red Iron Oxide (b) (4)	USP/NF, JP			
Yellow Iron Oxide (b) (4)	USP/NF, JP			
Titanium Dioxide (b) (4)	USP/NF, Ph Eur., JP			
Cap (b) (4)				
Gelatin	USP/NF, Ph Eur., JP	(b) (4)		
Red Iron Oxide (b) (4)	USP/NF, JP			
Yellow Iron Oxide (b) (4)	USP/NF, JP			
Titanium Dioxide (b) (4)	USP/NF, Ph Eur., JP			
Approximate Weight of Capsule Shell				
<b>Print Ink<sup>4</sup></b>				
Approximate Weight of Ink on Capsule Shell				(b) (4)

N/A is not applicable; HG is hard gelatin; qsp is sufficient quantity

1 (b) (4)

2 (b) (4)

3 (b) (4)

4 The body is pre-printed with "PBC 75" and the cap pre-printed with "Pfizer" with (b) (4) White. Printing ink contains Shellac (b) (4) in (b) (4) (USP/NF, Ph Eur., JP), Titanium Dioxide (USP, FCC, Ph Eur., JP) E171, (b) (4) (USP, Ph Eur., JP), Ammonium Hydroxide (b) (4), Propylene Glycol (USP, FCC, Ph Eur., JP, JSFA) (b) (4) and Simethicone USP/Simeticone Ph Eur. (b) (4)

5 (b) (4)

**2.4 Comments on Novel Excipients: N/A**

**2.5 Comments on Impurities/Degradants of Concern:**

The CMC review team requested input from the pharmacology/toxicology team regarding the proposed drug product acceptance limit for degradation product (b) (4) at NMT (b) (4)%, and specifications for two drug substance impurities, (b) (4) and (b) (4), proposed at NMT (b) (4)% and (b) (4)%, respectively.

The proposed acceptance limit at NMT (b) (4)% for the degradation product (b) (4) is acceptable from a pharmacology/toxicology perspective, as (b) (4) is a metabolite formed in rats and humans. In addition, there were no severe toxicities observed in rats with a systemic exposure of (b) (4) (b) (4) times higher than

expected in humans at the recommended therapeutic dose at a specification of (b) (4) % of (b) (4). (b) (4) was tested in an in vitro bacterial reverse mutation (Ames) assay and in an in vitro micronucleus assay. (b) (4) was not mutagenic in the Ames test, but was positive in the micronucleus assay, and subsequently found to be aneugenic based on the results of a fluorescent in situ hybridization test. Therefore, (b) (4) is an aneugenic impurity.

The amount of (b) (4) in the batch of drug substance used to conduct the in vivo micronucleus study in rats with palbociclib was (b) (4) %, and the NOEL in that study was 50 mg/kg/day. Therefore, the NOEL for aneugenicity of (b) (4) in the in vivo rat micronucleus study was (b) (4) mg/kg/day, or (b) (4) mg/m<sup>2</sup>/day. The amount of (b) (4) that would be administered to a patient in the to be marketed formulation of palbociclib at the proposed specification of (b) (4) % is (b) (4) mg/day, or (b) (4) mg/m<sup>2</sup>/day ((b) (4) mg/kg/day) for a 60 kg patient. Therefore, the amount of (b) (4) administered to rats in the in vivo micronucleus assay was higher than the maximum amount that patients would receive from the recommended dose of palbociclib. Given that aneugens have a threshold for inducing their effects, the proposed specification of (b) (4) (NMT (b) (4) %) in the palbociclib drug substance presents little concern for inducing clastogenic effects and subsequent secondary malignancies in patients. The amount of (b) (4) administered to rats in the 15-week repeat-dose toxicity study at the no observed adverse effect level (NOAEL) was (b) (4) times higher than the amount of (b) (4) that would be administered to a 50 kg patient receiving the recommended dose of 125 mg/day palbociclib. The proposed specification for (b) (4) is acceptable for the proposed indication of this NDA in patients with advanced breast cancer.

The structure of (b) (4) was similar to palbociclib, which is aneugenic. (b) (4) was negative in a bacterial reverse mutation (Ames) assay. The amount of (b) (4) in a batch of palbociclib used for a 3-week repeat-dose toxicity study was (b) (4) %. At a dose level of 50 mg/kg/day, which did not result in severe toxicity in that study, rats received a dose of (b) (4) mg/kg/day, or (b) (4) mg/m<sup>2</sup>/day. The amount of (b) (4) that would be administered to a patient in the to be marketed formulation of palbociclib at the proposed specification of (b) (4) % is (b) (4) mg/day, or (b) (4) mg/m<sup>2</sup>/day ((b) (4) mg/kg/day) for a 60 kg patient. Therefore, the amount of (b) (4) administered to rats at a dose level resulting in an acceptable level of toxicity was (b) (4) times greater than the maximum amount that patients would receive from the recommended dose of palbociclib.

The proposed specifications for (b) (4) and (b) (4) are acceptable from a nonclinical perspective based on safety, since these levels are qualified by toxicology studies. The CMC review team will determine the final acceptance of the proposed control criteria for these drug substance impurities according to the batch analysis data and manufacturing capabilities.

## **2.6 Proposed Clinical Population and Dosing Regimen**

Proposed clinical population:

Postmenopausal women with estrogen receptor (ER) positive, human epidermal growth factor receptor 2 (HER2)-negative advanced breast cancer who have not received previous systemic treatment for their advanced disease.

Proposed dose and dose regimen:

125 mg, once daily for 21 consecutive days followed by 7 days off treatment with food and in combination with letrozole 2.5 mg once daily given continuously.

Route of administration: oral

### 3 Studies Submitted

#### Studies Reviewed

##### Secondary Pharmacodynamics

	Title	Study no.	Folder/file name
1	In Vitro Pharmacology: Study of PF-00080665-73	7570744	M4.2.1.2
2	In Vitro Pharmacology: Pfizer 2009 profile study of PF-05089326-00	75760087	M4.2.1.2

##### Safety Pharmacology

	Title	Study no.	Folder/file name
1	Effect of PD-0332991-0054 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	130729.QHJ	M4.2.1.3
2	Effects OF PD-0332991 on action potentials recorded from dog isolated purkinje fibres in vitro	PD332991/IC/001/02	M4.2.1.3
3	Safety Pharmacology - Neurofunctional Evaluation of PD 332991-54 in Sprague-Dawley Rats	745-03890	M4.2.1.3
4	Safety Pharmacology - Effect of PD 332991-54 on Pulmonary Function in Beagle Dogs	745-03892	M4.2.1.3
5	Safety Pharmacology – Blood Pressure, Heart Rate, and Cardiac Rhythm Effects of PD 332991-2B in Beagle Dogs	745-03696	M4.2.1.3
6	Safety pharmacology-cardiovascular assessment of oral PD-0332991 in telemetry instrumented male beagle dogs	13GR248	M4.2.1.3

##### ADME

	Title	Study no.	Folder/file name
Absorption			
1	Single dose pharmacokinetics, dose proportionality, and oral (PO) bioavailability of PD 0332991 in rats following intravenous administration of PD 0332991-0002C and	764-04175	M4.2.2.2

	PO administration of PD 0332991-0054		
2	Single dose pharmacokinetics and oral bioavailability of PD 0332991 in male beagle dogs following intravenous administration of PD 0332991-0002C and oral administration of PD 0332991-0054	764-04166	M4.2.2.2
3	Single dose pharmacokinetics and oral bioavailability of PD 0332991 in male Cynomolgus monkeys following intravenous administration of PD 0332991-0002C and oral administration of PD 0332991-0054	764-04200	M4.2.2.2
Distribution			
1	Tissue distribution of [14C]PD 0332991 in Long-Evans male rats	DM2005-0332991-001	M4.2.2.3
2	Protein binding of PD-0332991 in rabbit plasma	141526	M4.2.2.3
3	Protein binding of PF-05089326 in mouse, rat, dog and human plasma	104347	M4.2.2.3
4	In vitro protein binding of PD 0332991 to plasma proteins of mouse, rat, dog, and human	764-04174	M4.2.2.3
5	Definitive red blood cell (RBC) distribution of PD 0332991-0054 in whole blood of mouse, rat, dog, monkey, and human	764-04302	M4.2.2.3
Metabolism			
1	Reaction phenotyping of PD-0332991 using human hepatocyte relay method	095443	M4.2.2.4
2	Metabolism of PD-0332991 in rat, dog and human hepatocytes	193503	M4.2.2.4
3	Metabolism and excretion of PD-0332991 in sprague Dawley rats following a single oral dose of 50 mg/kg [14C]PD-0332991 and identification of circulating and excretory metabolites	135758	M4.2.2.4
4	Metabolism of PD-0332991 following a single oral dose of 1.97 mg/kg [14C]PD-0332991 in beagle dog	204619	M4.2.2.4
Excretion			
1	Absorption and excretion of [14C]PD-0332991 following oral administration to rats	8273768	M4.2.2.5
2	Absorption and excretion of [14C]PD-0332991 following oral administration to dogs	8276000	M4.2.2.5
Drug interactions			
1	Effect of PD-0332991 on human drug metabolizing enzymes in vitro	181141	M4.2.2.6
2	In vitro evaluation OF PF-00080665 (PD-0332991) as an inhibitor of UDP-glucuronosyltransferase (UGT) enzyme activities in human liver microsomes	165849	M4.2.2.6

Toxicology studies

## Repeat dose

	Title	Study no.	Folder/file name
1	A 15-week toxicity study of PD-0332991 by oral gavage administration in rats with a 4-week recovery period	20026125	M4.2.3.2
2	27-week oral gavage chronic toxicity and toxicokinetic study with PD-0332991 in rats with a 12-week recovery phase	8282224	M4.2.3.2
3	A 15-week toxicity study of PD-0332991 by oral gavage administration in Dogs with a 4-Week Recovery Period	20026126	M4.2.3.2

## Genotoxicity studies

	Title	Study no.	Folder/file name
1	Bacterial Mutagenicity Assay of PD 332991-54	745-03858	M4.2.3.3
2	Chromosomal Aberrations In Cultured Human Peripheral Blood Lymphocytes With PD 332991-0054	25093-0-449OECD	M4.2.3.3
3	Genetic toxicology–bacterial mutagenicity assay of (b) (4)	2013-1QA-GN	M4.2.3.3
4	In vitro micronucleus assay of (b) (4)	eTK62013-5	M4.2.3.3
5	Genetic toxicology–bacterial mutagenicity assay of an impurity (b) (4) of parent compound PD-0332991	eAMES2013-15	M4.2.3.3

## Reproductive and development toxicity studies

	Title	Study no.	Folder/file name
1	A Fertility and Early Embryonic Development Study of PD-0332991 by Oral (Gavage) Administration in Female Rats	20037853	M4.2.3.5
2	An Embryo-Fetal Development Study of PD-0332991 by Oral (Gavage) in Rats	20039574	M4.2.3.5
3	An Embryo-Fetal Development Study of PD-0332991 by Oral (Gavage) in Rabbits	20039575	M4.2.3.5

**Studies summarized**Pharmacology

	Title	Study no.	Folder/file name
1	Palbociclib (PD-0332991) biochemical potency evaluation toward DK4/6 and expanded human kinome selectivity analysis	PD-0332991_12Dec13_133827	M4.2.1.1
2	Summary of palbociclib (PD-0332991) combination with anti-estrogen therapeutics in ER+ breast cancer	PD-332991_07Jan14_110019	M4.2.1.1
3	PK/PD Modeling of Tumor Growth and Biomarker Response to PD 0332991 in a Human Tumor Xenograft Mouse Model	RR 764-04338	
4	Summary of the preclinical pharmacology for PD 0332991	REG 700-00180	M4.2.1.1

Toxicology studies

## Single dose studies\*

	Title	Report no.	Folder/file name
1	Acute oral toxicity study of PD332991-54 in rats	RR745-03742	M4.2.3.1
2	Oral escalating dose toxicity study of PD332991-2B in dogs	RR745-03662	M4.2.3.1

\*The studies reviewed under IND 69324

## Repeat dose

	Title	Study no.	Folder/file name
1*	2-Week Oral Dose Range-Finding Study of PD 332991-54 in Rats	745-03781	M4.2.3.2
2*	3-Week Oral Toxicity Study of PD 332991-54 in Rats	(b) (4) 3107 (74503731)	M4.2.3.2
3*	2-week oral dose range-finding study of PD 332991-54 in dogs	745-03765	M4.2.3.2
4*	3-Week Oral Toxicity Study of PD 332991-54 in Dogs	745-03871	M4.2.3.2
5	39-week oral gavage chronic toxicity and toxicokinetic study with PD-0332991 in dogs with a 12-week recovery phase	8282225	M4.2.3.2

\*The studies reviewed under IND 69324

## Local tolerance study

	Title	Study no.	Folder/file name
1	Local vascular tissue irritation study of PD-0332991 in new zealand white rabbits	12LJ094	M4.2.3.6

## Phototoxicity study

	Title	Study no.	Folder/file name
1	Determination of the phototoxic potential of PD-0332991 in the 3T3 Neutral Red uptake (NRU) assay	08SAN-07_3	M4.2.3.7

## Other

	Title	Study no.	Folder/file name
1	Exploratory Kinetochore In Vitro Micronucleus Analysis in CHO-WBL Cells	PD-0332991-0054	M4.2.3.3

**Studies submitted, but not reviewed**

## ADME

	Title	Study no.	Folder/file name
Analytical methods and validation reports			
1	Partial Validation of a Method for the Determination of PD-0332991 in Rabbit Plasma (K2-EDTA) by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)	1400238	M4.2.2.1
2	Partial Validation of a Method for the Determination of PD-0332991 in Rat Plasma (K2-EDTA) by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)	1400243	M4.2.2.1
3	Validation of a Method for the Determination of PD-00332991 and Metabolite PF-05089326 in Rat Plasma (K2-EDTA) by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)	143180	M4.2.2.1
4	Partial Validation of a Method for the Determination of PD-00332991 and Metabolite PF-05089326 in Dog Plasma (K2-EDTA) by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)	143181	M4.2.2.1
5	Method Validation Report: LC/MS/MS Analysis of PD 0332991 in EDTA Monkey, Mouse, Rabbit, and Rat Plasma	764-04143	M4.2.2.1
6	Method Validation Report: LC/MS/MS Analysis of PD 0332991 in EDTA Dog Plasma	764-04144	M4.2.2.1

	Calibration Curve Range of 10.0 to 10,000 ng/mL and 1.00 to 900 ng/mL		
Absorption			
1	Permeability and transport evaluation of PD 0332991	142925	M4.2.2.2
2	PF-00080665 (PD-0332991) in vitro assessment of uptake and biliary excretion assay in sandwich culture human hepatocytes (SCHH)	111932	M4.2.2.2
Metabolism			
1	Identification of excretory and circulating metabolites in six healthy human volunteers following a single 125 mg [ <sup>14</sup> C]PD-0332991 oral dose	113146	M4.2.2.4
2	In vitro recombinant sulfotransferase (sult) reaction phenotyping of PD-0332991 (PF-00080665) sulfonation and enzyme kinetic parameters in human liver S9, cytosol and sult2A1	170350	M4.2.2.4
Excretion			
1	Radioactivity analysis of urine, and fecal samples from healthy human male subjects following a single oral dose of [ <sup>14</sup> C]-PD-0332991 in support of clinical protocol number A5481011	A5481011	M4.2.2.5
Drug interactions			
1	Effect of PF-05089326, a metabolite of PD-332991, on human drug metabolizing enzymes in vitro	130322	M4.2.2.6
2	The in vitro study of MDR1 (ABCB1) inhibition by PF00080665 (PD-0332991) in MDCKII-MDR1 cells	163603	M4.2.2.6
3	In vitro investigation of the potential for PF-00080665 to induce cytochrome P450 (CYP1A2, CYP2B6, CYP2C8 AND CYP3A4) in cultured cryopreserved human hepatocytes	(b) (4) 123065	M4.2.2.6
4	In vitro studies of selected test articles with the human BSEP (ABCB11/sPgp) transporter in the vesicular transport inhibition assay	12_03731	M4.2.2.6
5	In vitro interaction studies of selected test articles with human renal uptake transporters	(b) (4) 128429	M4.2.2.6
6	<i>In vitro</i> interaction studies of PF-00080665 (PD-0332991) with the human OATP1B1 and OATP1B3 uptake transporters	(b) (4) 128430	M4.2.2.6
7	The in vitro study of BCRP (ABCG2) inhibition by PF-00080665-73-0006 (PD-0332991) in MDCKII-BCRP cells	(b) (4) 128431	M4.2.2.6

## Genotoxicity studies

	Title	Study no.	Folder/file name
1	Genetic toxicology–bacterial mutagenicity assay of (b) (4) a drug product degradant of parent compound PD-332991	eAMES2014-8	M4.2.3.7

## Reproductive and development toxicity studies

	Title	Study no.	Folder/file name
1	A Dose Range-Finding Embryo-Fetal Development Study of PD-0332991 by Oral (Gavage) in Rats	20036581	M4.2.3.5
2	A Dose Range-finding Embryo-fetal Development Study of PD-0332991 by Oral (Gavage) in Rabbits	20036582	M4.2.3.5

## Other

	Title	Study no.	Folder/file name
1	PD-0332991: study report for the in vitro compatibility with rabbit blood	12LJ093	M4.2.3.7
2	PD-0332991: study report for the in vitro compatibility with human blood	12LJ095	M4.2.3.7
3	Exploratory fish (fluorescent in situ hybridization) analysis in TK6 cells	2013ETK6-5 FISH	

## 4 Pharmacology

### 4.1 Primary Pharmacology

#### Primary pharmacodynamics

Title: Summary of the Preclinical Pharmacology for PD 0332991

Research report no.: RR-REG 700-00180

Test facility: Pfizer global research & development

Ann arbor laboratories

Ann arbor, Michigan

Report date: February 20, 2004

#### Methods and Results

The following tables and figures were copied from the Applicant's submission.

#### Inhibition of Cdk4/Cdk6 and enzyme selectivity

Table 1. Inhibition of Cyclin-Dependent Kinases by PD 0332991

<b>Table 1. Inhibition of Cyclin-Dependent Kinases by PD 0332991</b>		
Cdk I	$C_{50}^a$	$K_i$ ( $\mu$ M) <sup>b</sup>
Cdk4/cyclinD <sub>1</sub> 0.011		0.002
Cdk4/cyclinD <sub>3</sub> 0.009		0.001
Cdk6/cyclinD <sub>2</sub> 0.015		ND
Cdk2/cyclinA >5		ND
Cdk1/cyclinB >5		ND
Cdk5/p25 >5		ND

<sup>a</sup> Concentration of PD 0332991 necessary to inhibit activity by 50%.

<sup>b</sup> Kinetic inhibition constant calculated by tight binding inhibition analysis. ND = not determined.

**Summary:** PD 0332991 is an inhibitor of Cdk4/cyclinD1 kinase activity with an  $IC_{50}$  of 11 nM and  $K_i$  of 2 nM. PD 0332991 is an inhibitor of Cdk6/cyclinD2 kinase activity with an  $IC_{50}$  of 15 nM. PD 0332991 did not show activity against Cdk1, 2 or 5, or other protein kinases tested (data submitted, but not captured in this review).

APPEARS  
THIS WAY  
ON  
ORIGINALInhibition of Retinoblastoma Phosphorylation in Tumor Cells

Table 2. Effect of PD 0332991 on the Phosphorylation of Retinoblastoma at Cdk4-Specific Amino Acid Sites in the MDA-MB-435 Human Breast Carcinoma Cell Line

Retinoblastoma phosphorylation site	IC <sub>50</sub> (μM) <sup>b</sup>
Serine 780	0.066
Serine 795	0.063

<sup>a</sup> Cells were exposed to varying concentrations of PD 0332991 for 24 hours. The phosphorylation status of retinoblastoma was determined by western blot.

<sup>b</sup> Concentration of PD 0332991 necessary to reduce phosphorylation by 50%.

**Summary:** PD 0332991 treatment resulted in reduction of Rb phosphorylation at serine-780 and 795 in the MDA-MB-435 Human Breast Carcinoma cells.

Antiproliferative effects of PD 0332991 in vitroTable 4. Inhibition of Thymidine Incorporation into DNA in human tumor and normal cell lines treated with PD 0332991<sup>a</sup>

Cell Line	Cell Type	IC <sub>50</sub> (μM) <sup>b</sup>
MDA-MB-435 B	reast Carcinoma	0.16
ZR-75-1 B	reast Carcinoma	0.17
T-47D B	reast Carcinoma	0.04
MCF-7 B	reast Carcinoma	0.10
H1299 L	ung Carcinoma	0.12
Colo-205 Colon	Carcinoma	0.13
CRRF-CEM	Acute Lymphoblastic Leukemia	0.25
K562 Chronic	Myelogenous Leukemia	0.40
MCF-10A	Normal Breast Epithelial Cell	0.09
HS-27 Normal	Human Fibroblast	0.19

<sup>a</sup> Cells were exposed to varying concentrations of PD 0332991 for 24 hours.

<sup>b</sup> Concentration of PD 0332991 necessary to inhibit cell proliferation by 50%.

Table 5. Inhibition of cell proliferation in human tumor cell lines treated with PD 0332991

Tumor T	ype	IC <sub>50</sub> (μM) <sup>a</sup>
MDA-MB-435 B	reast Carcinoma	0.032
Colo-205 Colon	Carcinoma	0.089

<sup>a</sup> Concentration of PD 0332991 necessary to inhibit cell proliferation by 50%.

**Summary:** PD 0332991 inhibited thymidine incorporation into the DNA of a panel of Rb-positive human breast, colon, and lung carcinoma cells, with IC<sub>50</sub> values ranging from 0.040 to 0.17 μM. PD 0332991 inhibited cell proliferation of breast carcinoma cells and colon carcinoma cells with IC<sub>50</sub> values of 0.032 to 0.89 μM, respectively.

A role in growth arrest

Table 5. Effect of PD 0332991 on cell cycle phase distribution of the MDA-MB-453 human breast carcinoma cells<sup>a</sup>

PD 0332991 ( $\mu$ M) <sup>b</sup>	% Cells in each Phase of the Cell Cycle	
	G <sub>1</sub> S	G <sub>2</sub> /M
0.59	30	11
0.0254	33	12
0.0474	22	4
0.0889	7	3
0.1181	16	5
0.3386	12	3
0.6286	11	3
1.087	11	2
1.2584	14	2
2.587	8	5
3.089	9	2
5.081	17	2
10.081	16	4

<sup>a</sup> DNA content as assayed by flow cytometry, was used to determine the relative distributions of cells in each phase of the cell cycle.

<sup>b</sup> Cells were treated for 24 hours with the indicated concentrations of PD 0332991.

*Summary:* MDA-MB-453 breast carcinoma cells that were exposed to varying concentrations of PD 0332991 for 24 hours showed a significant increase in the percentage of cells in G<sub>1</sub> in the presence of as little as 0.04  $\mu$ M PD 0332991 with the maximum accumulation of cells in G<sub>1</sub> at 0.08  $\mu$ M.

Comparison of EC<sub>50</sub> and IC<sub>50</sub> on pharmacologic effects

Table 6 Correlation between concentrations of PD 0332991 that modulate target and produce anticipated biological effects

Biological Effect	EC <sub>50</sub> ( $\mu$ M) <sup>a</sup>
Inhibition of Cellular Proliferation	0.032
Inhibition of Rb Phosphorylation (serine-780)	0.066
Significant G <sub>1</sub> Arrest	0.040

<sup>a</sup> Concentration of PD 0332991 necessary to elicit 50% of the response.

*Summary:* Similar concentrations of PD 0332991 were required to inhibit cell proliferation and phosphorylation of Rb, and to cause G<sub>1</sub> arrest in MDA MB-453 cells.

Table 7. Inhibition of Thymidine Incorporation into DNA by PD 0332991 in Rb-Positive or Rb-Negative Tumors

Tumor T	ype	Rb Status	IC <sub>50</sub> (μM) <sup>a</sup>
MDA-MB-435 B	reast Carcinoma	Positive	0.16
MDA-MB-468 B	reast Carcinoma	Negative	>3
H1299 L	ung Carcinoma	Positive	0.12
H2009 L	ung Carcinoma	Negative	>3

<sup>a</sup> Concentration of PD 0332991 necessary to inhibit by 50%.

**Summary:** PD 0332991 had no antiproliferative activity on Rb-negative tumor cells when assayed at concentrations up to 3 μM.

#### In vivo activity

PD 0332991 was evaluated for activity against a panel of Rb-positive tumor models and several Rb-negative tumor models. The phosphorylation status of serine-780 on Rb in tumor tissue was monitored over time to study the correlation between activity and reduction of tumor retinoblastoma phosphorylation in vivo.

**Summary:** The submitted data suggested that PD 0332991 had antitumor activity against multiple tested human tumor xenograft models in SCID mice with Rb-Positive Tumors. However, MDA-MB-468 breast carcinoma and DU-145 prostate tumor models, both Rb-negative, did not respond to PD 0332991. The observed antitumor activity in Rb-positive tumor models was correlated with the percentage reduction of tumor retinoblastoma phosphorylation in vivo.

#### **Conclusion**

PD 0332991 is an inhibitor of Cdk4 and Cdk6 kinase activity. The compound exhibited IC<sub>50</sub> values for inhibition of these enzymes in the 10 nM range. PD 0332991 is an antiproliferative agent against Rb-positive tumor cells in vitro, preventing cells from entering the S phase of the cell cycle and causing a G1 arrest. Cells exposed to this compound exhibited reduction in Rb-phosphorylation at the Cdk4 phosphorylation sites, serine-780, and serine-795. The in vivo pharmacology indicated that PD 0332991 had anti-tumor effects on multiple Rb-positive tumors with tumor regression or tumor stasis. The phosphorylation status of serine-780 on Rb was detected in tumor tissue and correlated with antitumor response.

#### Drug activity related to proposed indication

**Title:** Summary of palbociclib (PD-0332991) combination with anti-estrogen therapeutics in ER+ breast cancer

Test facility: not provided

Affiliation of principal scientist (author): Oncology Research Unit, WRD, La Jolla, USA

Final approval date: May 27, 2014

Method: The mode of action (MOA) of the combination of PD-0332991 with ER antagonists in enhancing activity of treating ER+ breast cancer cells was studied *in vitro* and *in vivo* by the following methods:

1. Evaluation of the effects on a signaling pathway at different downstream points;
2. Evaluation the effects on DNA replication, cell proliferation, and senescence;
3. Evaluation of *in vivo* anti-cancer activity.

Note: the brief experimental methods are presented in the results section with the experimental data.

Results: The following table and figures were copied from the Applicant's submission.

Inhibitory effects on proliferation and DNA synthesis by PD-0332991 in combination with anti-ER therapeutic agents and the molecular mechanism underlying the combination potentiation

T47D and MCF7 lines were treated with PD-0332991, fulvestrant (ER downregulator) or tamoxifen (ER antagonist), or with the combination of PD-0332991/fulvestran or PD-0332991/tamoxifen. MCF7-CYP19 cells (an MCF7 cell line that was engineered to stably overexpress the aromatase enzyme via transfection with the CYP19 cDNA to isolate effects of letrozole on aromatase) were treated with letrozole (aromatase inhibitor). After 7 days of cell growth, cell viability was assessed using Cell Titer-Blue viability reagent and fluorescence measurement.

Figure 1. Inhibition of Proliferation by PD-0332991 and Fulvestrant

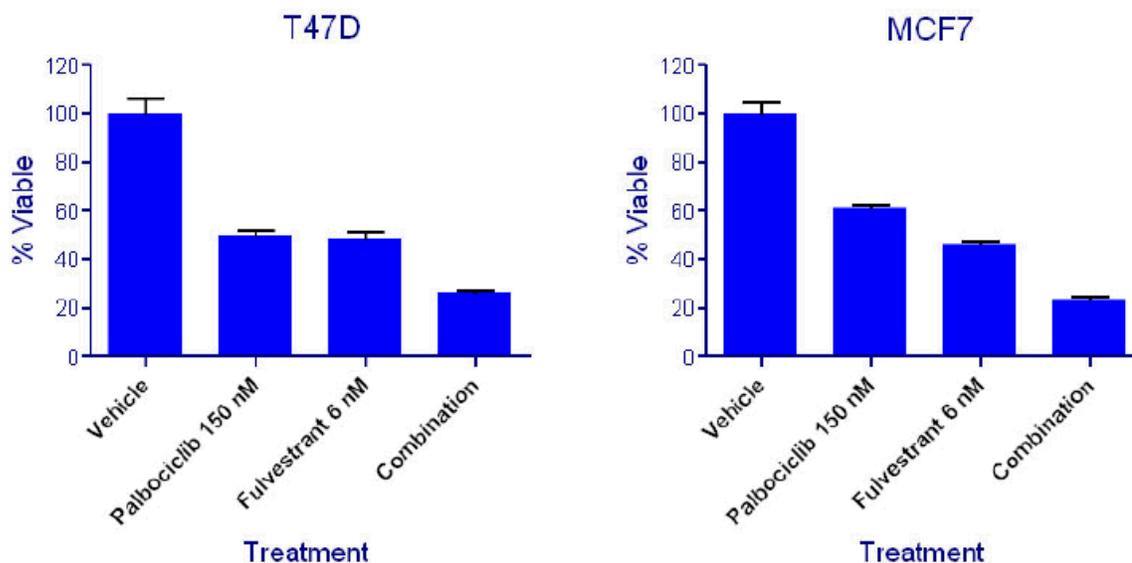


Figure 2. Inhibition of Proliferation by PD-0332991 and Tamoxifen

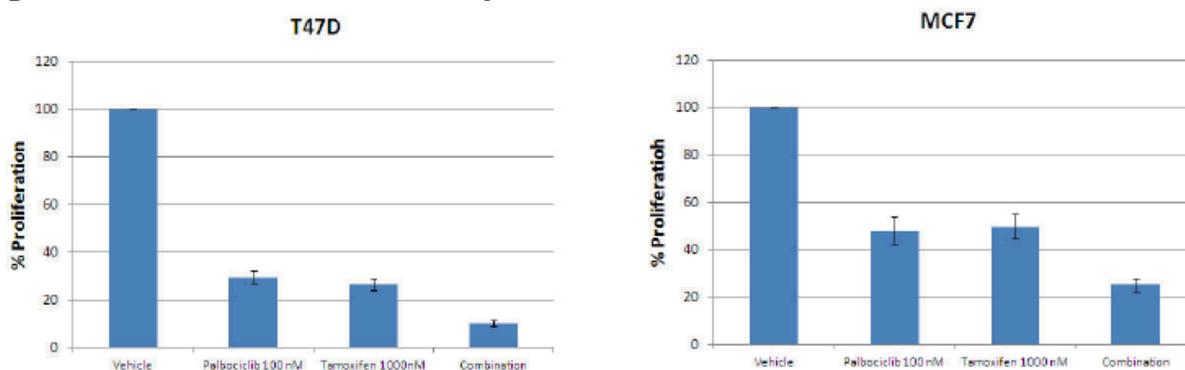
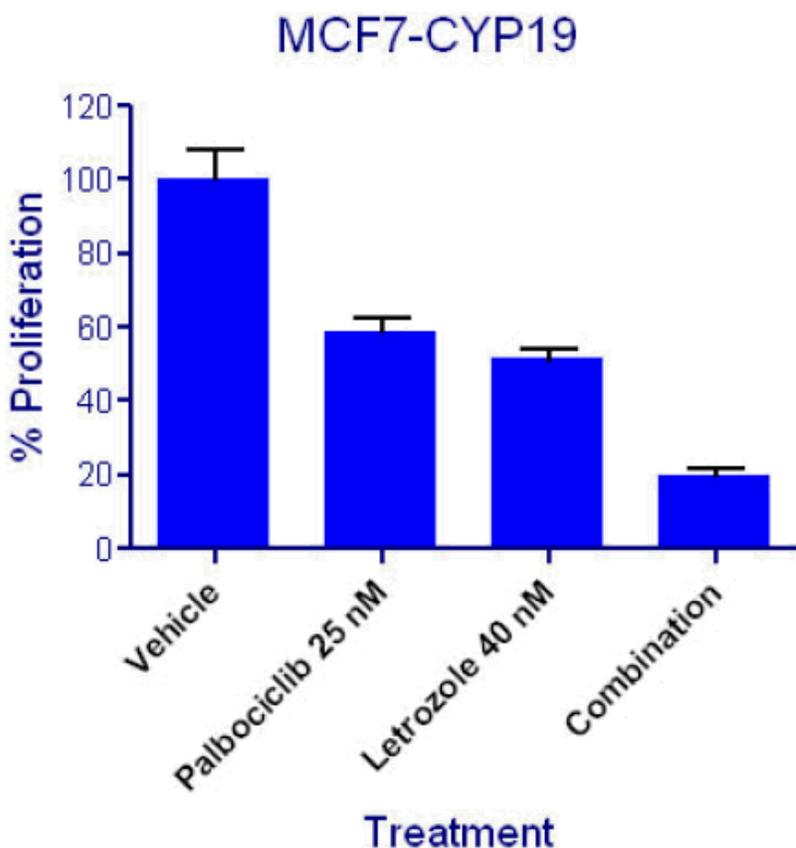


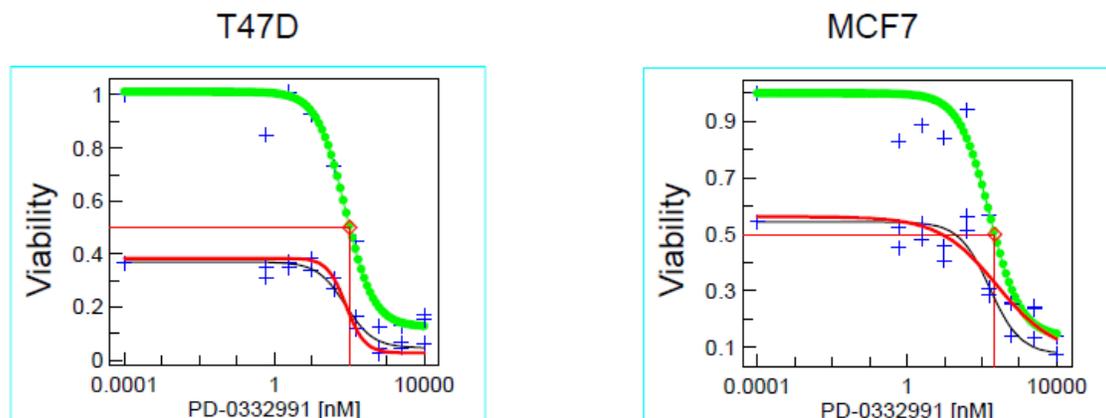
Figure 3. Inhibition of Proliferation by PD-0332991 and Letrozole



*Summary:* When compared to PD-0332991 alone, the combination of PD-0332991 with fulvestrant or tamoxifen resulted in an additive inhibition on cell viability in T47D cells (an additional inhibition of 23.3% and 19.4% respectively), and in MCF7 cells (an additional inhibition of 37.8%, 22.9% respectively). The combination of PD-0332991 and letrozole (40 nM) provided an additional inhibition of cell proliferation of 39% in MCF7-CYP19 cells.

Cell proliferation assays were conducted and analyzed with the BLISS additivity algorithm, which calculates the theoretical activity if the combination of two drugs were additive.

Figure 4. BLISS Additivity Curves of T47D and MCF7 Cells Treated with PD-0332991 and Fulvestrant



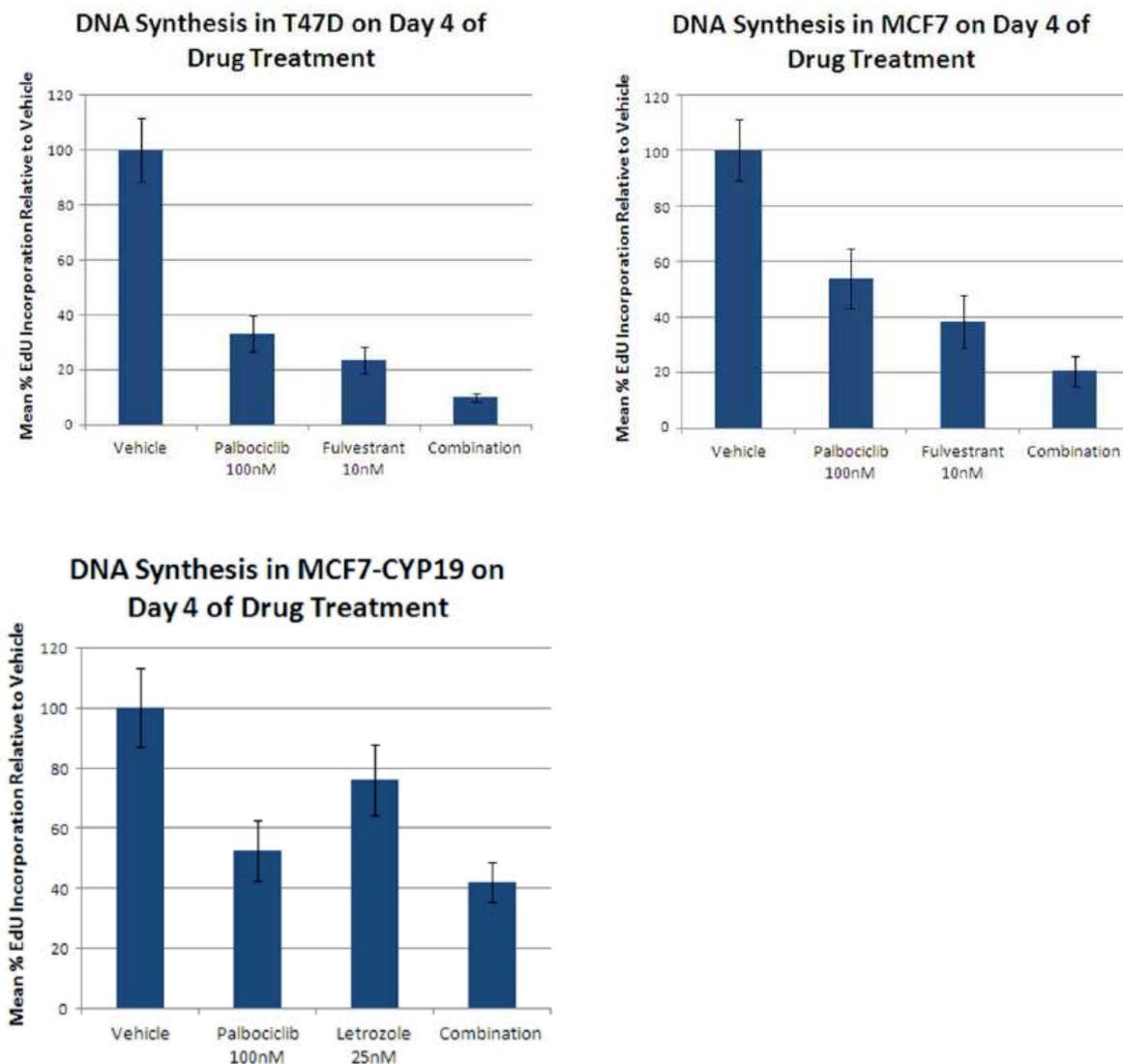
(copied from Applicant's submission)

Single agent PD-0332991 (full titration viability curve: green curve with solid circles); predicted BLISS additivity curve of titrated PD-0332991 in combination with fulvestrant held constant at 8 nM (black dashed curve); actual observed viability of titrated PD-0332991 in combination with fulvestrant held constant at 8 nM (thick red solid curve). Overlap of the BLISS curve and observed curve signifies additivity. Wide dashed lines indicate  $IC_{50}$  values for single agent PD-0332991: T47D: 103 nM; MCF7: 219 nM.

**Summary:** The study results suggested that in both T47D and MCF7 cell lines, the combination of PD-0332991 and fulvestrant provided the additive benefit compared to either single agent activity.

DNA synthesis was measured by Click-iT® EdU (5-ethynyl-2'-deoxyuridine) incorporation assays.

Figure 5. Effect of PD-0332991 and anti-estrogen agent combinations on DNA synthesis

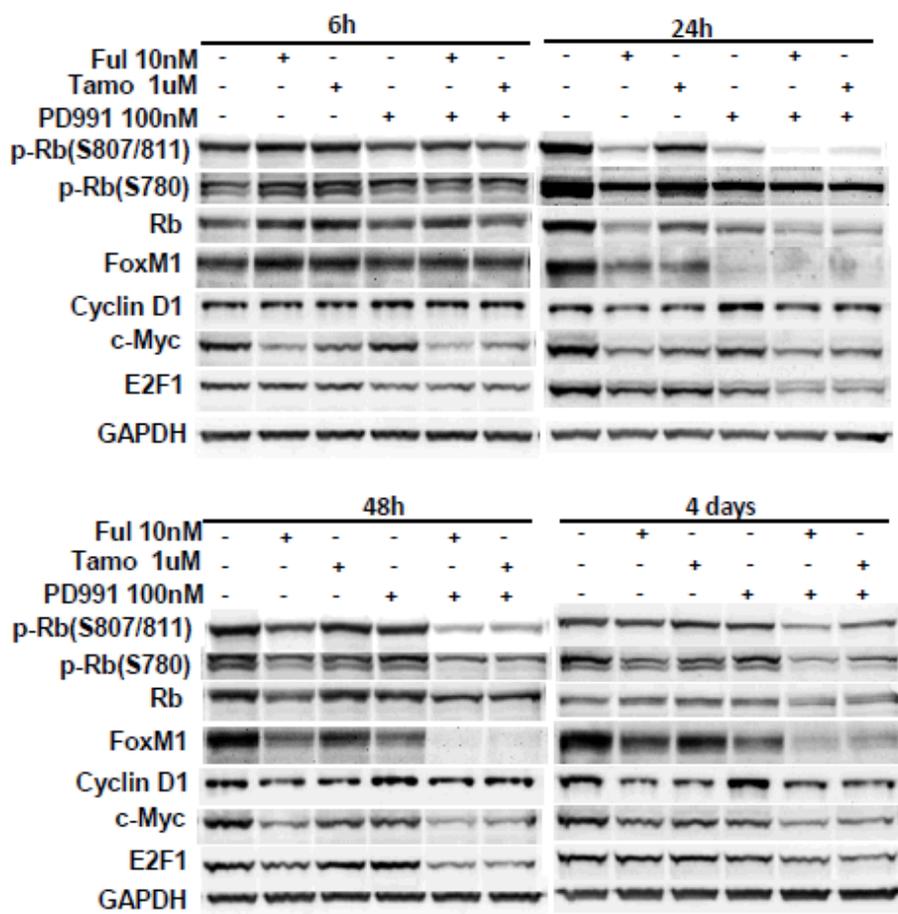


(copied from Applicant's submission)

**Summary:** Treatment of T47D cells with PD-0332991 for 4 days inhibited DNA synthesis by 66.9% and addition of fulvestrant yielded an additional 23% inhibition. A 4 day treatment with PD-0332991 inhibited DNA synthesis by 46% in MCF7 cells, and addition of fulvestrant enhanced this inhibition by 33.4%. A 4 day treatment with PD-0332991 inhibited DNA synthesis by 47.5% in MCF7-CYP19 cells and addition of letrozole enhanced this inhibition by an additional 10.5%.

To better understand the mechanism of action underlying the potentiation effects of ER signaling modulators, effectors and targets of both CDK4/6 and ER signaling were evaluated. A series of time-course experiments were conducted with T47D and MCF7-CYP19 cells treated with PD-0332991 in combination with anti-estrogen therapeutics at 6h, 24h, 48h, and 4 days.

Figure 6. Inhibition of cell cycle and ER signaling as measured by phosphoprotein levels by PD-0332991 and anti-estrogen therapeutics (tamoxifen or fulvestrant) in T47D cells

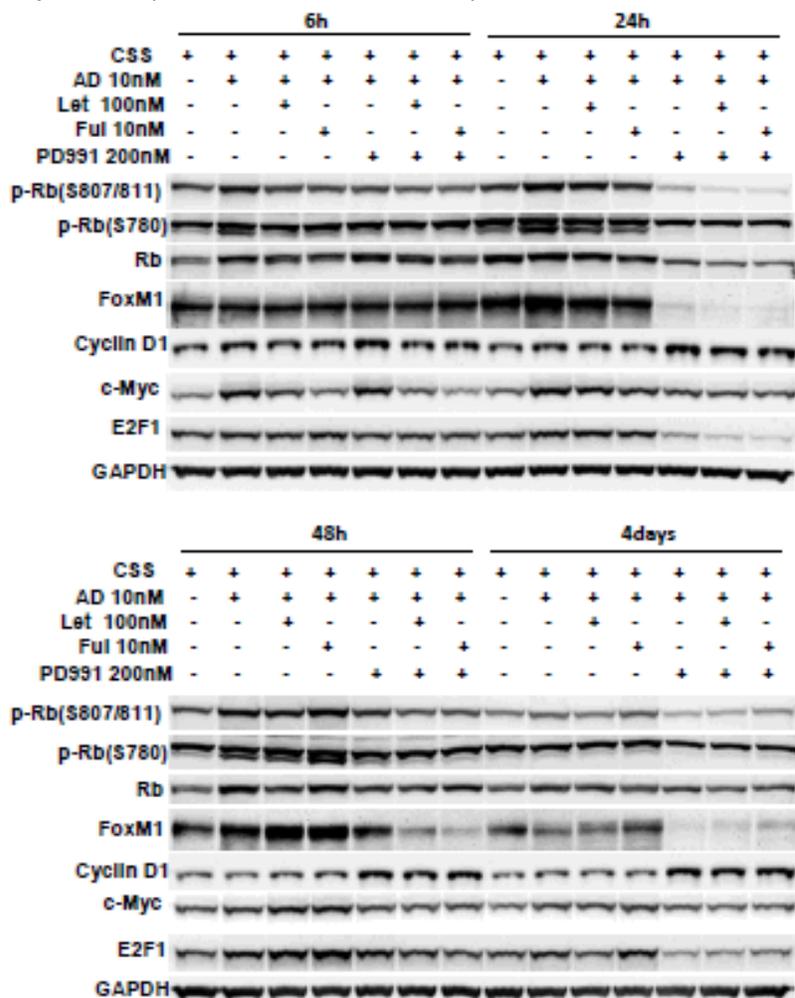


(copied from Applicant's submission)

**Summary:** Maximal changes in PD readouts were observed at the 24h timepoint in T47D cells exposed to drugs. Phosphorylation of Rb (S807/811) was inhibited by tamoxifen (45%), fulvestrant (80%) and PD-0332991 (81 %) as single agents. However, combination treatment with PD-0332991 and either anti-estrogen agents yielded near complete inhibition (>95% by either combination) and sustained inhibition for up to 4 days with a minimal restoration of signaling. A consequence of Rb hypophosphorylation induced by either PD-0332991 or anti-estrogen agents is the inactivation of the E2F transcription factors (E2F1, 2, and 3). Combination treatments of PD-0332991 and

either fulvestrant or tamoxifen provided an additional 40% and 29% decrease in E2F1 expression levels, respectively, compared with the most active of any single agent.

Figure 7. Inhibition of cell cycle and ER signaling by PD-0332991 and anti-estrogen therapeutics (letrozole or fulvestrant) in MCF7-CYP19 cells



(copied from Applicant's submission)

**Summary:** In MCF7-CYP19 cells, the most robust modulation of signaling occurred after 24 hours of drug treatment. At this timepoint, phosphorylation of Rb (S807/811) was inhibited 14% by letrozole, 32% by fulvestrant, and 80% by PD-0332991 single agent treatment. Combination treatment with PD-0332991 and either anti-estrogen agent yielded approximately 13% more inhibition of phosphorylated Rb S807/S811. This translated to similar levels of E2F1 repression. As in T47D cells, PD-0332991 had the greatest impact on FoxM1 downregulation. Combination with either anti-estrogen agent provided minimal additional repression.

Assessment of c-Myc levels in MCF7-CYP19 revealed that only anti-estrogen agents reduced c-Myc levels as single agents, with the greatest level of inhibition occurring at 6

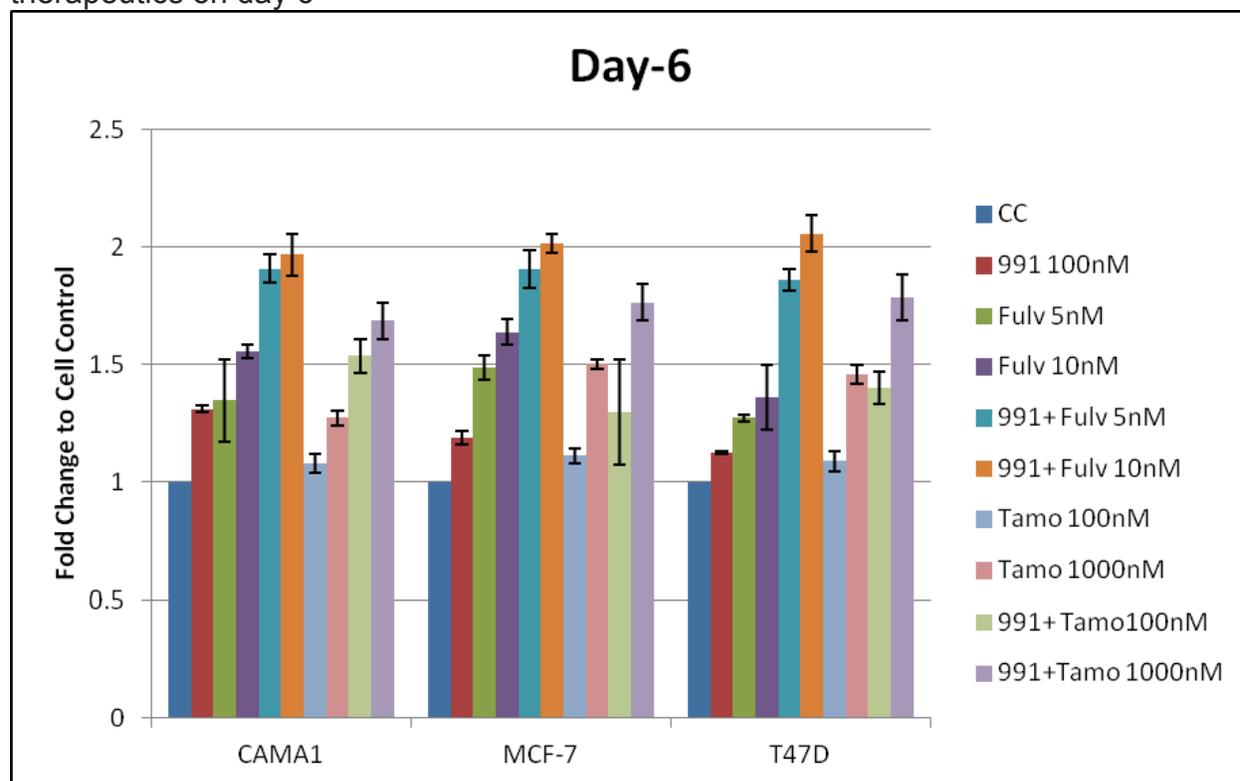
hours. C-Myc was restored to normal levels by 48 hours and the rebound in expression levels was prevented by combination treatment with PD-0332991.

(note for abbreviations: AD: androstenedione; CSS: Charcoal Stripped Serum)

#### Induction of senescence by PD-0332991 and enhancement by anti-estrogen therapeutics

Senescence associated- $\beta$ -galactosidase (SA- $\beta$ -gal) accumulation in lysosomes is one of the hallmarks of senescent cells. ER+ breast cancer lines (CAMA1, MCF7 and T47D) were treated with PD-0332991 (100 nM), fulvestrant (5 or 10 nM), and tamoxifen (100 or 1000 nM) as single agents or in combination for 6 days, and analyzed with a fluorometric SA- $\beta$ -gal assay.

Figure 8. Senescence is enhanced by a combination of PD-0332991 and anti-estrogen therapeutics on day 6



(copied from Applicant's submission)

**Summary:** Single agent treatment with either PD-0332991 or anti-estrogen agents caused a slight elevation in SA- $\beta$ -gal activity normalized to the vehicle control group. However, combination treatment significantly increased senescence.

Combination of PD-0332991 and anti-estrogen therapeutics provided more durable responses

To assess if combinations can provide a more durable response after drug removal, a series of drug wash out experiments were performed. ER+ breast cell lines were treated with PD-0332991 or anti-estrogen agents for up to seven days, after which drugs were washed out and the cells were allowed to grow for another 3 days.

Figure 9. Immunoblot analysis of signal modulation in T47D cells following drug removal

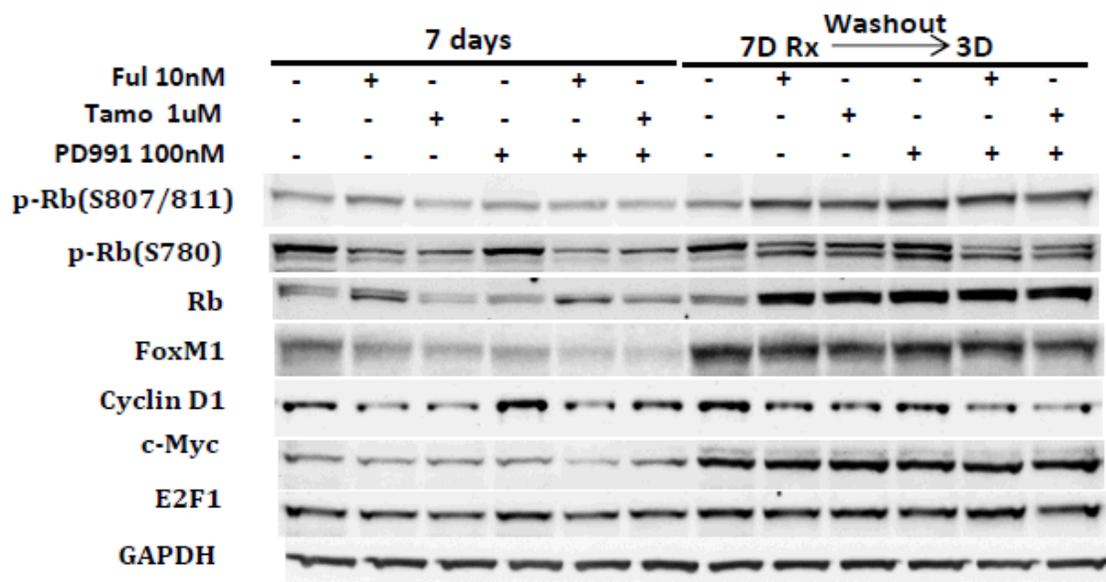


Figure 10. Immunoblot Analysis of Signal Modulation in MCF-CYP19 Following Drug Removal

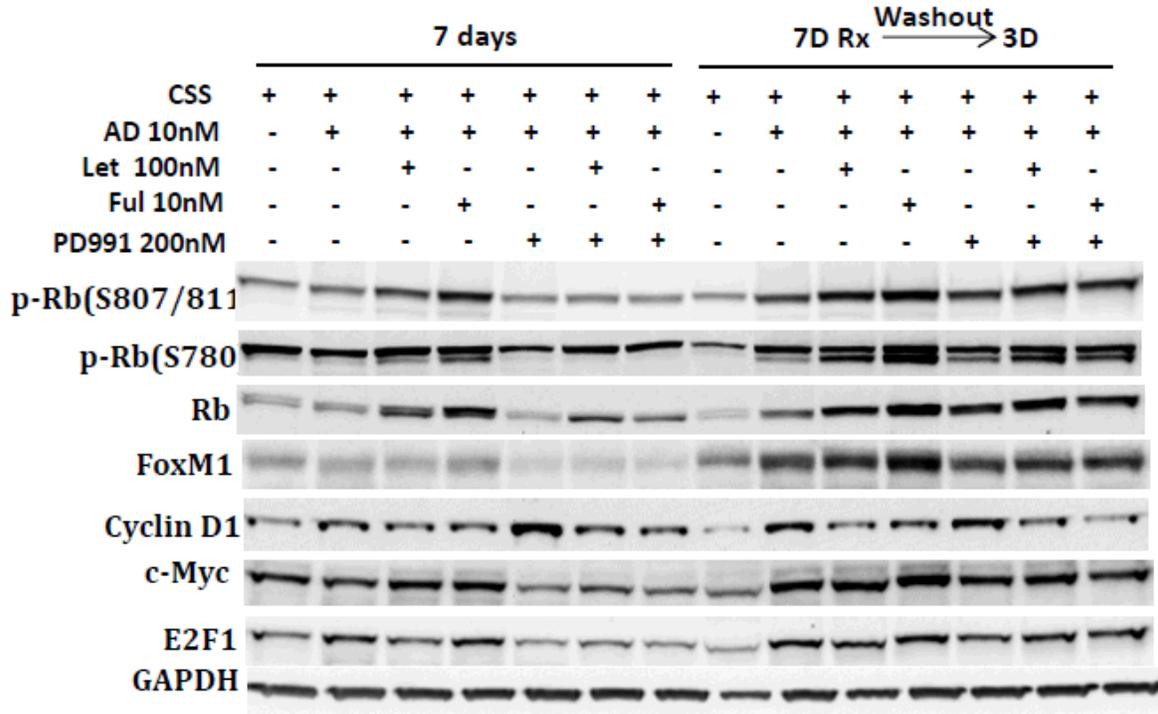


Figure 11. Senescence Effects Persist Following Drug Removal in T47D Cells

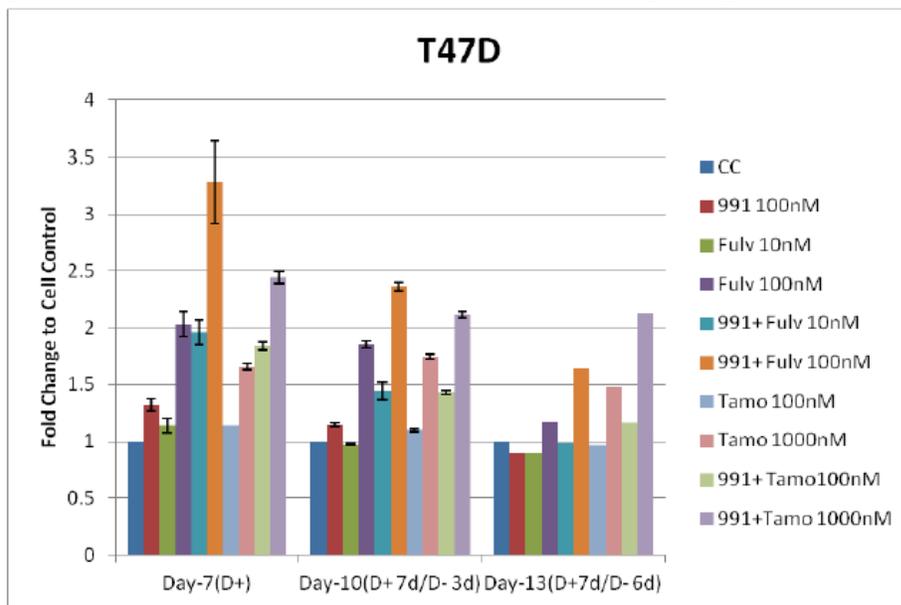
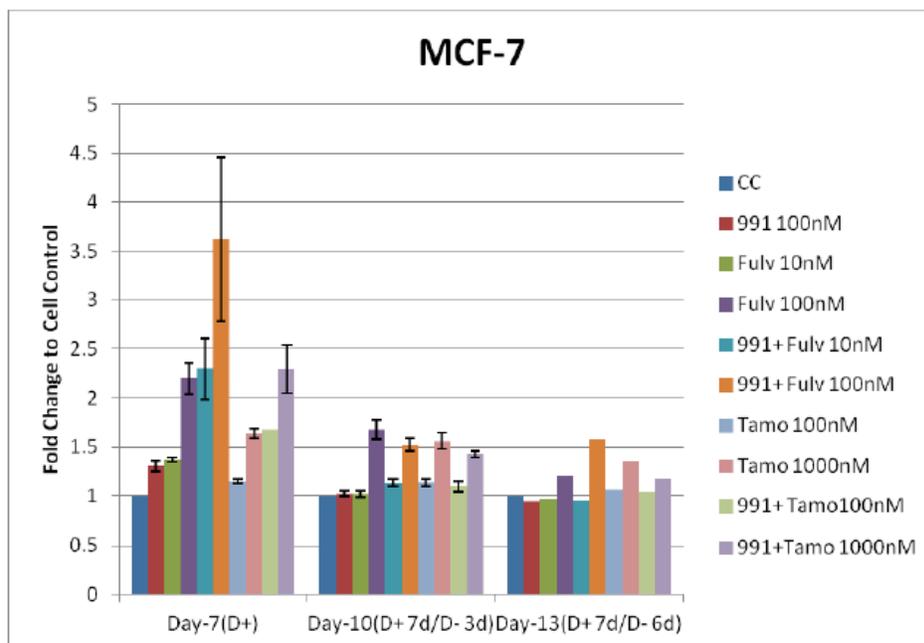


Figure 12. Senescence Response is not Maintained Following Drug Removal in MCF7 Cells

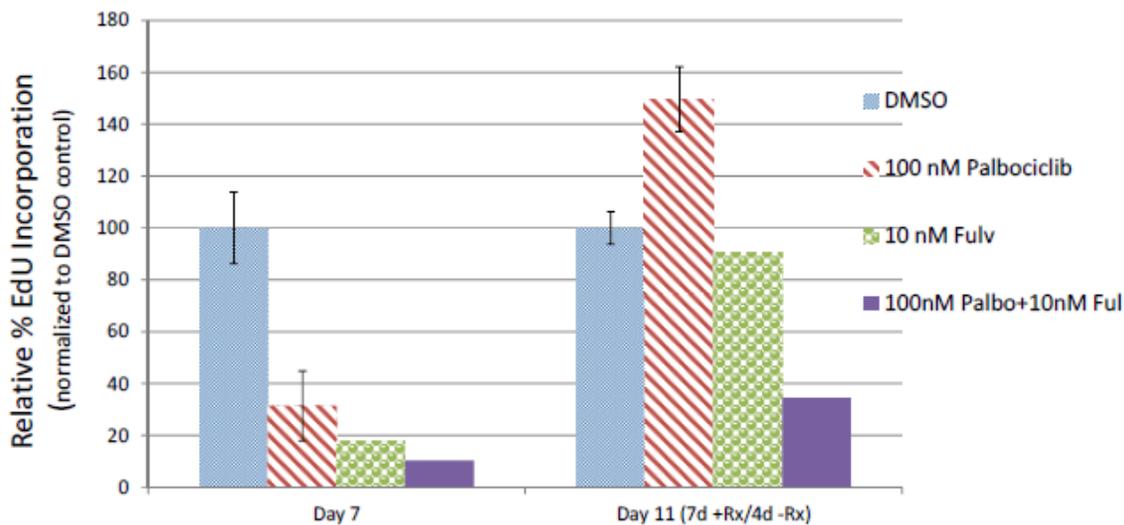


(copied from Applicant's submission)

*Summary:* Phosphorylated Rb (S780) and cyclin D1 displayed a durable response following removal of a combination of PD-0332991 and anti-estrogen agents in T47D cells (figure 9) and in the MCF7-CYP19 cells (figure 10). In the MCF7-CYP19 cells, only cyclin D1 levels remained repressed following removal of the PD 0332991/anti-estrogen agent combination (Figure 10), and there was lack of durable senescence induction in MCF7 cells after drug removal (Figure 12). In contrast, combination treatments provided a more durable level of senescence in T47D cells, which persisted for up to 6 days after drug washout (Figure 11)

EdU incorporation was used as a measure of new DNA synthesis. T47D cells were treated with PD-0332991, fulvestrant or the combination for 7 days and then released from drug exposure for an additional 4 days of culture. Rates of new DNA synthesis were measured after 7 days on drug, and following washout and culture in the absence of drug for 4 days.

Figure 13. Combination Treatment Increases the Durability of DNA Synthesis Inhibition Following Drug Removal in T47D Cells



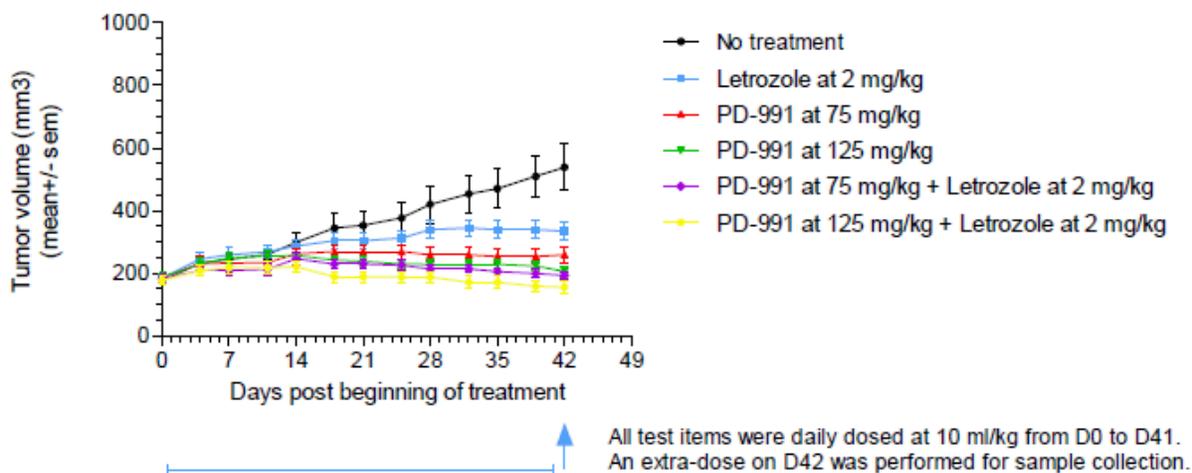
(copied from Applicant's submission)

*Summary:* Treatments produced 68.6%, 82% and 89.6% inhibition of EdU incorporation by PD-0332991 (100 nM), fulvestrant (10 nM) or the combination of the two, respectively after 7 days of drug treatment. After 4 days of culture in the absence of drug, post-washout, T47D cells treated with single agent PD-0332991 or fulvestrant displayed new DNA synthesis rates equivalent to control cultures. In contrast, cultures treated with the combination of agents and released from drug for 4 days retained significant inhibition of new DNA synthesis compared to vehicle treated cultures – DNA synthesis remained inhibited by >65% in T47D cells treated with the combination of PD-0332991 and fulvestrant, as compared to control and single agent treated cultures.

In vivo study: anti-tumor efficacy of PD-0332991 and Letrozole combination in HBCx-34

An estrogen-dependent patient derived xenograft breast cancer model (HBCx-34) was used to: 1) evaluate PD-0332991 and letrozole anti-tumor activity as single agents and in combination; 2) assess pharmacodynamic (PD) modulation of cell cycle and ER signaling by these agents in vivo; 3) assess induction of senescence and inhibition of proliferation.

Figure 14. Anti-tumor Activity of PD-0332991 and Letrozole in Estrogen-dependent Breast Cancer Xenograft Model: HBCx-34



(copied from Applicant's submission)

*Summary:* After 42 days, letrozole (2 mg/kg) demonstrated 38% inhibition over control. PD-0332991 dosed at 75 and 125 mg/kg demonstrated approximately 52% and 62% inhibition over control, respectively. Combination of PD-0332991 at 75 and 125 mg/kg with letrozole provided an additional 12% and 9% inhibition over control, respectively. The combination of letrozole with PD-0332991 was statistically different from either agent alone.

A secondary pharmacodynamic (PD) study was performed in the HBCx-34 model treated for 6 hours (on day 0), or 6, 13 and 20 days of continuous daily dosing, with sample collection at 6 hours after the final dose.

Figure 15 Pharmacodynamic Modulation by PD-0332991 and Letrozole in HBCx-34 on Day 13

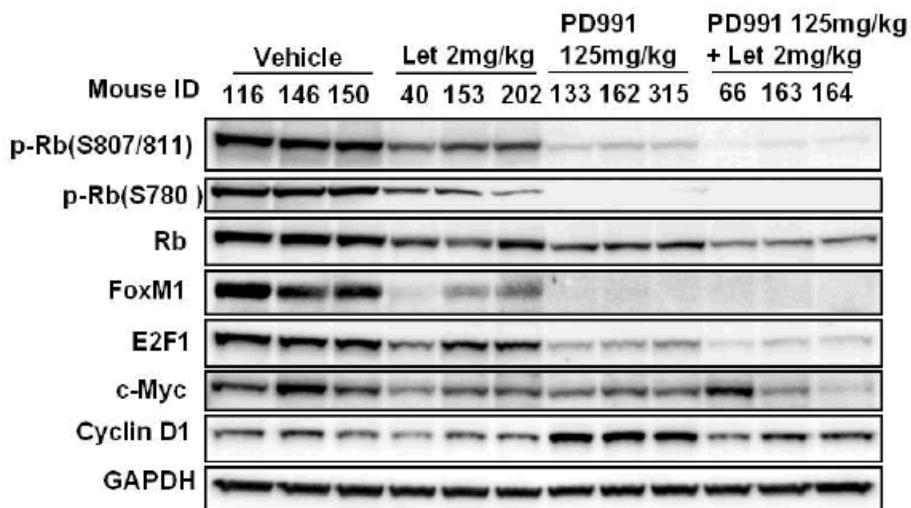
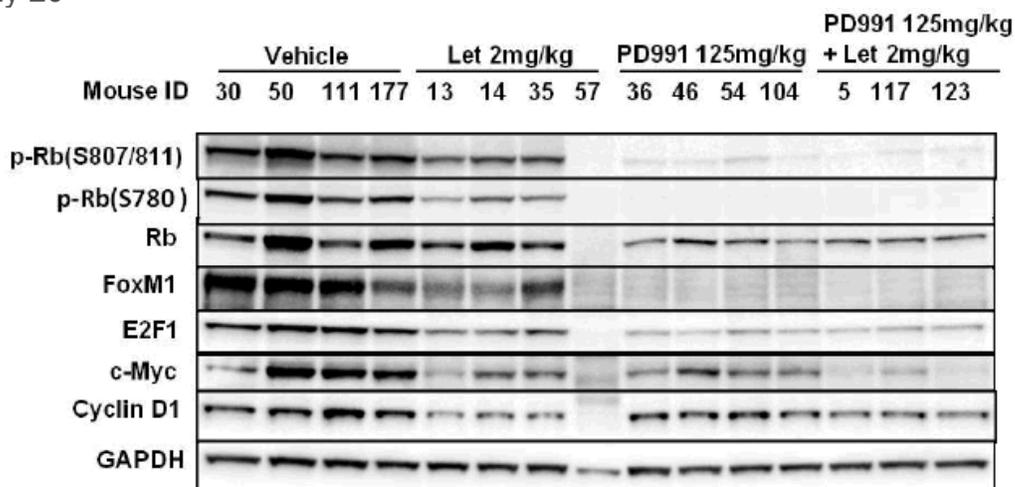


Figure 16. Pharmacodynamic modulation by PD-0332991 and Letrozole in HBCx-34 on Day 20

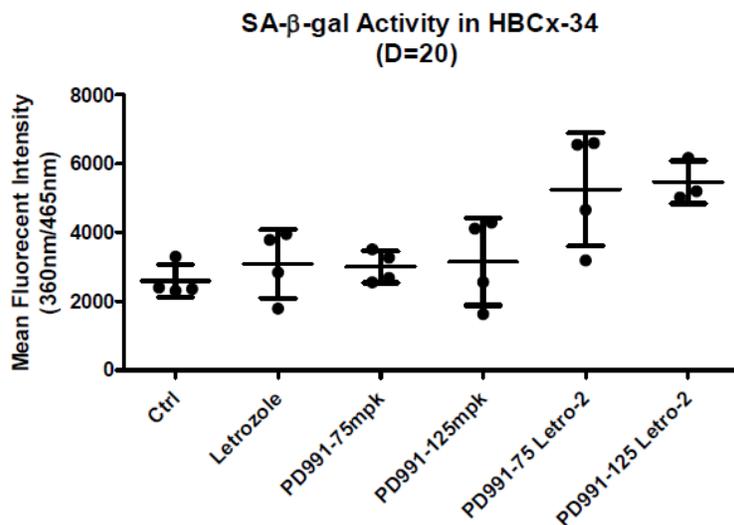


(copied from Applicant's submission)

*Summary:* After 13 days of daily dosing, letrozole (2 mg/kg) inhibited phosphorylation of Rb S807/S811 by 19%; PD-0332991 (125 mg/kg) inhibited S807/S811 by an average of 66%, and the combination inhibited S807/811 by an average of 81%. FoxM1 levels were decreased an average of 38% by letrozole, an average of 56% by PD-0332991 (125 mg/kg), and an average of 65% by the combination on day 13. Similar levels of repression of FoxM1 levels were observed on day 20.

Induction of senescence was evaluated in tumor samples from various treatment arms of the HBCx-34 pharmacodynamic study by measuring 5249 and 5462 mean fluorescent intensity for SA- $\beta$ -gal activity.

Figure 17. Induction of Senescence by PD-0332991 and Letrozole in HBCx-34 on Day 20



(copied from Applicant's submission)

**Summary:** At day 20, average SA- $\beta$ -gal activity was statistically higher in the two combination treatment arms compared with their single agent counterparts.

Tumors harvested from the HBCx-34 study were stained with a Ki67 antibody to assess inhibition of cell proliferation by PD-0332991 and/or letrozole. Inhibition of cell proliferation was assessed at 6 hours (on day 0), or following 6 and 13 days of treatment. Induction of apoptosis was also evaluated in these studies by staining for cleaved caspase 3.

Table 8. Evaluation of anti-proliferative and apoptotic effects of PD 0332991 and Letrozole by IHC

Treatment	Ki-67 <sup>a</sup>			Cleaved Caspase 3 <sup>b</sup>		
	Day 0	Day 6	Day 13	Day 0	Day 6	Day 13
Untreated	4.00	4.00	4.00	1.00	1.00	1.50
Letrozole 2 mg/kg	3.67	3.33	3.33	1.00	1.00	1.00
PD 991 75 mg/kg	4.00	3.00	3.00	1.00	1.33	2.00
PD 991 125 mg/kg	3.67	1.33	1.33	1.33	1.67	2.00
PD 991 75 + Letro 2	3.67	1.33	1.00	1.00	1.67	1.67
PD 991 125 + Letro 2	3.67	1.00	1.00	1.33	2.33	2.00

a. Ki-67 Score: 0=<1 % immunoreactive; 1=1-3% immunoreactivity; 2=4-15% immunoreactivity; 3=16-30% immunoreactivity; 4=>30% immunoreactivity

b. Cleaved Caspase 3 Score: 0=no or rare immunoreactive tumor cells; 1=occasional scattered immunoreactive cells; 2=low moderate scattered immunoreactive cells; 3=high moderate scattered immunoreactive cells; 4=>5% immunoreactivity

Study Report 14LJ006

(copied from Applicant's submission)

**Summary:** Letrozole (2 mg/kg) alone did not significantly inhibit proliferation. A dose dependent inhibition of proliferation was observed following treatment with PD-0332991 at 75 mg/kg and 125 mg/kg on day 6 and 13. Furthermore, the combination of PD-0332991 (75 mg/kg) and letrozole enhanced the anti-proliferative effects of either single agent. Increases in cleaved caspase staining were minimal, which corroborated the lack of apoptosis observed in the in vitro studies.

### Conclusion

Combination of PD-0332991 with anti-estrogen agents such as letrozole, tamoxifen and fulvestrant yielded enhanced activity to induce cell cycle arrest, inhibit cell proliferation, and inhibit tumor growth. Mechanistically, inhibition of cell cycle and ER signaling converged at Rb phosphorylation. Furthermore, PD-0332991 showed greater effects on FoxM1 and anti-estrogen agents showed greater effects on c-Myc repression. Both FoxM1 and cMyc functions have been implicated in cellular senescence. Evaluation of ER+ breast cell lines displayed increased induction of senescence. Lastly, in an ER+ PDX breast cancer model, PD-0332991 and letrozole treatment recapitulated in vitro findings.

## 4.2 Secondary Pharmacology

### In Vitro Pharmacology: Study of PF-00080665-73

**Study no:** 7570744

**Volume #, and page #:** electronic submission, Module 4

M4\421-pharmacology\4212-secondary pharmacodynamics\Study 7570744-7,

Page 1-49

**Conducting laboratory and location:**



**Date of study initiation:** May 10, 2007

**GLP compliance:** no

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** PF-00080665-73

Batch No.: PF-00080665-73-0002

Purity: not provided

**Methods:** PF-00080665-73, the isethionate salt form of palbociclib, was evaluated against a panel of receptors, ion channels, transporters, and enzymes at a concentration of 10  $\mu$ M.

*In Vitro* Pharmacology binding assays and enzyme and uptake assays were performed and the data collection were analyzed using the following methods (copied from the Applicant's submission)

In Vitro Pharmacology: Binding Assays

The results are expressed as a percent of control specific binding

$$\frac{\text{measured specific binding}}{\text{control specific binding}} * 100$$

and as a percent inhibition of control specific binding

$$100 - \left( \frac{\text{measured specific binding}}{\text{control specific binding}} * 100 \right)$$

obtained in the presence of PF-00080665-73.

*In Vitro* Pharmacology: Enzyme and Uptake Assays

The results are expressed as a percent of control specific activity

$$\frac{\text{measured specific activity}}{\text{control specific activity}} * 100$$

and as a percent inhibition of control specific activity

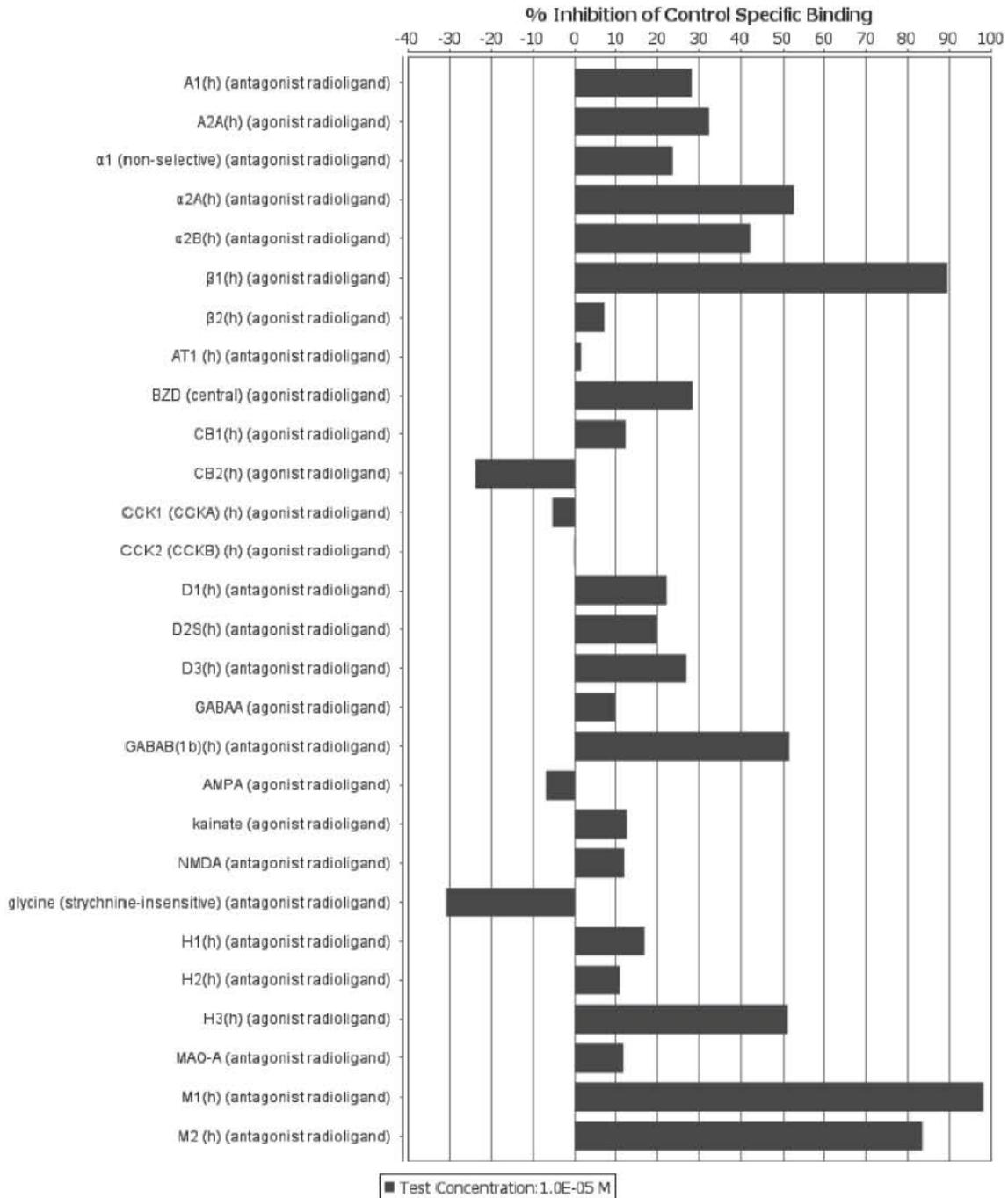
$$100 - \left( \frac{\text{measured specific activity}}{\text{control specific activity}} * 100 \right)$$

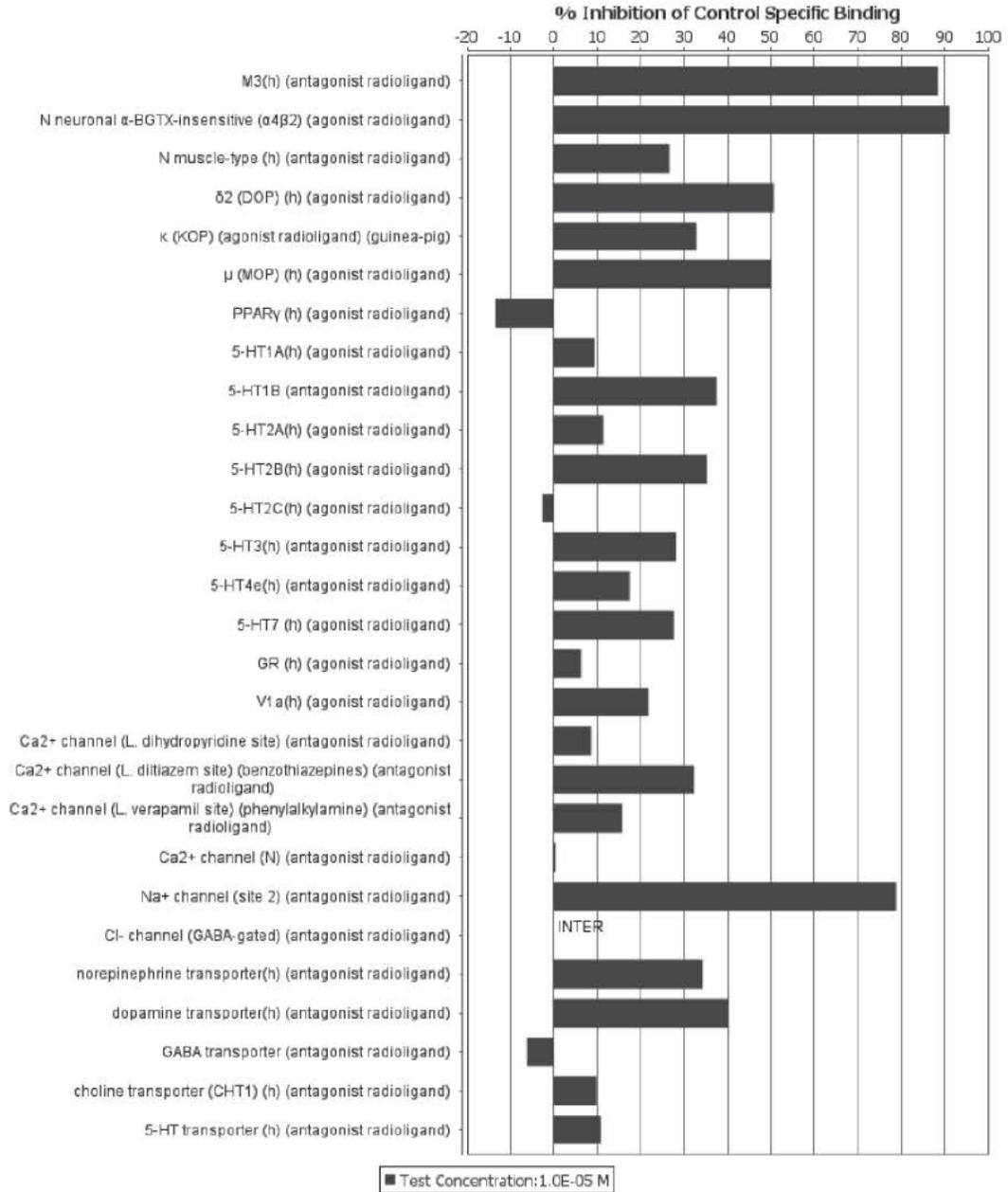
obtained in the presence of PF-00080665-73.

**Results:** The following figures are copied from the Applicant's submission.

**In Vitro Pharmacology: Binding Assays**

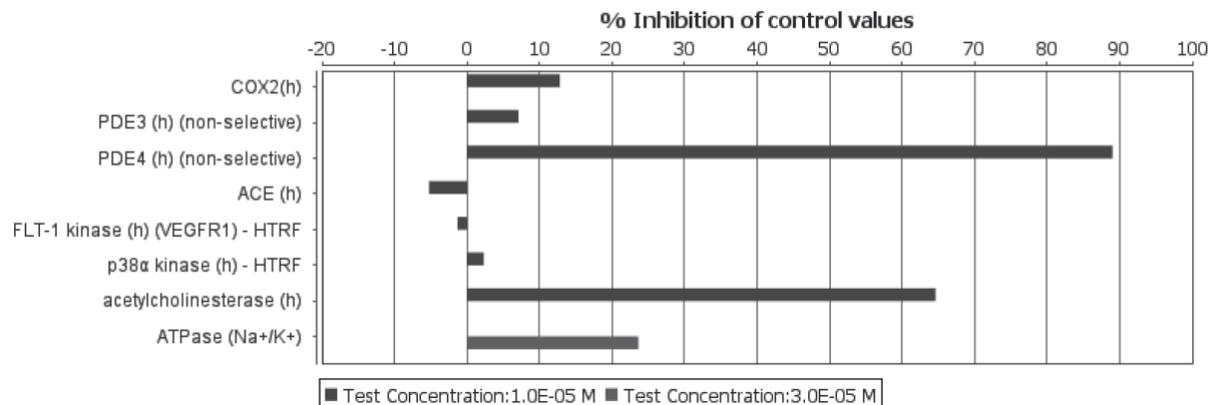
Figure 18. % inhibition of Control Specific Binding by PF-00080665-73





*In Vitro* Pharmacology: enzyme and uptake assays

Figure 19. % inhibition of control values by PF-00080665-73



**Summary:** The most potent binding affinity (with  $\geq 50\%$  inhibition) was for the rat neuronal nicotinic receptor ( $K_i$  290 nM) (note: an approximate 7-fold margin compared to the geometric mean unbound steady-state maximal plasma concentration ( $C_{max}$ ) associated with the recommended human dose of 125 mg QD). Weaker binding affinities ( $\geq 18$ -fold margins compared to the  $C_{max}$  at the recommended human dose) were displayed for the human beta1-adrenoceptor, muscarinic M1 receptor, phosphodiesterase (PDE) 4, muscarinic M3 receptor, muscarinic M2 receptor, sodium channel, histamine H3 receptor, mu opioid receptor, GABA $\beta$ (1b) receptor, adrenergic alpha2a receptor, delta opioid receptor (delta2), and acetylcholinesterase.

**Study title:** In Vitro Pharmacology: Pfizer 2009 Profile  
Study of PF-05089326-00

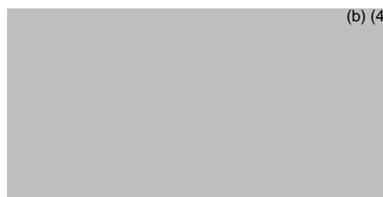
**Study no:** 75760087

**Volume #, and page #:** electronic submission, Module 4

M4\421-pharmacology\4212-secondary pharmacodynamics\Study 75760087,

Page 1-50

**Conducting laboratory and location:**



**Date of study initiation:** June 30, 2009

**GLP compliance:** no

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** PF-05089326-00

Batch No.: PF-05089326-00-0001

Purity: not provided

**Methods:** PF-05089326 (M17), a minor human metabolite, was evaluated against a panel of receptors, ion channels, transporters, and enzymes at a concentration of 10  $\mu$ M. *In Vitro* Pharmacology binding assays and enzyme and uptake assays were

performed, and the data collection were analyzed using the following methods (copied from the Applicant's submission)

*In Vitro* Pharmacology: Binding Assays

The results are expressed as a percent of control specific binding

$$\frac{\text{measured specific binding}}{\text{control specific binding}} * 100$$

and as a percent inhibition of control specific binding

$$100 - \left( \frac{\text{measured specific binding}}{\text{control specific binding}} * 100 \right)$$

obtained in the presence of PF-00080665-73.

*In Vitro* Pharmacology: Enzyme and Uptake Assays

The results are expressed as a percent of control specific activity

$$\frac{\text{measured specific activity}}{\text{control specific activity}} * 100$$

and as a percent inhibition of control specific activity

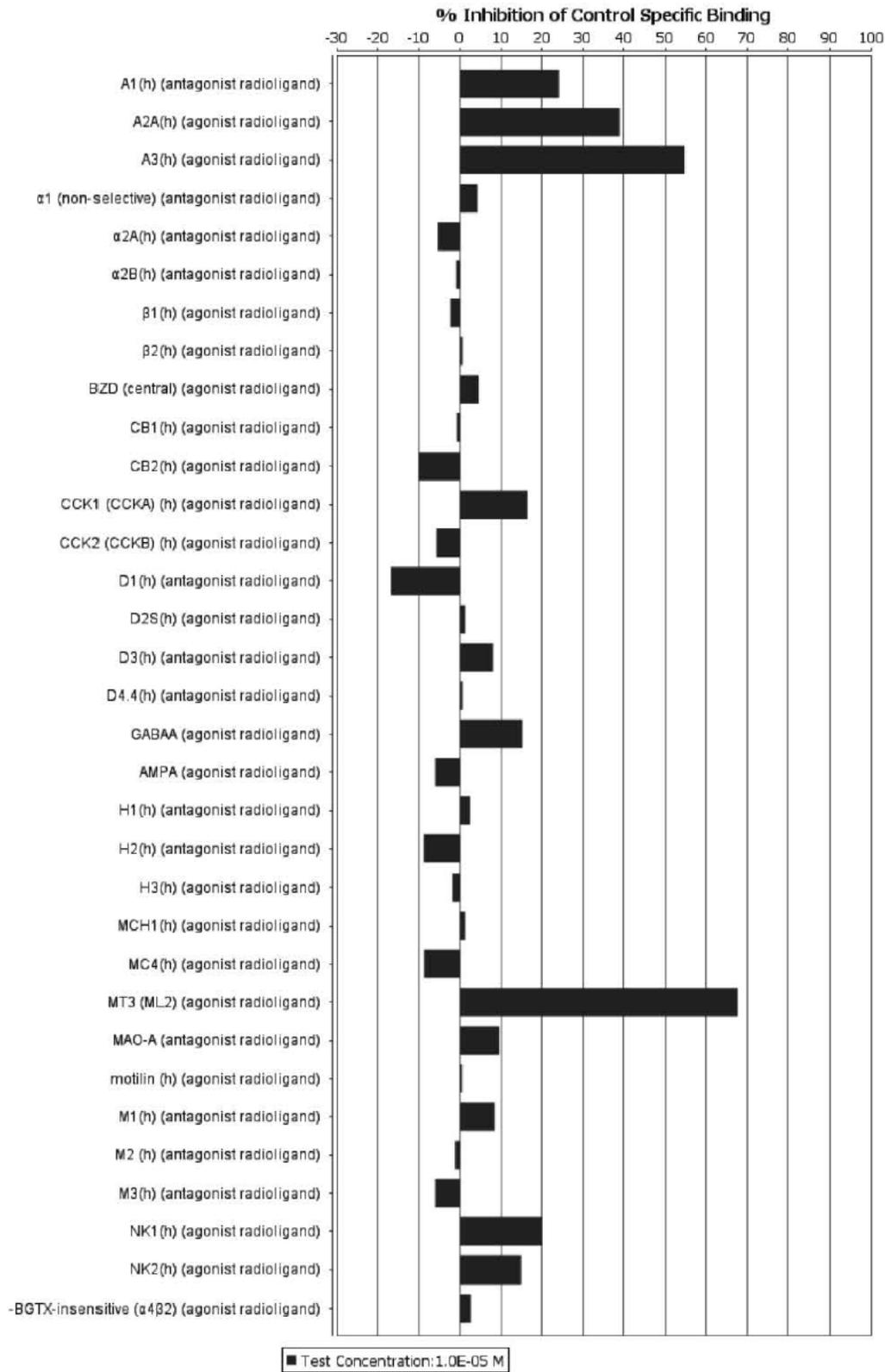
$$100 - \left( \frac{\text{measured specific activity}}{\text{control specific activity}} * 100 \right)$$

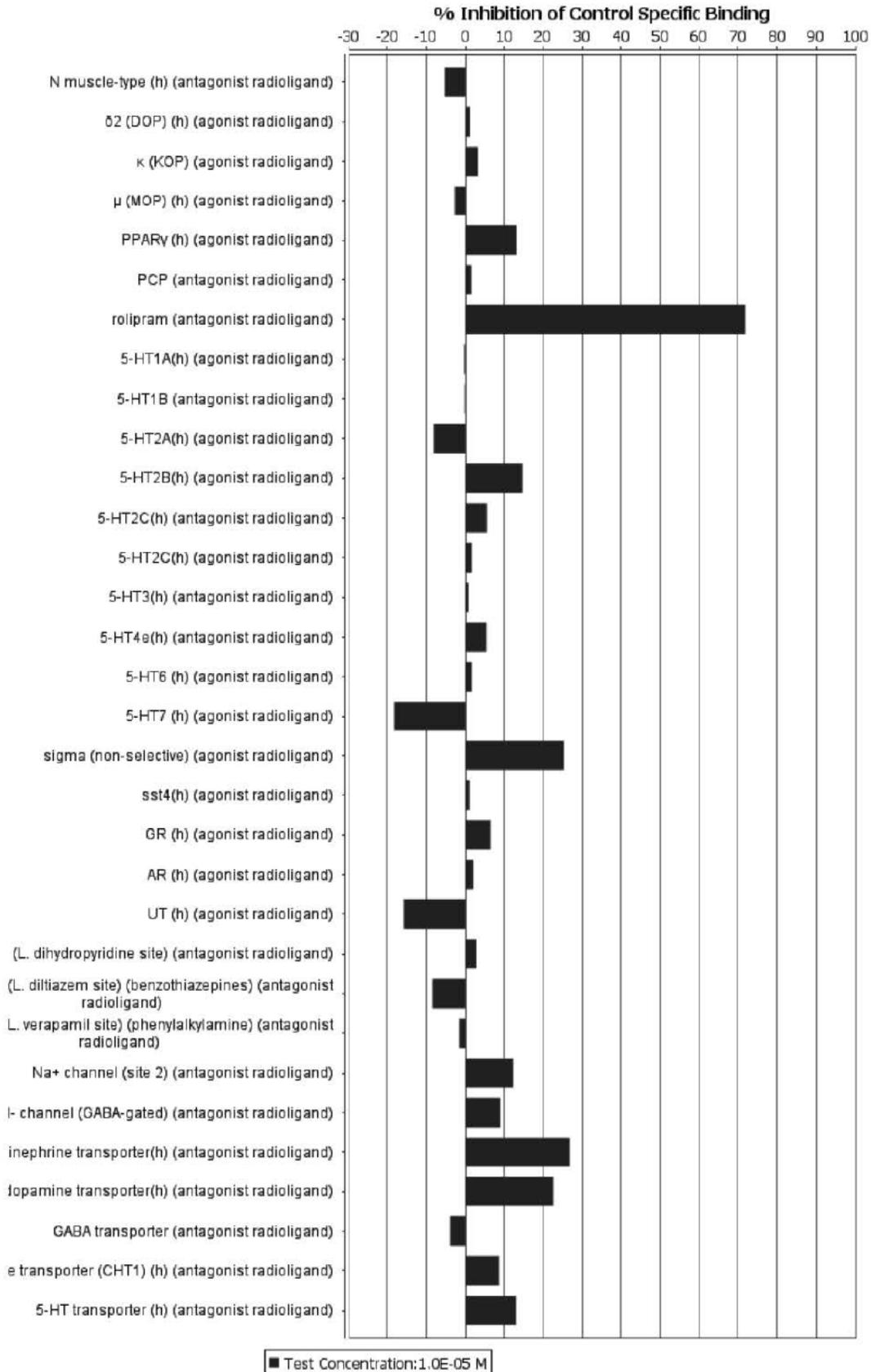
obtained in the presence of PF-00080665-73.

**Results:** The following figures are copied from the Applicant's submission.

*In Vitro* Pharmacology: Binding Assays

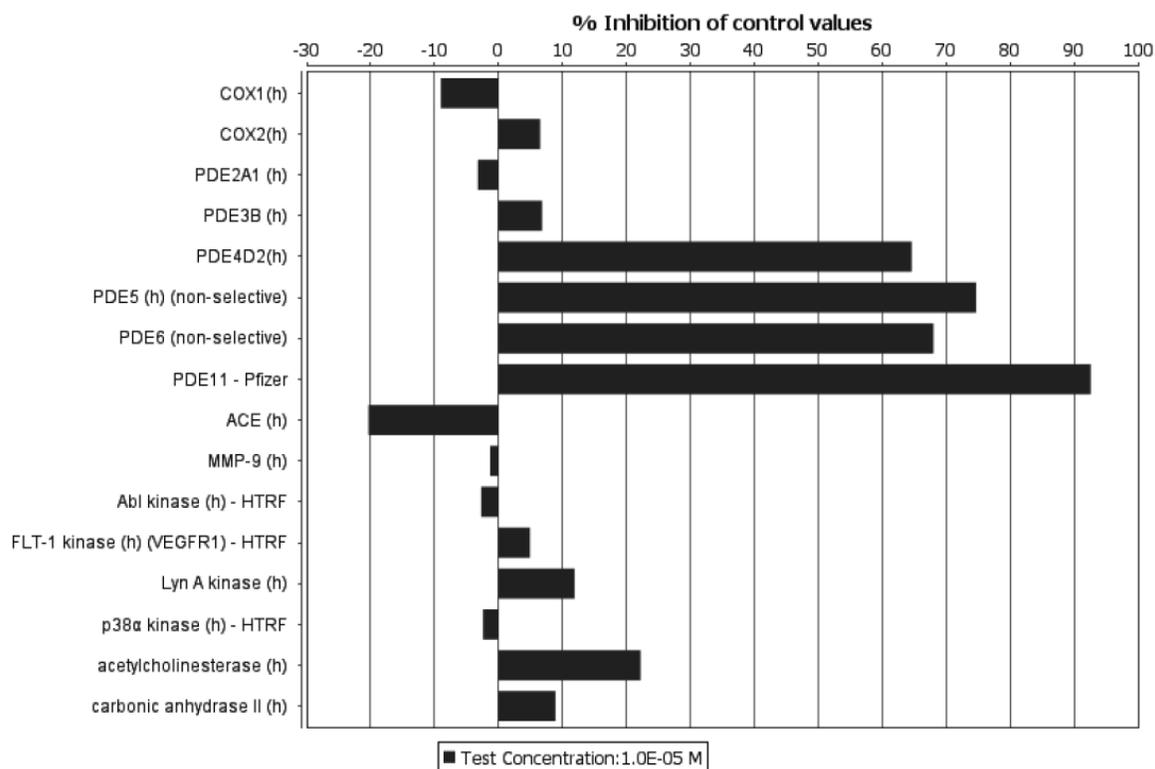
Figure 20. % inhibition of Control Specific Binding by PF-05089326





*In Vitro* pharmacology: enzyme and uptake assays

Figure 21. % inhibition of control values by PF-05089326



**Summary:** Secondary (off-target) pharmacology related to PF-05089326 is considered unlikely due to its high plasma protein binding and presence as a minor metabolite in humans.

### Conclusion

Palbociclib and PF-05089326 (M17), a minor human metabolite, did not demonstrate significant secondary (off-target) activities and are not likely to significantly contribute either to the clinical efficacy or safety of palbociclib, based on the concentrations at which binding activity occurs.

### 4.3 Safety Pharmacology

#### Neurological effects:

**Study title:** Safety Pharmacology - Neurofunctional Evaluation of PD 332991-54 in Sprague-Dawley Rats

**Study no:** research report #RR 745-03890, Study protocol #3108

**Volume #, and page #:** electronic submission, Module 4

M4\421-pharmacol\4213-safety-pharmacol\Study RR-745-03890, Page 1-151

**Conducting laboratory and location:** Pfizer global research & development  
Ann Arbor Laboratories

Ann Arbor, Michigan

**Date of study initiation:** May 6, 2003**GLP compliance:** yes**QA report:** yes ( x ) no ( )**Drug, lot #, radiolabel, and % purity:** PD 0332991-0054, PF-00080665-73

Lot No.: LCD-4245

Purity: 74.9% (drug potency)

**Formulation/vehicle:** deionized water

**Methods:** The effect on the neurological function was studied in male Sprague-Dawley rats following a single oral dose of vehicle (deionized water) or PD 332991-54 at 30 or 300 mg/kg. Animals were observed for clinical signs of toxicity approximately every 15 minutes for 60 minutes, 23 hours postdose. Animals were evaluated for appearance, exploratory behavior, gait and general activity for approximately 5 minutes in an open-field environment, 24 hours postdose. Neurofunctional testing was performed at 24 hours postdose. The parameters evaluated were air-righting reflex, visual placing, acoustic startle, grip strength, catalepsy, hindlimb foot splay, analgesia, and coordination. During reflex testing, corneal and pupillary reflexes were evaluated following a 10-minute dark adaptation, and body temperature was measured. Total distance and number of vertical movements in the automated activity monitor were recorded for 30 minutes.

Subsequent to neurofunctional evaluation, 16 vehicle-control rats were reassigned to new dose groups for determination of plasma drug concentrations and calculation of toxicokinetic parameters of PD 332991. Blood samples were collected at approximately 1, 4, 7, 12, 24, and 30 hours postdose from 4 rats/group/time point given either 30 or 300 mg/kg.

See the details for the study design in the table below (copied from the Applicant's submission)

Table 1. Experimental Groups

Group	No. of Rats	Dose (mg/kg)	Animal No.
<b>Observational Assessment</b>			
1	8	0 (vehicle) <sup>a</sup>	111690-111697
2	8	30	111698-111705
3	8	300	111706-111713
<b>Neurofunctional Tests</b>			
4	8	0 (vehicle) <sup>a</sup>	111714-111721
5	8	30	111722-111729
6	8	300	111730-111737
<b>Reflex Testing/Activity Monitoring</b>			
7	8	0 (vehicle) <sup>a</sup>	111738-111745
8	8	30	111746-111753
9	8	300	111754-111761
<b>Toxicokinetic Assessment<sup>b</sup></b>			
1	8	30	111690-111697
4	8	300	111714-111721

<sup>a</sup> Vehicle = Deionized water

<sup>b</sup> Subsequent to completion of neurofunctional evaluations, control animals in Groups 1 and 4 were reassigned new dose

### Dosing:

Species/strain: male Sprague-Dawley [CrI:CD@(SD)IGS BR] rats

#/sex/group or time point: 8/group

Age: 43 days old

Weight: 159 to 187 g

Doses in administered units: 30, 300 mg/kg (dose selection was based on the study results from single dose study in rats)

Route, form, volume, and infusion rate: oral gavage, at a dose volume of 10 mg/mL

### Results:

#### *Observational assessment*

30 mg/kg: 2 animals had decreased exploratory behavior during the open-field assessment.

300 mg/kg: 1 animal had reduced feces and was hypoactive with decreased exploratory behavior during the open-field assessment.

#### *Neurofunctional testing*

Unremarkable

#### *Reflex testing/activity monitoring*

The decreases in activity, in the total distance habituation rate profile, and in the initial vertical activity profile was observed at 300 mg/kg 24 hours postdose.

Mean total distance and number of vertical movements were 19% and 18% less than controls, respectively. The total distance habituation rate profile and the initial vertical activity profile were ~26% and ~18% respectively compared to the vales at control group. The observed changes were not statistically significant.

Note: An unanticipated  $T_{max}$  at 30 and 300 mg/kg occurred 4 hours and 24 hours postdose, respectively; therefore, assessment at 24 hours postdose may result in the lack of change detected in locomotor activity at 30 mg/kg.

### Plasma Drug Concentrations

$C_{max}$  values of 1.15 and 6.34  $\mu\text{g/mL}$  at 30 and 300 mg/kg were achieved at 4 and 30 hours postdose, respectively (Table 3). Corresponding  $\text{AUC}(0-30)$  values were 16.3 and 80.5  $\mu\text{g}\cdot\text{hr/mL}$ , respectively;  $\text{AUC}(0-24)$  values were 16 and 50.7  $\mu\text{g}\cdot\text{hr/mL}$ , respectively. The following summary table was copied from the Applicant's submission.

Table 3. Mean Plasma Concentration ( $\mu\text{g/mL}$ )<sup>a</sup>

Dose (mg/kg)	Time Postdose					
	1 hr	4 hrs	7 hrs	12 hrs	24 hrs	30 hrs
30	0.595 $\pm$ 0.179	1.15 $\pm$ 0.22	0.897 $\pm$ 0.163	0.85 $\pm$ 0.155	0.0774 $\pm$ 0.0434	0.0365 $\pm$ 0.0165
300	1.25 $\pm$ 0.31	1.77 $\pm$ 0.405	1.45 $\pm$ 0.316	1.82 $\pm$ 0.157	3.59 $\pm$ 0.929	6.34 $\pm$ 1.4

<sup>a</sup> Data presented as group mean  $\pm$  standard deviation; n = 4 animals/time point.

**Summary:** PD 332991-54 induced a mild decrease in activity at the doses tested  
Respiratory effects:

**Study title:** Safety Pharmacology - Effect of PD 332991-54 on Pulmonary Function in Beagle Dogs

**Study no:** Research report# RR 745-03892, Study #3117

**Volume #, and page #:** electronic submission, Module 4

M4\421-pharmaco\4213-safety-pharmacol\Study RR- 745-03892, Page 1-98

**Conducting laboratory and location:** Pfizer global research & development  
Ann arbor laboratories  
Ann arbor, Michigan

**Date of study initiation:** May 21, 2003

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** PD 0332991-0054, PF-00080665-73  
Lot No.: LCD-4245  
Purity: 74.15%

**Formulation/vehicle:** 5% dextrose.

**Methods:** The effect of PD 0332991-0054 on the respiratory system was studied in male beagle dogs following a single intravenous (IV) administration of PD 332991-54 using a 3-period crossover design with each animal serving as its own control. Following 20 minutes of baseline data collection, animals were administered vehicle (5% dextrose) or PD 332991-54 at 1 or 5 mg/kg intravenously. Pulmonary function data were collected for 60 minutes postdose. Primary response variables were minute volume, resistance, and compliance. Secondary parameters included peak expiratory flow, peak inspiratory flow, respiratory rate, and tidal volume. Blood was collected from all animals at 5, 15, 30, and 60 minutes after the start of IV administration for determination of plasma drug concentrations. There was a 7-day interval between treatments.

See the details of the study design in the table below (copied from the Applicant's submission).

Table 1. Treatment Regimen

Animal No.	Sex	Dose (mg/kg)		
		Treatment 1	Treatment 2	Treatment 3
5752	M	Vehicle <sup>a</sup>	1	5
5753	M	1	5	Vehicle
5754	M	5	Vehicle	1
5755	M	Vehicle	5	1

<sup>a</sup> Vehicle = 5% Dextrose.

**Dosing:**

Species/strain: male beagle dogs

#/sex/group or time point: 4/dose

Age: approximately 3 to 5 years old

Weight: 8.6 to 13 kg

Doses in administered units: 1, 5 mg/kg (based on the conducted single dose study in dogs)

Route, form, volume, and infusion rate: IV, a dose volume at 1 mL/kg and the infusion rate at 10 mL/minute.

**Results:** The following figures were copied from the Applicant's submission.

*Pulmonary assessments*

Figure 22. Effect of PD 332991-54 on minute volume

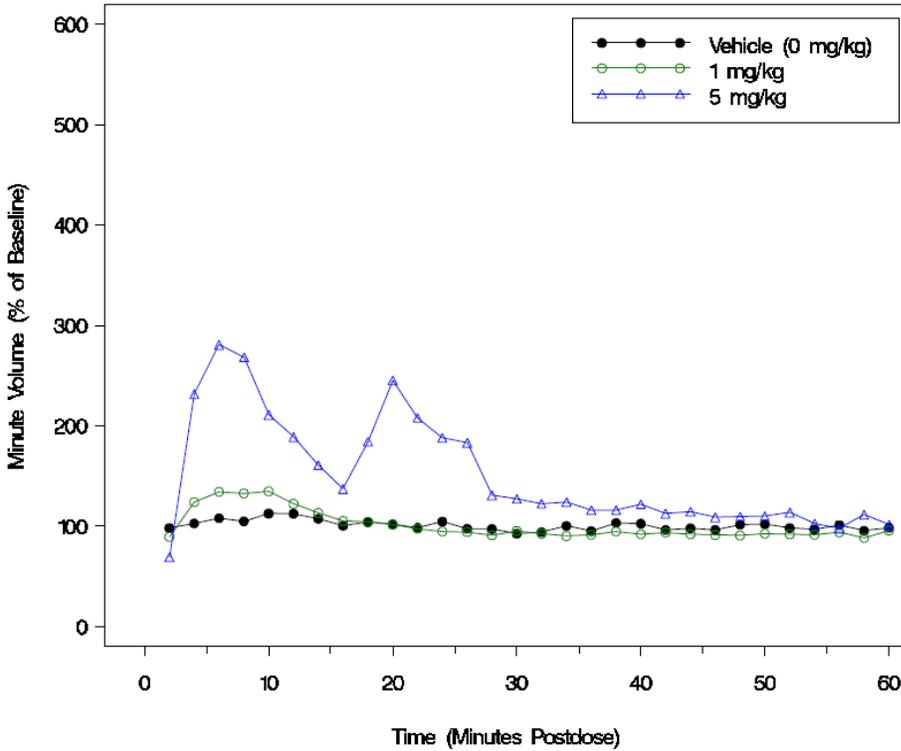


Figure F-1. Effect of PD 332991-54 on Minute Volume (% of Baseline)

Figure 23. Effect of PD 332991-54 on pulmonary resistance

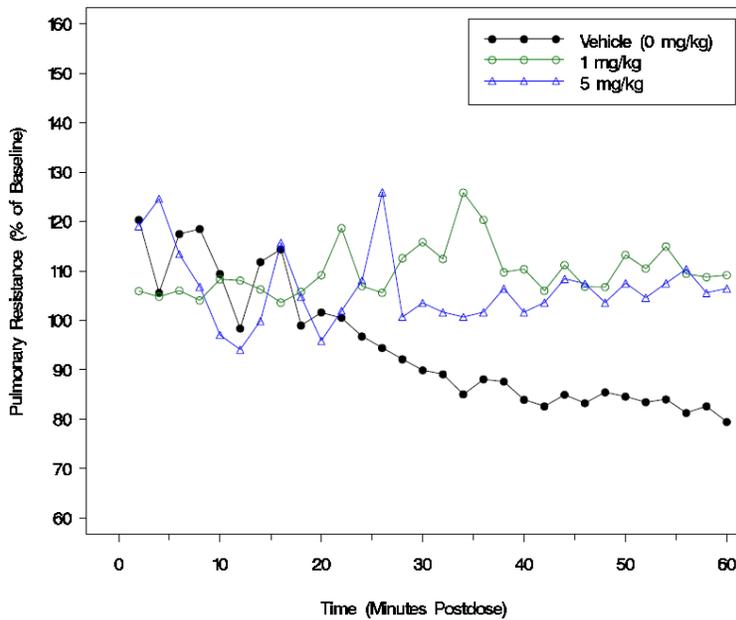


Figure F-2. Effect of PD 332991-54 on Pulmonary Resistance (% of Baseline)

Figure 24. Effect of PD 332991-54 on pulmonary compliance

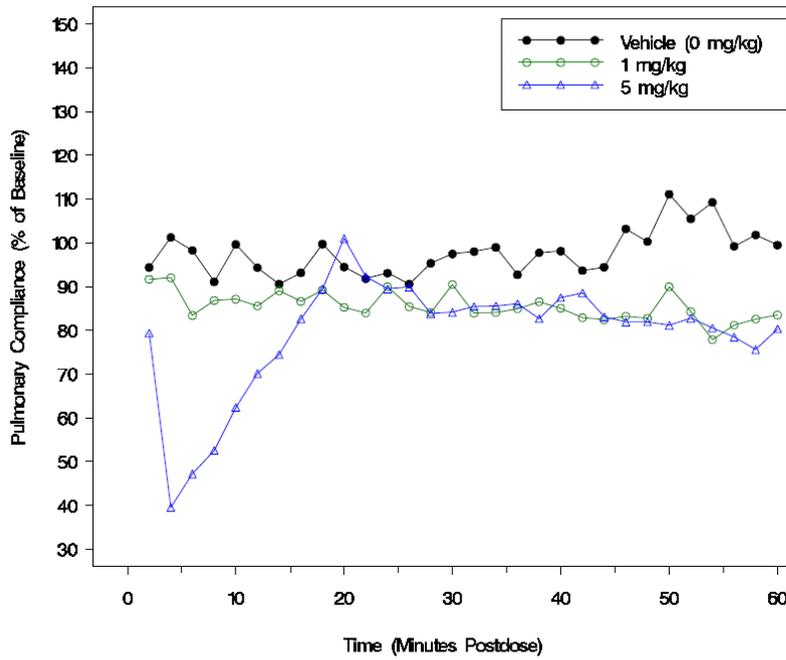


Figure F-3. Effect of PD 332991-54 on Pulmonary Compliance (% of Baseline)

Figure 25. Effect of PD 332991-54 on peak expiratory flow

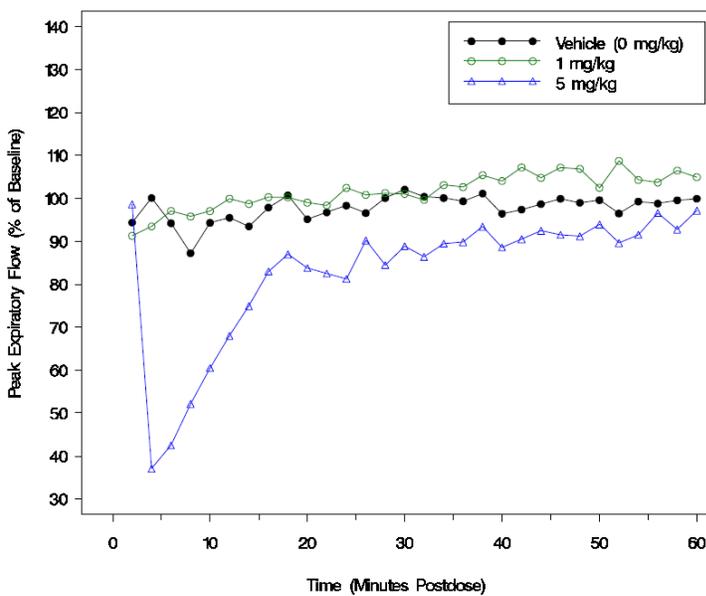


Figure F-4. Effect of PD 332991-54 on Peak Expiratory Flow (% of Baseline)

Figure 26. Effect of PD 332991-54 on peak inspiratory flow

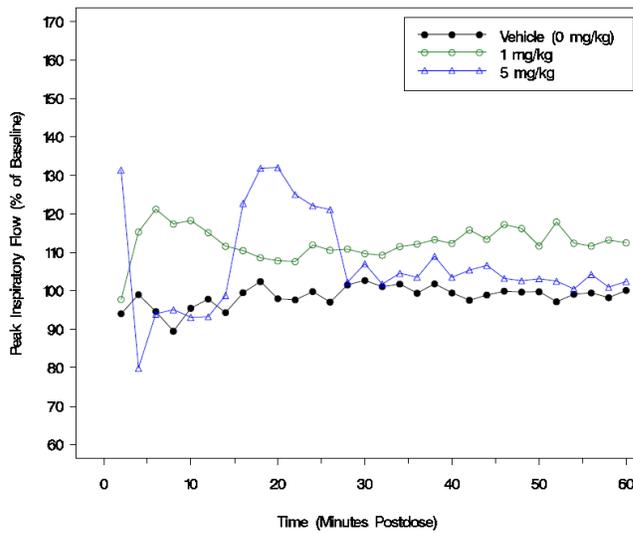


Figure F-5. Effect of PD 332991-54 on Peak Inspiratory Flow (% of Baseline)

Figure 27. Effect of PD 332991-54 on peak respiratory rate

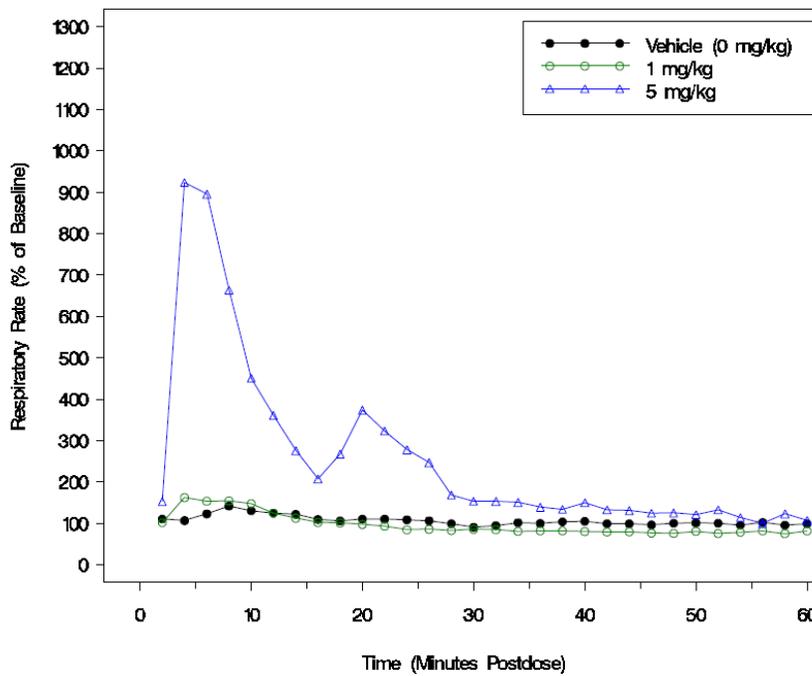


Figure F-6. Effect of PD 332991-54 on Respiratory Rate (% of Baseline)

Figure 28. Effect of PD 332991-54 on tidal volume

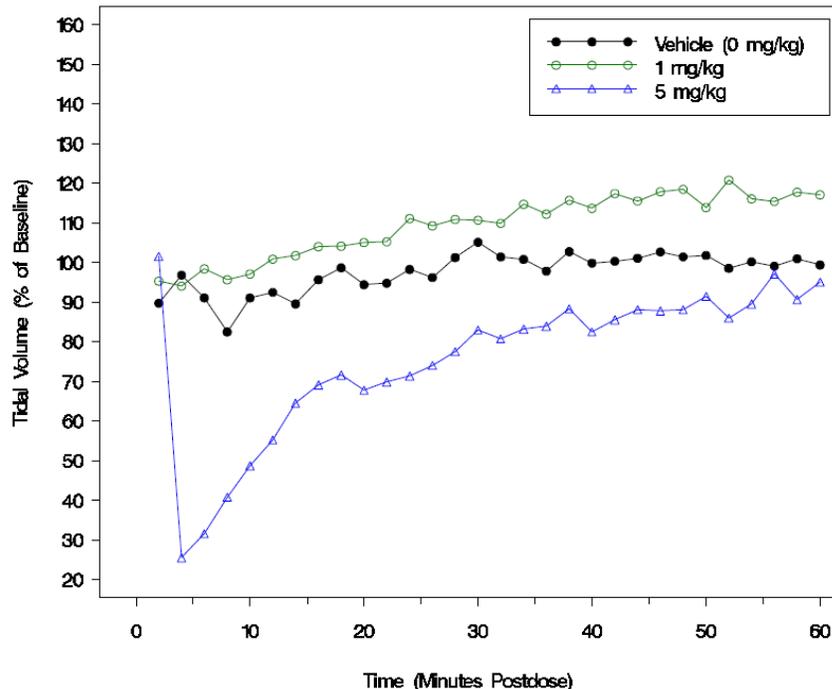


Figure F-7. Effect of PD 332991-54 on Tidal Volume (% of Baseline)

Note: 1) Two of 4 animals administered PD 332991-54 at 5 mg/kg stopped breathing less than 2 minutes after initiation of drug infusion. Blood oxygen saturation in these animals decreased 58% and 33% relative to predose values. Dosing was discontinued in 1 animal and repeated attempts to administer oxygen and continue with data collection in both of these animals were ultimately unsuccessful. Although both dogs recovered, no additional pulmonary data were collected from these 2 animals at this dose.

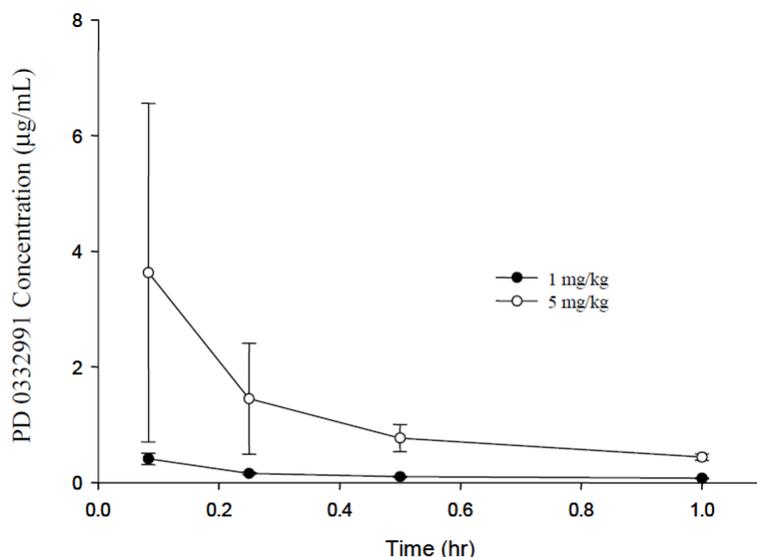
The other 2 animals dosed at 5 mg/kg had 27% to 31% reductions in pO<sub>2</sub> within 2 minutes of drug infusion along with 30% to 42% reductions in heart rate, but data collection continued without interruption. Blood oxygen saturation began to increase within 5 minutes of drug-infusion and was at predose levels within 36 to 43 minutes postinfusion.

2) The observed pulmonary effects associated with an interaction of PD 332991 and the anesthetic cannot be excluded, as a central respiratory depressant used per protocol may induce respiratory depression.

**Summary:** Postdose minute volume and respiratory rate were significantly increased 0.6- to 4.6-fold relative to the controls. Significant decreases in compliance, peak expiratory flow, and tidal volume ranging from 17% to 72% were also noted in the 5 mg/kg animals between 4 and 12 minutes postdose.

*Plasma drug concentration analysis*

Figure 29. Mean plasma concentration of PD 332991

Figure 1. Mean ( $\pm$ SD) Plasma Concentrations of PD 332991

Note: Mean plasma drug concentrations 5 minutes after the start of dosing were 0.412 and 3.63  $\mu$ g/mL at 1 and 5 mg/kg, respectively.

Summary: A single IV dose of PD 332991-54 in anesthetized dogs at 5 mg/kg caused significant effects on pulmonary parameters including increases in minute volume and respiratory rate, and decreases in compliance, peak expiratory flow, and tidal volume.

Note: The effects were transient, appeared related to peak-plasma concentrations of drug ( $\geq 2.04$   $\mu$ g/mL), and were consistent with respiratory depression. The effect on respiratory function was observed at an unbound  $C_{max}$  of  $\geq 842$  ng/mL, approximately 50-times the human clinical exposure at 125 mg QD based on mean unbound  $C_{max}$  (17 ng/mL).

Cardiovascular effects:

**Study title:** Effects of PD 332991 on action potentials recorded from dog isolated purkinje fibres *in vitro*

**Study no:** PD332991/IC/001/02

**Volume #, and page #:** electronic submission, Module 4  
M4\4.2.1.3 PD332991/IC/001/02, Page 1-33

**Conducting laboratory and location:** Candidate Research Group Laboratories  
Pfizer Global Research and Development  
Sandwich Laboratories  
Ramsgate Road  
Kent, CT13 9NJ, United Kingdom

**Date of study initiation:** February 26, 2002

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** PD332991  
Lot No.: Lot Q,  
Purity: not provided

**Formulation/vehicle:** 0.5% (w/v) methylcellulose

**Methods:** The effects of PD332991 on action potentials were evaluated in dog isolated Purkinje fibers *in vitro*. PD332991 was tested at assay concentrations of 0.1µM, 1µM and 10µM. Data obtained from seven fibers from six dogs were reported in this study, five fibers were treated with PD332991 and two fibers were treated with vehicle. The vehicle control was 0.1% v/v DMSO. All action potentials generated were continuously recorded and analyzed by Notocord-Hem.

**Results:**

PD332991 had no effect on the resting membrane potential, action potential amplitude,  $V_{max}$  or action potential duration at 50% repolarization. PD332991 had no effect on APD90 at 0.1µM or 1µM. PD332991 at 10µM caused a statistically significant increase on APD90  $8.2 \pm 2.0\%$  ( $p=0.032$ ).

**Study title:** Effect of PD-0332991-0054 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

**Study no:** Applicant Study # 13GR243; Test study# 130729.QHJ

**Volume #, and page #:** electronic submission, Module 4  
M4\4.2.1.3 . 13GR243, Page 1-77

**Conducting laboratory and location:**

(b) (4)

**Date of study initiation:** October 2, 2013

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** PD-0332991-0054  
Lot No.: GR05574 (E010012644)  
Purity: 77.9%

**Formulation/vehicle:** 0.5% (w/v) methylcellulose

**Methods:** In this assay, hERG potassium channels expressed in a human embryonic kidney (HEK293) cell line were used to examine the in vitro effects of PD-0332991-0054 on the hERG (human ether-à-go-go-related gene) channel current. Four concentrations of PD-0332991-0054 (0.3, 1, 3, 10  $\mu\text{M}$ ) were tested to evaluate the concentration-response relationship. Each concentration was tested in at least three cells ( $n \geq 3$ ). The steady state before and after test article application was used to calculate the percentage of current inhibited at each concentration. Percent inhibition at each concentration in the test group was compared with the vehicle control group using one-way ANOVA followed by Dunnett's multiple comparison test (JMP Version 5.0.1, SAS Institute, Cary, NC).

Vehicle control: a HEPES-buffered physiological saline (HB-PS) solution composed of (in mM): NaCl, 137; KCl, 4.0; CaCl<sub>2</sub>, 1.8; MgCl<sub>2</sub>, 1; HEPES, 10; Glucose, 10; pH adjusted to 7.4 with NaOH (refrigerated until used) and supplemented with 0.3% DMSO

Positive control: terfenadine

Reference Substance: E-4031

### Results:

Table 9. Mean percent inhibition compared to vehicle control values

Concentration ( $\mu\text{M}$ )	Mean	N
0	0.4%	3
0.3	*8.3%	3
1	*23.4%	4
3	*48.1%	3
10	*76.7%	3

- Value is statistically different from vehicle alone ( $P \leq 0.05$ )

*Summary:* PD-0332991-0054 inhibited hERG current under the testing condition. The  $\text{IC}_{50}$  for the inhibitory effect of PD-0332991-0054 on hERG potassium current was 3.2  $\mu\text{M}$  (Hill coefficient = 1.0).

**Study title:** Safety Pharmacology Blood Pressure, Heart Rate, and Cardiac Rhythm Effects of PD 332991-2B in Beagle Dogs

**Study no:** Research report# RR 745-03696; study#2889

**Volume #, and page #:** electronic submission, Module 4

M4\4.2.1.3.13rr-745-03696, Page 1-70

**Conducting laboratory and location:** Pfizer Global Research & Development  
Ann Arbor Laboratories  
Ann Arbor, Michigan

**Date of study initiation:** September 13, 2013

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** PD-0332991  
Lot No.: E010014102  
Purity: > 98.6%

**Formulation/vehicle:** 0.5% (w/v) methylcellulose

**Methods:** PD-0332991 was administered to conscious, unrestrained, radio-telemetry implanted male beagle dogs as a single dose via oral gavage at dose levels of 3, 10 and 30 mg/kg. Telemetered data were obtained from each animal beginning ~ 1 hour predose through ~ 24 hours postdose to evaluate the effects of PD-0332991 on heart rate (HR), blood pressure (BP), electrocardiographic (ECG) parameters, and activity. Plasma levels of PD-0332991 were measured in the study. Plasma samples for determination of PD-0332991 concentrations were obtained predose and ~4 HPD during the CV phase. Following completion of the CV phase, all animals received a single oral dose of PD-0332991 at 3 and 30 mg/kg (Treatments 5 and 6, respectively) for the provision of a toxicokinetic profile (TK phase). Plasma samples for determination of PD-0332991 concentrations during the TK phase of the study were obtained predose and ~1, 2, 4, 7, 12, and 24 HPD for both Treatments 5 and 6. Clinical signs were assessed at least once daily beginning upon receipt of animals to study and ended when animals were returned to colony.

### **Dosing:**

Species/strain: male Beagle dogs

#/sex/group or time point: n=4\*

\* a washout period of at least 2 weeks between doses.

Age: 1.5-3 years

Weight: 8-13 kg

Doses in administered units: 3, 10, 30 mg/kg

Route, form, volume, and infusion rate: oral gavage ay dose volume of 2 mL/kg

### **Results:**

#### Clinical signs

Emesis was observed at doses  $\geq$  30 mg/kg from around 4 hours postdose to 24 hours postdose.

#### Cardiovascular parameters and ECG and heart rate measurements

The table below summarizes test article-related changes from vehicle.

Table 10. Test article-related cardiovascular effects by time period and parameter

parameter	Dose (mg/kg)	0.5- 3.5 h Postdose	4.5- 9.25 h Postdose	9.5- 20 h Postdose	20.25- 24 h Postdose
Activity (arbitrary units)	3				
	10				
	30				
Systolic blood pressure (mm Hg)	3				
	10		+5.75	+4.25	
	30			+3.25	
Diastolic blood pressure (mm Hg)	3				
	10				
	30				
Mean blood pressure (mm Hg)	3				
	10				
	30				
Heart Rate (beats per minute (bpm))	3				
	10		-7.75		
	30		-5.5		
PR-Interval (msec)	3				
	10				
	30				
QRS-Interval (msec)	3				
	10				
	30				
QT-Interval (msec)	3				
	10	+5.75	+12.25	+6.25	+6.00
	30	+7.5	+13.5	+7.75	+8.25
QTc-Interval (msec)	3		+5.00		
	10		+7.00	+4.50	+5.00
	30	+5.50	+9.25	+7.5	+7.00
RR-Interval (msec)	3				
	10		+55.25		
	30				

Blank: unremarkable

+ = Indicates a finding with increased value over vehicles.

- = Indicates a finding with decreased value over vehicles.

Shaded coloring indicates statistically significant finding.

**TK:** The following summary was excerpted from the Applicant's submission.

**Summary:** The mean plasma concentrations ( $\pm$ SD) of PD-0332991 at 4 hours postdose (HPD) during the CV phase of the study were 12, 340, and 365 ng/mL, for the 3, 10, and 30 mg/kg dose groups, respectively. For the 3 mg/kg TK phase of the study, mean T<sub>max</sub> was at 10 HPD; mean C<sub>max</sub> and AUC<sub>24</sub> were 83 ng/mL and 1310 ng•h/mL, respectively. For the 30 mg/kg TK phase of the study, mean T<sub>max</sub> was at 11 HPD; mean C<sub>max</sub> and AUC<sub>24</sub> were 516 ng/mL and 6670 ng•h/mL, respectively. The mean

concentrations of PD-0332991 at 4 HPD at 3 mg/kg and 30 mg/kg during the TK phase were 66 ng/mL and 267 ng/mL, which were 46% and 27% lower than exposures during the CV phase.

*Summary:* Oral administration of PD-0332991 at  $\geq 3$  mg/kg resulted in increases in the QTc-interval that correlated with individual animal PD-0332991 plasma concentrations. Specifically, a 5 msec increase in QTc was associated with a plasma concentration of 162 ng/mL. At  $\geq 10$  mg/kg, PD-0332991 produced decreases in HR, and increases in the QT-interval, RR-interval, and systolic blood pressure.

**Study title:** Safety pharmacology-cardiovascular assessment of oral PD-0332991 in telemetry instrumented male beagle dogs

**Study no:** 13GR248

**Volume #, and page #:** electronic submission, Module 4  
M4\4.2.1.3.13GR248, Page 1-94

**Conducting laboratory and location:** Pfizer Worldwide Research & Development  
Drug Safety Research & Development  
Eastern Point Road  
Groton, CT 06340 USA

**Date of study initiation:** September 13, 2013

**GLP compliance:** yes

**QA report:** yes ( x ) no ( )

**Drug, lot #, radiolabel, and % purity:** PD-0332991  
Lot No.: E010014102  
Purity: > 98.6%

**Formulation/vehicle:** 0.5% (w/v) methylcellulose

**Methods:** PD-0332991 was administered to conscious, unrestrained, radio-telemetry implanted male beagle dogs as a single dose via oral gavage at dose levels of 3, 10, 30 mg/kg. Telemetered data were obtained from each animal beginning ~ 1 hour predose through ~ 24 hours postdose to evaluate the effects of PD-0332991 on heart rate (HR), blood pressure (BP), electrocardiographic (ECG) parameters, and activity. Plasma levels of PD-0332991 were measured in the study. Plasma samples for determination of PD-0332991 concentrations were obtained predose and ~4 HPD during the CV phase. Following completion of the CV phase, all animals received a single oral dose of PD-0332991 at 3 and 30 mg/kg (Treatments 5 and 6, respectively) for the provision of a toxicokinetic profile (TK phase). Plasma samples for determination of PD-0332991 concentrations during the TK phase of the study were obtained predose and ~1, 2, 4, 7, 12, and 24 HPD for both Treatments 5 and 6. Clinical signs were assessed at least once daily beginning upon receipt of animals to study and ended when animals were returned to colony.

**Dosing:**

Species/strain: male Beagle dogs

#/sex/group or time point: n=4\*

\* a washout period of at least 2 weeks between doses.

Age: 1.5-3 years

Weight: 8-13 kg

Doses in administered units: 3, 10, 30 mg/kg

Route, form, volume, and infusion rate: oral gavage ay dose volume of 2 mL/kg

**Results:**Clinical signs

Emesis was observed at doses  $\geq 30$  mg/kg from around 4 hours postdose to 24 hours postdose.

Cardiovascular parameters and ECG and heart rate measurements

The table below summarizes test article-related changes from vehicle.

Table 11. Test article-related cardiovascular effects by time period and parameter

parameter	Dose (mg/kg)	0.5- 3.5 h Postdose	4.5- 9.25 h Postdose	9.5- 20 h Postdose	20.25- 24 h Postdose
Activity (arbitrary units)	3				
	10				
	30				
Systolic blood pressure (mm Hg)	3				
	10		+5.75	+4.25	
	30			+3.25	
Diastolic blood pressure (mm Hg)	3				
	10				
	30				
Mean blood pressure (mm Hg)	3				
	10				
	30				
Heart Rate (beats per minute (bpm))	3				
	10		-7.75		
	30		-5.5		
PR-Interval (msec)	3				
	10				
	30				
QRS-Interval (msec)	3				
	10				
	30				
QT-Interval (msec)	3				
	10	+5.75	+12.25	+6.25	+6.00
	30	+7.5	+13.5	+7.75	+8.25
QTc-Interval (msec)	3		+5.00		
	10		+7.00	+4.50	+5.00

parameter	Dose (mg/kg)	0.5- 3.5 h Postdose	4.5- 9.25 h Postdose	9.5- 20 h Postdose	20.25- 24 h Postdose
	30	+5.50	+9.25	+7.5	+7.00
RR-Interval (msec)	3				
	10		+55.25		
	30				

Blank: unremarkable

+ = Indicates a finding with increased value over vehicles.

- = Indicates a finding with decreased value over vehicles.

Shaded coloring indicates statistically significant finding.

TK: The following summary was excerpted from the Applicant's submission.

The mean plasma concentrations ( $\pm$ SD) of PD-0332991 at 4 hours postdose (HPD) during the CV phase of the study were 12, 340, and 365 ng/mL, for the 3, 10, and 30 mg/kg dose groups, respectively. For the 3 mg/kg TK phase of the study, mean  $T_{max}$  was at 10 HPD; mean  $C_{max}$  and  $AUC_{24}$  were 83 ng/mL and 1310 ng•h/mL, respectively. For the 30 mg/kg TK phase of the study, mean  $T_{max}$  was at 11 HPD; mean  $C_{max}$  and  $AUC_{24}$  were 516 ng/mL and 6670 ng•h/mL, respectively. The mean concentrations of PD-0332991 at 4 HPD at 3 mg/kg and 30 mg/kg during the TK phase were 66 ng/mL and 267 ng/mL, which were 46% and 27% lower than exposures during the CV phase.

*Summary*: Oral administration of PD-0332991 at  $\geq$ 3 mg/kg resulted in increases in QTc-interval that correlated with individual animal PD-0332991 plasma concentrations. At  $\geq$ 10 mg/kg, PD-0332991 produced decreases in HR, and increases in the QT-interval, RR-interval, and systolic blood pressure.

Renal effects: no study conducted

Gastrointestinal effects: no study conducted

Abuse liability: no study conducted

### Conclusion

No clinically relevant adverse effects of PD 332991-54 on neurological function were observed in the conducted safety pharmacology studies. PD 332991-54 showed adverse effects on cardiovascular or pulmonary functional parameters in the conducted safety pharmacology studies.

**PHARMACOLOGY TABULATED SUMMARY**

Table 12. Comparison of Biochemical Activity (enzymatic activity) In Vitro

Biochemical Activity (enzymatic activity) In Vitro	IC50
CDK4/cyclinD1	11 nM
CDK6/cyclinD2	15 nM
CDK7/cyclinH/MAT1	> 3 $\mu$ M
CDK9/cyclinT1	0.4 $\mu$ M
Cdc7/ASK	> 3 $\mu$ M

Table 13. Overview of primary pharmacodynamics studies in vitro

Tested cell types	IC <sub>50</sub> , nM				
	RB Phosphorylation			Functional Properties	
	S780	S795	S807/S811	DNA Synthesis	Cell Proliferation
MCF7 breast carcinoma	9		19	100	200
T47D breast carcinoma	30		21	40	95
MDA-MB435 breast carcinoma	66	63		160	32
Colo205 colon carcinoma				130	89
NCIH1299 lung carcinoma				120	
MDA-MB468 Rb- breast carcinoma	-	-	-		> 3000
NCIH2009 Rb- lung carcinoma	-	-	-		> 3000

**5 Pharmacokinetics/ADME/Toxicokinetics****5.1 PK/ADME****Methods of Analysis:**

Liquid chromatography/tandem mass spectrometry (LC-MS/MS) methods were used to determine concentrations of palbociclib in plasma samples from pharmacokinetic studies conducted in rats, dogs, and monkeys.

**Absorption**

**Study title:** Single dose pharmacokinetics, dose proportionality, and oral (PO) bioavailability of PD 0332991 in rats following intravenous administration of PD 0332991-0002C and PO Administration of PD 0332991-0054

**Study no.:** 764-04175

**Volume #, and page #:** electronic submission, Module 4, page 1-21

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** November 2001

**GLP compliance:** no

**QA report:** yes ( ) no (x )

**Drug, lot #, and % purity:** PD 0332991-0000

Lot number: P

Purity: 100% (IV), 99.18% (oral)

### Methods

LC/MS/MS analytical method was used to determine the pharmacokinetic profile of PD 0332991 in Sprague-Dawley rats following administration of single 1- or 5 mg/kg IV dose of PD 0332991-0002C (HCl salt) and 5-, 20-, 50-, or 200-mg/kg PO dose of PD 0332991-0054.

Species/strain: male Sprague-Dawley rats

#/sex/group: IV-3 animals/dose, oral- 4 animals/dose

Schedule: single

Doses in administered units: IV-1, 5, mg/kg, 0.25 mL/kg,  
oral- 5, 20, 50, 200 mg/kg, 0.83 mL/kg

Route: IV or oral gavage

Blood samples collection:

IV- Predose (0), 0.083 (end of infusion), 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 hours

oral- Predose (0), 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30, 48 hours

The following tables are copied from the Applicant's submission.

	<b>Intravenous Infusion</b>	<b>PO Gavage</b>
Formulation Type and Composition:	Solution 5% DMA/25% Propylene Glycol/70% D5W	Suspension 5%:95% PEG200/0.5% methylcellulose
Formulation Concentration (mg/mL):	2.0	6.0
Dose Volume (mL/kg):	0.25	0.83
Dose Administered (mg/kg):	1.0 and 5.0	5, 20, 50 and 200
Compound Administered:	PD 0332991-0002C	PD 0332991-0054
Lot No.:	P	P
Percent Purity:	100%	99.18%
Percent Parent:	77.2%	78.01%
Compound Form:	HCL Salt	Isethionate Salt

	<b>Intravenous Infusion</b>	<b>PO Gavage</b>
Species/Strain:	Sprague-Dawley rats	Sprague-Dawley rats
No. of Animals:	3	4
Crossover Design (Y/N):	N	N
Gender:	Male	Male
Dose (mg/kg):	1.0 and 5.0	5, 20, 50, and 200
Single Dose (Y/N):	Y	Y
Sampling Times:	Predose (0), 0.083 (end of infusion), 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 hours	Predose (0), 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30, 48 hours
Fasted Overnight Before Dosing, 4 hours Following Dosing:	Y	Y
Sample Storage:	-20°C	-20°C

**Results:**

Table 14. Pharmacokinetic parameters of PD 0332991 in Sprague-Dawley rats following administration of single 1.0- or 5.0-mg/kg IV dose of PD 0332991-0002C and 5-, 20-, 50-, or 200-mg/kg PO dose of PD 0332991-0054

Dose (mg/kg)	C <sub>max</sub> (ng/mL)	Dose normalized C <sub>max</sub>	AUC <sub>0-t</sub> (ng.hr/mL)	Dose Normalized AUC <sub>0-t</sub>	T <sub>max</sub> (hr)	T <sub>1/2</sub> (hr)	CL (mL/min/kg)	Vd (mL/kg)
<b>IV</b>								
1	899	899	421	421	0.08	2.22	38	5650
5	2507	501	2160	432	0.08	2.60	37	7070
<b>PO</b>								
5	178	36	1140	228	3.5	2.3	-	-
20	1108	55	10700	535	5	2.8	-	-
50	1655	33	22900	458	4.5	4.92	-	-
200	2242	11	76800	384	30	-	-	-

**Summary:****IV**

- C<sub>max</sub> and AUC generally increased proportionately with dose escalation;
- Clearance values were similar at 1 or 5 mg/kg;
- The mean volume of distribution of PD 0332991 was 5.7 to 7.0 L/kg, which is at least 8-fold greater than total body water in rats (0.693 L/kg) indicating extensive tissue distribution.

**PO**

- C<sub>max</sub> and AUC values increased approximately proportionately with dose from 5 mg/kg to 50 mg/kg, but increased less than proportionately with dose increase from 50 mg/kg to 200 mg/kg;
- The mean terminal elimination half-life ranged from 2.3 to 4.9 hours;
- T<sub>max</sub> ranged from 3.5 to 30 hours;

- PD 0332991-0054 showed good bioavailability (53%) as a PO suspension-calculated based on mean AUC(0-t) after PO administration to the mean AUC(0-t) after IV administration at 5 mg/kg.

**Study title:** Single dose pharmacokinetics and oral bioavailability of PD 0332991 in male beagle dogs following intravenous administration of PD 0332991-0002C and oral administration of PD 0332991-0054

**Study no.:** RR 764-04166 (research report)

**Volume #, and page #:** electronic submission, Module 4, page 1-13

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** November 2001

**GLP compliance:** no

**QA report:** yes ( ) no (x )

**Drug, lot #, and % purity:** PD 0332991-0000  
Lot number: P  
Purity: 100% (IV), 99.18% (oral)

### Methods

LC/MS/MS analytical method was used to determine the pharmacokinetic profile of PD 0332991 following intravenous (IV) administration of PD 0332991-0002C and oral (PO) administration of PD 0332991-0054 to male beagle dogs.

Species/strain: male beagle dogs

#/sex/group: n=3

Schedule: single

Doses in administered units: IV-1mg/kg, 0.169 mL/kg,  
oral- 20 mg/kg, 1 capsule/dog

Route: IV (5-minute infusion) or oral

Blood samples collection:

IV- Predose (0), 0.083 (end of infusion), 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 hours

oral- Predose (0), 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30, 48 hours

The following tables are copied from the Applicant's submission.

	Intravenous Infusion	Oral Gavage
Species/Strain:	Beagle dogs	Beagle dogs
No. of Animals:	3	3
Crossover Design (Y/N):	Y	Y
Gender:	Male	Male
Dose (mg/kg):	1.0	20
Single Dose (Y/N):	Y	Y
Sampling Times:	Predose (0), 0.083 (end of infusion), 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 hours	Predose (0), 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30, 48 hours
Fasted Overnight Before Dosing, 4 Hours Following Dosing:	Y	Y
Sample Storage:	-20°C	-20°C

**Results:**Table 15. The mean ( $\pm$ SD) PK parameters of PD 0332991 (copied from the Applicant's submission)

Route	Dose (mg/kg)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	t <sub>1/2</sub> (hr)	AUC(0- $\infty$ ) (ng·hr/mL)	AUC(0-t) (ng·hr/mL)	CL (mL/min/kg)	V <sub>dss</sub> (L/kg)	F (%)
IV	1.0	--	--	10.8 $\pm$ 0.3	2330 $\pm$ 258	1860 $\pm$ 222	7.22 $\pm$ 0.85	6.2 $\pm$ 0.8	--
PO	20	664 $\pm$ 247	8.7 $\pm$ 3.1	20.7 $\pm$ 5.7	NR	17400 $\pm$ 6900	--	--	36.9 $\pm$ 12.4 <sup>a</sup>

NR = Not reported due to extrapolation &gt;20% in 2 of 3 dogs.

<sup>a</sup> F was calculated based on AUC(0-48 hr) for PO data.**Summary:**

- PD 0332991 has a low clearance in dogs (~21% of hepatic blood flow)
- A large apparent volume of distribution suggested extensive tissue distribution of PD 0332991 in dogs;
- The mean terminal elimination half-life was 10.8 hours following IV administration, and 20.7 hours following oral administration;
- C<sub>max</sub> occurred around 9 hours after oral dosing;
- The absolute oral bioavailability of PD 0332991-0054 calculated by the Applicant using extrapolation method was 36.9%.

**Study title:** Single dose pharmacokinetics and oral bioavailability of PD 0332991 in male Cynomolgus monkeys following intravenous administration of PD 0332991-0002C and oral administration of PD 0332991-0054

**Study no.:** RR 764-04200 (research report)**Volume #, and page #:** electronic submission, Module 4, page 1-12**Conducting laboratory and location:** (b) (4)**Date of study initiation:** November 2001**GLP compliance:** no**QA report:** yes ( ) no (x )

**Drug, lot #, and % purity:** PD 0332991-0002C (IV), PD 0332991-0054 (oral)  
 Lot number: P  
 Purity: 100% (IV), 99.18% (oral)

**Methods**

LC/MS/MS analytical method was used to determine the pharmacokinetic profile of PD 0332991 following intravenous (IV) administration of PD 0332991-0002C and oral (PO) administration of PD 0332991-0054 to male cynomolgous monkeys.

Species/strain: male cynomolgous monkeys.

#/sex/group: n=3

Schedule: single

Doses in administered units:

IV-0.5mg/kg, 0.25 mL/kg in 5% DMA/25% propylene glycol/70% D5W ,  
 oral- 2.66 mg/kg, 0.83 mL/kg in 5% /95% PEG200/0.5% methylcellulose

Route: IV (5-minute infusion) or oral gavage

Blood samples collection:

IV- Predose (0), 0.083 (end of infusion), 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 hours

oral- Predose (0), 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 hours

The following tables are copied from the Applicant's submission.

	<b>Intravenous Infusion</b>	<b>Oral Gavage</b>
Species/Strain:	Cynomolgous monkeys	Cynomolgous monkeys
No. of Animals:	3	3
Crossover Design (Y/N):	Y	Y
Gender:	Male	Male
Dose (mg/kg):	0.5	5/2.66*
Single Dose (Y/N):	Y	Y
Sampling Times:	Predose (0), 0.083 (end of infusion), 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 hours	Predose (0), 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 hours
Fasted Overnight Before Dosing, 4 Hours Following Dosing:	Y	Y
Sample Storage:	-20°C	-20°C

\* Nominal/actual dose

## Results:

Table 16. The mean ( $\pm$ SD) PK parameters of PD 0332991 (copied from the applicant's submission)

Route	Dose (mg/kg)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	t <sub>1/2</sub> (hr)	AUC(0-∞) (ng·hr/mL)	CL (mL/min/kg)	V <sub>dss</sub> (L/kg)	F (%)
IV	0.5	--	--	4.7 $\pm$ 1.4	624 $\pm$ 43	13.4 $\pm$ 0.9	5.1 $\pm$ 1.0	--
PO	2.66	86 $\pm$ 31	2.7 $\pm$ 1.2	5.3 $\pm$ 0.9	768 $\pm$ 150	--	--	23.1 $\pm$ 3.6

## Summary:

- PD 0332991 has a low clearance in cynomolgous monkeys (~30% of hepatic blood flow)
- A large apparent volume of distribution suggested extensive tissue distribution of PD 0332991 in monkeys;
- The mean terminal elimination half-life was 4.7 hours following IV administration, and 5.3 hours following oral administration;
- C<sub>max</sub> occurred around 3 hours after oral dosing;
- The absolute oral bioavailability of PD 0332991-0054 calculated by the Applicant using extrapolation method was 23%.

## Distribution

**Study title:** Tissue Distribution of [<sup>14</sup>C]PD 0332991 in Long-Evans Male Rats

### Key Study Findings:

- Radioequivalents were widely distributed into tissues and fluids, particularly in uvea, meninges, bile, harderian gland, preputial gland, liver, lacrimal gland, lung, thyroid, and spleen, with levels consistently greater than those observed in blood radioequivalents from 5 to 15 h;

- Radioequivalents in most CNS tissues were below that observed for mean blood except for meninges, pituitary, choroid plexus, and CSF, which were anatomically located outside the blood brain barrier.
- In most tissues, at least 90% of the radioactivity was eliminated compared to the respective  $C_{max}$  values at 168 hours postdose.

**Study no:** DM Study Number: DM2005-0332991-001

**Volume #, and page #:** electronic submission, page 1-15

**Conducting laboratory and location:**

Department of Pharmacokinetics, Dynamics and Metabolism  
Pfizer Global Research and Development  
Pfizer Inc,  
Groton, Connecticut 06340

**Date of study initiation:** not provided

**GLP compliance:** no

**QA report:** yes ( ) no (x )

**Drug, lot #, radiolabel, and % purity:** [ $^{14}\text{C}$ ]PD 0332991

Lot No.: 100342-019-ZJ000A

Purity: > 99%

**Formulation/vehicle:** a sterile, isotonic, ready to administer solution,  
no other details provided.

**Methods:** [ $^{14}\text{C}$ ]PD 0332991 radioequivalents were evaluated in 50 tissues and 4 fluids at 2.5, 5, 7.5, 10, 15, 24, 72, and 168 h following a single oral dose of [ $^{14}\text{C}$ ]PD 0332991 to male rats. Drug radioequivalents (mg eq/g) were calculated by averaging tissue concentrations measured at different sectioning levels and/or from replicate cryosections obtained from the same sectioning level.

**Species/strain:** Male Long-Evans rats

**# /time point:** 1 /time point

**Weight:** 241 - 256 g

**Doses in administered units:**  $20.3 \pm 0.5$  mg/kg and  $306 \pm 7$  mCi/kg

**Route, form, volume:** oral, [ $^{14}\text{C}$ ]PD 0332991 in sterile water at dose volume of  $1.08 \pm 0.03$  mL

**Results:** The following figures and tables are copied from Applicant's submission.

Table 17. Tissue concentrations of drug radioequivalents (mg eq/g) after oral administration of [<sup>14</sup>C]PD 0332991 (20.3 mg/kg) to Long-Evans male rats

Tissue	2.5 h	5 h	7.5 h	10 h	15 h	24 h	72 h	168 h
Adipose: Brown	3.1	6.0	3.1	1.7	0.70	0.28	---- <sup>b</sup>	----
Adipose: White	0.58	1.4	0.46	0.43	0.21	----	----	----
Adrenal Gland	17	25	7.3	11	1.8	0.69	----	----
Bile	90	121	82	39	----	----	----	----
Blood: Hepatic	2.2	3.0	1.3	0.78	0.32	0.12	----	----
Blood: Myocardial	0.71	1.6	0.72	0.42	0.16	----	----	----
Blood: Vena Cava	0.71	1.4	0.61	0.42	0.17	----	----	----
<b>Mean Blood</b>	<b>1.20</b>	<b>1.99</b>	<b>0.89</b>	<b>0.54</b>	<b>0.22</b>	----	----	----
<b>SD Blood</b>	<b>0.70</b>	<b>0.71</b>	<b>0.32</b>	<b>0.17</b>	<b>0.07</b>	----	----	----
Bone Marrow: Sternum	5.1	16	6.2	5.0	0.77	0.30	----	----
Bone Marrow: Vertebral	4.4	10	4.8	3.3	0.83	0.18	----	----
Buccal Gland	5.0	15	6.2	4.4	0.79	0.21	----	----
Cerebellum	----	0.14	----	----	----	----	----	----
Cerebrospinal Fluid	1.5	2.4	1.4	1.3	0.40	----	----	----
Cerebrum	----	0.15	----	0.15	----	----	----	----
Choroid Plexus	1.5	3.0	1.7	1.4	1.5	0.19	----	----
Epididymis	1.7	1.7	1.3	4.9	0.71	0.19	----	----
Harderian Gland	6.6	28	28	21	13	9.1	0.70	0.15
Intervertebral Disc	0.39	1.1	0.70	0.48	0.21	----	----	----
Kidney	10	24	9.3	6.0	1.7	0.70	0.17	----
Lacrimal Gland: Exorbital	4.5	30	13	10	2.2	0.80	0.20	0.14
Lacrimal Gland: Intraorbital	6.7	32	26	17	4.5	1.2	0.82	0.15
Liver	19	32	13	8.7	3.1	1.4	0.46	0.19
Lung	13	43	14	8.2	1.7	0.44	----	----
Lymph Node	4.7	14	10	7.3	1.9	0.66	0.12	----
Medulla Oblongata	0.11	0.16	----	0.12	----	----	----	----
Meninges	2.4	5.1	9.2	6.8	11	8.4	5.2	4.4
Mesencephalon	----	0.16	----	0.13	----	----	----	----
Mucosa: Gastric	4.7	15	5.6	2.0	0.40	0.43	----	----
Mucosa: Intestinal	4.3	12	9.4	2.2	1.2	0.29	----	----
Muscle	1.3	3.5	1.4	0.94	0.18	----	----	----
Myocardium	3.1	6.1	2.8	1.8	0.41	----	----	----
Olfactory Bulb	----	----	----	0.20	----	----	----	----
Pancreas	6.0	14	5.2	3.4	0.86	0.29	----	----
Parotid Gland	5.2	14	3.8	3.7	0.65	----	----	----
Pineal Gland	5.5	11	7.0	3.3	----	----	----	----
Pituitary	6.1	20	13	8.2	3.3	----	----	----
Pons	0.12	0.17	----	----	----	----	----	----
Preputial Gland	5.5	20	11	10	5.4	2.8	dnos <sup>c</sup>	3.6
Prostate	4.3	14	17	6.3	3.1	0.55	----	----
Renal Cortex	9.8	21	8.9	5.4	1.7	0.63	0.18	----
Renal Medulla	12	26	11	7.2	2.0	1.1	0.16	----

Tissue	2.5 h	5 h	7.5 h	10 h	15 h	24 h	72 h	168 h
Seminal Vesicle	1.3	3.8	2.3	1.3	0.43	0.12	----	----
Skin: Flank	1.4	5.0	3.1	2.0	1.1	3.8	0.22	----
Spinal Cord	----	0.18	----	0.10	----	----	----	----
Spleen	12	33	14	9.9	2.1	0.71	0.18	----
Sublingual Salivary Gland	6.3	23	7.1	5.2	0.92	0.31	----	----
Submaxillary Salivary Gland	7.7	24	10	6.8	1.1	0.36	----	----
Testis	0.43	1.2	1.1	1.3	0.98	0.65	0.24	----
Thoracic Duct Fluid	1.3	3.1	3.9	5.1	dnos	1.2	----	----
Thymus	3.3	12	6.9	5.2	1.3	0.36	----	----
Thyroid	7.6	25	8.9	13	2.1	1.1	0.21	----
Urine: Bladder	9.2	dnos	8.9	9.0	1.5	0.13	----	----
Urine: Renal Pelvis	8.4	24	4.3	5.4	0.76	----	----	----
Uvea	13	48	49	45	29	75	31	24
Vitreous Body	----	1.2	1.1	0.82	0.65	0.75	0.64	0.56
Whole-Body	24	26	29	21	16	0.89	0.24	0.12

<sup>a</sup>The lloq was 0.10 µg eq/g.

<sup>b</sup>-----: Drug radioequivalents in tissue were below the lloq.

<sup>c</sup>dnos: Did not obtain sample for tissue at this time point.

Table 18. Pharmacokinetics of [<sup>14</sup>C]PD 0332991 in Male Long-Evans Rats

Tissue	AUC <sub>(0-Tlast)</sub> (μg eq·h/g)	t <sub>1/2</sub> (h)	C <sub>max</sub> (μg eq/g)	T <sub>last</sub> (h)	T <sub>max</sub> (h)
Adipose: Brown	43	5.6	6	24	5
Adipose: White	8.1	6.4	1.4	15	5
Adrenal Gland	180	3.8	25	24	5
Bile	780	3.0	121	10	5
Blood: Hepatic	22	4.9	3	24	5
Blood: Myocardial	9.5	3.5	1.6	15	5
Blood: Vena Cava	8.9	4.0	1.4	15	5
Bone Marrow: Sternum	93	3.3	16	24	5
Bone Marrow: Vertebral	68	3.3	10	24	5
Buccal Gland	88	3.1	15	24	5
Cerebellum	0.18	---- <sup>b</sup>	0.14	5	5
Cerebrospinal Fluid	19	4.0	2.4	15	5
Cerebrum	0.55	----	0.15	10	5
Choroid Plexus	32	5.2	3.0	24	5
Epididymis	36	3.1	4.9	24	10
Harderian Gland	640	21	28	168	5
Intervertebral Disc	7.8	4.2	1.1	15	5
Kidney	170	19	24	72	5
Lacrimal Gland: Exorbital	220	45	30	168	5
Lacrimal Gland: Intraorbital	300	37	32	168	5
Liver	300	53	32	168	5
Lung	220	3.3	43	24	5
Lymph Node	140	15	14	72	5
Medulla Oblongata	0.83	----	0.16	10	5
Meninges	960	132	11	168	15
Mesencephalon	0.57	----	0.16	10	5
Mucosa: Gastric	77	3.7	15	24	5
Mucosa: Intestinal	82	4.7	12	24	5
Muscle	19	2.4	3.5	15	5
Myocardium	38	2.6	6.1	15	5
Olfactory Bulb	0.25	----	0.2	10	10
Pancreas	83	3.9	14	24	5
Parotid Gland	72	2.4	14	15	5
Pineal Gland	62	2.9	11	10	5
Pituitary	140	3.9	20	15	5
Pons	0.5	----	0.17	5	5
Preputial Gland	420	113	20	168	5
Prostate	140	3.9	17	24	7.5
Renal Cortex	150	20	20	72	5
Renal Medulla	200	16	26	72	5

Tissue	AUC <sub>(0-T<sub>last</sub>)</sub> (µg eq·h/g)	t <sub>1/2</sub> (h)	C <sub>max</sub> (µg eq/g)	T <sub>last</sub> (h)	T <sub>max</sub> (h)
Sublingual Salivary Gland	120	3.1	23	24	5
Submaxillary Salivary Gland	140	3.1	24	24	5
Seminal Vesicle	27	3.8	3.8	24	5
Skin: Flank	150	18	5.0	72	5
Spinal Cord	0.58	-----	0.18	10	5
Spleen	220	18	33	72	5
Testis	43	30	1.3	72	10
Thymus	87	3.7	12	24	5
Thyroid	206	18	25	72	5
Uvea	6200	97	75	168	24
Vitreous Body	108	-----	1.2	168	5
Whole-Body	440	54	29	168	7.5

<sup>a</sup> AUC<sub>(0-T<sub>last</sub>)</sub> values were calculated using linear trapezoidal approximation. The t<sup>1/2</sup> was calculated as 0.693/K<sub>el</sub>.

<sup>b</sup> -----The t<sub>1/2</sub> was not determined (nd) because a definitive elimination phase was not discernible.

### Summary:

- [<sup>14</sup>C]PD 0332991 radioequivalents distributed to 44 tissues at 2.5 h, 49 tissues at 5 h, 47 tissues at 7.5 h, 47 tissues at 10 h, 41 tissues at 15 h, 33 tissues at 24 h, 15 tissues at 72 h, and 8 tissues at 168 h;
- C<sub>max</sub> occurred in 44 tissues at 5 h;
- A decrease in the number of tissues presenting radioequivalents occurred from 5 to 168 h;
- By 168 h [<sup>14</sup>C]PD 0332991 radioactivity were still present in 8 tissues;
- Concentrations of [<sup>14</sup>C]PD 0332991 radioequivalents attained C<sub>max</sub> at 5 h for liver, myocardium and vena cava blood; [<sup>14</sup>C]PD 0332991 radioequivalents were sustained in myocardial and venous blood for at least 15 h and hepatic blood to 24 h;
- Concentrations of [<sup>14</sup>C]PD 0332991 radioequivalents in most CNS tissues except for cerebrospinal fluid, choroid plexus, meninges, and pituitary were below the concentration to those observed for mean blood over the time course of 2 to 15 h.

**Study title:** Protein binding of PD-0332991 in rabbit plasma**Key Study Findings:**

In pooled rabbit plasma at a concentration of 1  $\mu\text{M}$  and at 37°C for 4 hours, mean binding of PD-0332991 to rabbit plasma proteins, expressed as fraction unbound, was 0.0733.

**Study no:** 141526

**Volume #, and page #:** electronic submission, page 1-9

**Conducting laboratory and location:**

Pharmacokinetics, Dynamics and Metabolism, Pfizer, Groton,  
CT, US

**Date of study initiation:** June 10, 2013

**GLP compliance:** no

**QA report:** yes ( ) no (x )

**Drug, lot #, radiolabel, and % purity:** PF0080665-73

Lot No.: PF0080665-73-0007

Purity: > 99%

**Methods:** PD-0332991 (PF-00080665) was incubated with plasma from female rabbits at a concentration of 1  $\mu\text{M}$  (equivalent to 0.448  $\mu\text{g/mL}$ ) at 37°C for 4 hours. After the incubation, the samples were protein precipitated with an acetonitrile solution, and the supernatant was transferred to a new plate and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure the fraction unbound of PD-0332991 (PF-00080665).

**Results:** The table is copied from Applicant's submission.

Table 19. Mean fraction unbound (Fu) of PD-0332991 in rabbit plasma

Species	Gender	n	Tested Concentration		Fraction Unbound			
			$\mu\text{M}$	$\mu\text{g/mL}$	Geometric Mean	%CV	95% Confidence Interval	
							Lower	Upper
Rabbit	Female	12	1	0.448	0.0733	22.7	0.0634	0.0846

Fraction unbound values are calculated from n=12 individual replicates (4 replicates on each of 3 days).

Calculated molecular weight = 447.5 g/mole.

Abbreviations: CV = Coefficient of variation; n = Number of replicates.

**Study title:** Protein binding of PF-05089326 in mouse, rat, dog and human plasma

Note: PF-05089326 is the lactam metabolite of PD-0332991.

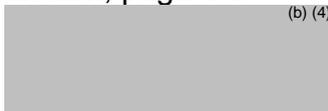
**Key study findings:**

Extent of plasma protein binding was concentration-independent over the concentration range evaluated in this study; the fraction unbound ranged from 0.035 to 0.042 in mice, from 0.044 to 0.051 in rats, from 0.24 to 0.56 in dogs, and 0.046 to 0.053 in humans.

**Study no:** 104347

**Volume #, and page #:** electronic submission, page 1-9

**Conducting laboratory and location:**



**Date of study initiation:** June 3, 2008

**GLP compliance:** yes

**QA report:** yes (x) no ( )

**Drug, lot #, radiolabel, and % purity:** PF-05089326

Lot No.: PF-05089326-00-0001

Purity: not provided

**Methods:** The extent of *in vitro* binding of PF-05089326 to plasma proteins from mice, rats, dogs and humans was conducted with the concentrations of PF-05089326 at 0.05, 0.25 and 1  $\mu\text{M}$  (equivalent to 0.0231, 0.115 and 0.462  $\mu\text{g/mL}$ , respectively) and determined by equilibrium dialysis.

**Results:** The following tables were copied from the Applicant's submission.

Table 20. Mean fraction unbound ( $F_u$ ) of PF-05089326 in mouse, rat, dog and human plasma

Species (Strain)	Gender	Wells (n)	Concentration Tested		Fraction Unbound (Mean $\pm$ SD)
			$\mu\text{M}$	$\mu\text{g/mL}$	
Mouse	Male and Female	3	0.05	0.0231	0.0350 $\pm$ 0.0015
Mouse	Male and Female	3	0.25	0.115	0.0418 $\pm$ 0.0011
Mouse	Male and Female	3	1	0.462	0.0398 $\pm$ 0.0014
Rat (Wistar Han)	Male and Female	3	0.05	0.0231	0.0439 $\pm$ 0.0056
Rat (Wistar Han)	Male and Female	3	0.25	0.115	0.0495 $\pm$ 0.0012
Rat (Wistar Han)	Male and Female	3	1	0.462	0.0514 $\pm$ 0.0019
Dog (Beagle)	Male and Female	3	0.05	0.0231	0.242 $\pm$ 0.0093
Dog (Beagle)	Male and Female	3	0.25	0.115	0.266 $\pm$ 0.0361
Dog (Beagle)	Male and Female	3	1	0.462	0.256 $\pm$ 0.0055
Human	Male and Female	3	0.05	0.0231	0.0467 $\pm$ 0.0010
Human	Male and Female	3	0.25	0.115	0.0530 $\pm$ 0.0008
Human	Male and Female	3	1	0.462	0.0474 $\pm$ 0.0012

Calculated molecular weight for PF-05089326 = 461.5 g/mol.

n = Number of determinations; SD = Standard deviation.

**Study title:** In vitro protein binding of PD 0332991 to plasma proteins of mouse, rat, dog, and human

**Key study findings:**

PD 0332991 protein binding in mouse and rat plasma was similar to that in human plasma. PD 0332991 was approximately 3x more free in dog plasma than in human plasma.

**Study no:** RR 764-04174

**Volume #, and page #:** electronic submission, page 1-14

**Conducting laboratory and location:** Pharmacokinetics, Dynamics, & Metabolism  
Pfizer global research & development  
Ann arbor laboratories  
Ann arbor, Michigan

**Date of study initiation:** September 23, 2002

**GLP compliance:** no

**QA report:** yes ( ) no (x )

**Drug, lot #, radiolabel, and % purity:** PD 0332991, PD 0332991-0054, A548  
Lot No.: P  
Purity: 99.18%

**Methods:** To determine the extent of in vitro binding of PD 0332991 to mouse, rat, dog, and human plasma and to human serum albumin (HSA) and  $\alpha$ 1-acid glycoprotein (AGP), plasma and protein solutions were fortified with PD 0332991 to achieve concentrations of 0.5, 1, 2.5, and 5  $\mu$ g/mL free base equivalents. After incubation at 37°C for 6 or 24 hours, PD 0332991 concentrations were determined by HPLC/MS/MS

**Results:** The following tables were copied from the Applicant's submission.

Table 21. Summary of PD 0332991 Protein Binding Data

Species or Protein	Mean % Free <sup>a</sup>	Mean % Bound <sup>a</sup>
Mouse	13.3 - 18.8%	81.2 - 86.7%
Rat	12.2 - 12.7%	87.3 - 87.8%
Dog	38.3 - 45.3%	54.7 - 61.7%
Human	13.7 - 16.1%	83.9 - 86.3%
HSA	47.5 - 73.5%	26.5 - 52.5%
AGP	58.3 - 72.4%	27.6 - 41.7%

<sup>a</sup> Values are lowest to highest across concentration range tested, not necessarily in order.

**Summary:** PD 0332991 protein binding was independent of drug concentration over a concentration range of 0.5 to 5  $\mu$ g/mL. Unbound PD 0332991 ranged from 12% to 19% in mice, rats, and humans, and ranged from 38 to 45% in dogs.

**Note:** The study results suggested that HSA and AGP does not account for the total binding in human plasma; therefore, other components, such as free fatty acids, may be contributing to the plasma binding of PD 0332991.

**Study title:** Definitive red blood cell (RBC) distribution of PD 0332991-0054 in whole blood of mouse, rat, dog, monkey, and human

**Key study findings:**

PD 0332991 preferentially distributes to red blood cells, relative to plasma, with Kp of 2.4 in humans. PD 0332991 is equally distributed between plasma and red blood cells with Kp ~1 in mice, rat, dog, and monkey.

**Study no:** RR 764-04302

**Volume #, and page #:** electronic submission, page 1-6

**Conducting laboratory and location:** Pharmacokinetics, Dynamics, & Metabolism  
Pfizer global research & development  
Ann arbor laboratories  
Ann arbor, Michigan

**Date of study initiation:** June 15, 2003

**GLP compliance:** no

**QA report:** yes ( ) no (x)

**Drug, lot #, radiolabel, and % purity:** PD 0332991-0054, PD 0332991-0000  
Lot No.: P  
Purity: 99.18%

**Methods:** To determine the RBC distribution of PD 0332991-0054 in whole blood of the mouse, rat, dog, monkey, and human. PD 0332991-0054 was incubated at 37°C with fresh whole blood from each specie to yield 2.5 µg/mL concentration for 2 hours. At the end of incubation, plasma separated from whole blood was analyzed by HPLC/MS/MS.

The Kp (partition coefficient) is determined by the following equation:

$$K_p = \frac{C_{RBC}}{C_p} = \frac{\left[ \left( \frac{C_b}{C_p} \right) - (1 - H) \right]}{H}$$

Where:  $C_{RBC}$  = Compound concentration in RBCs.  
 $C_p$  = Measured concentration in the plasma spun from whole blood.  
 $C_b$  = Measured concentration in the whole blood.  
 $H$  = Hematocrit value (expressed as a fraction).

**Results:** The following tables were copied from the Applicant's submission.

Table 22. Red blood cell distribution of PD 0332991-0054 at a nominal concentration of 2.5 µg/mL

Species	Replicate	C <sub>b</sub> (µg/mL)	C <sub>p</sub> (µg/mL)	Hematocrit (2 hr)	C <sub>b</sub> /C <sub>p</sub>	K <sub>p</sub>
Mouse	1	2280	2070	0.35		
	2	1966	2010	0.35		
	3	2640	2030	0.35		
	Mean	2295	2037	0.35	1.13	1.36
	SD	337.3	30.6			
	%CV	14.7	1.5			
Rat	1	2400	2550	0.51		
	2	2340	2530	0.51		
	3	2640	2540	0.51		
	Mean	2460	2540	0.51	0.97	0.94
	SD	158.7	10.0			
	%CV	6.5	0.4			
Dog	1	2420	2380	0.49		
	2	2400	2420	0.49		
	3	2420	2440	0.48		
	Mean	2413	2413	0.49	1.00	1.00
	SD	11.5	30.6			
	%CV	0.5	1.3			
Monkey	1	1528	1390	0.45		
	2	1380	1580	0.46		
	3	1490	1300	0.45		
	Mean	1466	1423	0.45	1.04	1.09
	SD	76.9	142.9			
	%CV	5.2	10.0			
Human	1	2560	1560	0.44		
	2	2460	1490	0.44		
	3	2360	1470	0.44		
	Mean	2460	1507	0.44	1.63	2.44
	SD	100.0	47.3			
	%CV	4.1	3.1			

**Summary:** The K<sub>p</sub> (partition coefficient) values for mouse, rat, dog, monkey, and human are: 1.36, 0.94, 1.0, 1.09, and 2.44, respectively.

**Metabolism**

**Study title:** Reaction phenotyping of PD-0332991 using human hepatocyte relay method

**Key Study Findings:**

PD-0332991 was mainly metabolized by CYP family, and CYP3A was primarily responsible for the oxidative metabolism of PD-0332991.

**Study number:** 095443

**Volume #, and page #:** Electronic submission; page 1-7

**Conducting laboratory and location:** Pharmacokinetics, Dynamics and Metabolism, Pfizer, Groton, CT, USA

**Date of study initiation:** not provided,

The approval of this research report was signed on April 4, 2013

**GLP compliance:** no,

**QA report:** yes ( x ) no (x)

**Drug, lot #, radiolabel, and % purity:** PD-0332991  
Lot: PF-00080665-73-0006  
Purity: not provided

**Methods:**

Human hepatocyte relay experiment was conducted using pooled human hepatocytes from 10 donors and chemical inhibitors of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A and a pan-CYP inhibitor ABT. The reaction mixture (500 µL) containing the individual inhibitors (see the table below, copied from the Applicant's submission), PD-0332991 or probe substrate positive controls, HHEP (0.5 million cells/mL) in WEM buffer (pH 7.4) were incubated at 37°C, 5% CO<sub>2</sub> and 75% RH. PD-0332991 concentrations were determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

## Preparation of Inhibitor Stock Solution

Enzymes	Inhibitors	1 <sup>st</sup> Stock Concentration (mM)	2 <sup>nd</sup> Stock Concentration (mM)	Final Concentration (µM)
1A2	Furafylline	1	0.1	1
2B6	Clopidogrel	30	3	30
2C8	Gemfibrozil Glucuronide	30	10	100
2C9	Tienilic acid	10	1	10
2C19/2B6	Ticlopidine	10	1	10
2D6	Paroxetine	2	0.2	2
3A	Mibefradil	10	1	10
Pan-CYP	1-Aminobenzotriazole	100	100	1000

**Results:** The Applicant only presented the summary table. The following summary tables are copied from the Applicant's submission.

Table 23. HHEP relay phenotyping results of PD-0332991

CYP Enzymes	Selective CYP Probe Substrate	Inhibitor (Positive Control)	% Contribution (Positive Control)	CL <sub>int,app,5</sub> (mL/min/kg) PD-0332991	% Contribution PD-0332991
HHEP	-	-	-	11.8	-
1A2	Tizanidine	Furafylline (1 µM)	81	14.1	-
2C9	Tolbutamide	Tienilic acid (10 µM)	>74	12.1	-
2D6	Timolol	Paroxetine (2 µM)	68	12.3	-
3A	Disopyramide	Mibefradil (10 µM)	>70	7.75	34
Pan-CYP	Disopyramide	ABT (1 mM)	>70	3.34	71
HHEP	-	-	-	16.0	-
2B6	Efavirenz	Clopidogrel (30 µM)	77	15.1	-
2C8	Rosiglitazone	Gemfibrozil Glucuronide (100 µM)	64	15.1	-
HHEP	-	-	-	17.3	-
2C19/2B6	S-Mephenytoin	Ticlopidine (10 µM)	78	18.2	-

**Summary:** The total CYP contribution to PD-0332991 metabolism is 71% based on the ABT inhibition data, leaving 29% of the metabolism of PD-0332991 via non-CYP mediated pathways. CYP3A contribution to PD-0332991 metabolism is 34%.

**Note:** It was stated in the submission that contributions from the other six CYPs (1A2, 2B6, 2C8, 2C9, 2C19 and 2D6) could not be determined quantitatively using this method. This may be attributable to the relatively minor contribution from each individual CYP isoform to the overall metabolism of PD-0332991.

**Study title:** Metabolism of PD-0332991 in rat, dog and human hepatocytes

**Key Study Findings:**

- The metabolites of PD-0332991 yielded after incubation of PD-0332991 with human hepatocytes were observed when PD-0332991 was incubated with rat hepatocytes.

**Study number:** 193503 (Report Number)

**Volume #, and page #:** Electronic submission; page 1-25

**Conducting laboratory and location:** Pharmacokinetics, Dynamics and Metabolism, Pfizer, Groton, CT, USA

**Date of study initiation:** not provided,

The approval of this research report was signed November 22, 2013

**GLP compliance:** no,

**QA report:** yes ( x ) no (x)

**Drug, lot #, radiolabel, and % purity:** not provided

**Methods:**

Cryopreserved hepatocytes from rat, dog and human were incubated at 37°C for 3 hours with PD-0332991 at a final concentration of 10 µM. The metabolites of PD-0332991 in hepatocyte incubations were detected with LC/MS techniques using an OrbiTrap mass spectrometer.

**Results:** The following summary table is copied from the Applicant's submission.

Table 24. Summary of metabolites observed following incubation of PD-0332991 in rat, dog and human hepatocytes

Molecular Ion (MH <sup>+</sup> )	Metabolites	Rat	Dog	Human
448	PD-0332991	Yes	Yes	Yes
464	M23e	Yes		
464	M23a	Yes		Yes
624	M22	Yes		Yes
422	M32	Yes	Yes	Yes
464	M23c	Yes		Yes
464	M23d	Yes		
446	M28			Yes
450	M14	Yes		Yes
462	M17	Yes		Yes
464	M23f	Yes		
528	M11	Yes		Yes
490	M12	Yes		

**Study title:** Metabolism and excretion of PD-0332991 IN Sprague Dawley rats following a single oral dose of 50 mg/kg [<sup>14</sup>C]PD-0332991 and identification of circulating and excretory metabolites

**Key Study Findings:**

- PD-0332991 was well absorbed and extensively metabolized as evidenced by approximately 4% of the dose identified as PD-0332991 in feces from both sexes.
- Sulfation of PD-0332991 was the primary metabolic clearance mechanism as M11 (the sulfamic acid of PD-0332991) the major metabolism in feces;
- M12 and M14 are the two major circulating metabolites.

**Study number:** 135758

**Volume #, and page #:** Electronic submission; page 1-43

**Conducting laboratory and location:**

Department of Pharmacokinetics, Dynamics and Metabolism  
Pfizer Worldwide Research and Development  
Pfizer Inc., Groton, Connecticut 06340

**Methods:** Samples of fecal homogenates, urine, bile, and plasma were obtained from (b) (4) Study Identification: 8273768). Refer to the Extraction section for the study methods. Liquid Chromatography-Mass Spectrometry was used for the analysis and quantitative assessment of excreted metabolites.

**Results:** Mass Balance and routes of excretion was evaluated in this study, but only the metabolic profiles in feces, urine, bile, and plasma, following a single 50 mg/kg (100 µCi/kg) oral dose of [<sup>14</sup>C]PD-0332991 administered to Sprague Dawley rats is summarized below.

Metabolic Profiles

The following tables are copied from the Applicant's submission.

Table 25. Q uantitative assessment of drug-derived material in rat feces as a percentage of administered dose

Drug substance	m/z	% of Dose Excreted in Feces	
		Male	Female
PD-0332991	448	3.6	4.2
M11	528	37.9	75.4
M12	490	3.3	1.5
M14	450	0.8	nd
M16	437	1.4	0.5
M17	462	1.2	0.2
M24	476	NQ	nd
M25	287	2.5	1.0
M26	476	NQ	0.5

nd = Not detected; NQ = detected by MS, not not quantitated by radioactivity.

Table 26. Quantitative assessment of drug-derived material in rat urine as a percentage of administered dose

Metabolites	m/z	% of Dose Excreted in Urine	
		Male	Female
PD-0332991	448	2.1	NQ
M11	528	0.1	NQ
M12	490	nd	nd
M14	450	nd	nd
M16	437	0.4	nd
M17	462	0.2	nd
M22	624	NQ	NQ
M23a/M23b	464	1.0	nd
M24	476	NQ	nd
M25	287	0.05	nd

nd = Not detected; NQ = detected by MS, not not quantitated by radioactivity.

Table 27. Quantitative assessment of drug-derived material in rat bile as a percentage of recovered radioactivity in bile

#### 8.5. Quantitative assessment of drug-derived material in rat bile as a percentage of recovered radioactivity in bile

Drug substance	m/z	% of radioactivity in bile	
		Male	Female
PD-0332991	448	<3.8	nd
M11	528	54.5	68.9
M12	490	3.6	nd
M14	450	nd	nd
M16	437	2.6	nd
M17	462	nd	nd
M22	624	nd	nd
M24	476	NQ	nd
M25	287	NQ	NQ

nd = Not detected; NQ = detected by MS, not not quantitated by radioactivity.

Table 28. Quantitative assessment of circulating drug-derived material in rat plasma as a percentage of circulating AUC of radioactivity

Drug substance	m/z	% of Radioactivity in Plasma	
		Male	Female
PD-0332991	448	40.3	NQ
M11	528	0.4	nd
M12	490	9.0	NQ
M14	450	9.6	NQ
M16	437	1.4	nd
M17	462	1.8	nd
M25	287	NQ	nd
M26	476	1.3	nd

nd = Not detected; NQ = detected by MS, not not quantitated by radioactivity.

**Summary:** In male and female rats, M11 constituted 37.9 % and 75.4% of the administered dose recovered in the feces. PD-0332991 is the primary drug-related material in circulation constituting approximately 42% of the radioactivity in the plasma AUC pool over 24 hours in the male rat. M12 and M14 are the two major circulating metabolites and constitute approximately 9% each to the circulating radioactivity.

**Note:** M11 is the sulfamic acid of PD-0332991, M12 is the acetamide of PD-0332991, and M14 is the reduced keto carbonyl of PD-0332991.

**Study title:** Metabolism of PD-0332991 following a single oral dose of 1.97 mg/kg [<sup>14</sup>C]PD-0332991 in beagle dog

**Key study findings:**

- Oxidation was the primary route of metabolism in the dog;
- The carboxylic acid (M16) was the major metabolite in the excreta;
- PD-0332991 was the primary drug-related material in circulation.

**Study no:** 204619

**Volume #, and page #:** electronic submission, page 1-30

**Conducting laboratory and location:** Pharmacokinetics, Dynamics and Metabolism, Pfizer, Groton, CT

**Date of study initiation:** not provided, the approval of the study report was signed on October 30, 2013

**GLP compliance:** no,

**QA report:** yes ( ) no (x)

**Drug, lot #, radiolabel, and % purity:** [<sup>14</sup>C]PD-0332991  
Lot # CFQ41521  
Purity: not provided

**Formulation/vehicle:** not provided

**Methods:** Beagle dogs (2 males and 2 females) were administered [<sup>14</sup>C] PD-0332991 (1.97 mg/kg, 19.6 μCi/kg). Liquid chromatography-mass spectrometry was used for analysis and quantitative assessment of excreted metabolites in urine, feces, and plasma.

**Results:** Mass Balance and routes of excretion were evaluated in this study, but only the metabolic profiles in feces, urine, bile, and plasma, following a single oral dose of [<sup>14</sup>C]PD-0332991 at a dose of 1.97 mg/kg (19.6 μCi/kg) is summarized below. The following tables are copied from the Applicant's submission.

#### Metabolic Profiles

Table 29. Percentage of Pooled Urinary Metabolites (0-72 hr) of PD-0332991 in Beagle Dogs After Oral Administration of 1.97 mg/kg [<sup>14</sup>C]PD-0332991 (Expressed as % Of Dose)

Metabolites	Retention Time (min)	m/z	% of Dose Excreted in Urine <sup>1</sup>		
			Male	Female	Mean
M23a	23.5	464	0.4	0.8	0.6
M22	23.5	624	ND	ND	ND
M35	24.6	640	0.4	1.1	0.8
M23b	25.2	464	ND	ND	ND
PD-0332991	30.3	448	5.1	5.0	5.1
M16	39.2	437	0.5	0.6	0.6

<sup>1</sup> The percentage of dose excreted in the urine following oral administration of [<sup>14</sup>C]PD-0332991 was 9.13% and 9.91 % from males and females, respectively.

**Summary:** Unchanged PD-0332991 accounted for 5.1% and 5.0% of the dose in urine for male and female dogs, respectively.

Table 30. Percentage of pooled fecal metabolites (0-72 hr) of PD-0332991 in beagle dogs after oral administration of 1.97 mg/kg [<sup>14</sup>C]PD-0332991 (Expressed as % Of Dose)

Metabolites	Retention Time (min)	m/z	% of Dose Excreted in Feces <sup>1</sup>		
			Male	Female	Mean
M23a	23.3	464	3.52	1.72	2.62
	24.3	Unknown <sup>2</sup>	<1	<1	<1
M23b	25.5	464	<1	1.95	1.95
	27.8	Unknown <sup>2</sup>	<1	1.18	1.18
PD-00332991	30.3	448	4.76	2.63	3.70
M37	32.3	480	2.29	2.62	2.46
M23c	35.0	464	2.71	2.63	2.67
M23d	37.5	464	3.56	2.69	3.13
M16	39.8	437	13.7	15.9	14.8
	42.5	Unknown <sup>2</sup>	2.35	3.65	3.00
	51.0	Unknown <sup>2</sup>	2.69	2.38	2.535
	52.3	Unknown <sup>2</sup>	<1	1.24	1.24

<sup>1</sup> The percentage of dose excreted in the feces following oral administration of [<sup>14</sup>C]PD-0332991 was 75.1% and 79.1% from males and females, respectively.

<sup>2</sup> The metabolites labeled as unknown could not be identified. The molecular ion could not be discerned due to ion suppression due to fecal matter.

**Summary:** The major component in the feces of the male and female dogs was the carboxylic acid metabolite (M16), which accounted for 13.7 and 15.9% of the dose in the feces of the male and female dogs, respectively. Unchanged PD-0332991 accounted for 4.8 % and 2.6% of the dose in feces for male and female dogs, respectively, and the remaining identified and unknown metabolites represented ~2 to 4% of the dose.

Table 31. Percentage of circulating metabolites (0-24 hr) of PD-00332991 in Beagle Dogs

After Oral Administration of 1.97 mg/kg [<sup>14</sup>C]PD-00332991 (Expressed as % of Radioactivity)

Metabolites	Retention Time (min)	m/z	% of Radioactivity in Plasma		
			Male	Female	Mean
PD-00332991	30.3	448	99	91	95
M16	39.7	437	trace	trace	trace

*Summary:* PD-0332991 was the only major peak observed in both male and female dogs and accounted for 99 and 91% of the total circulating radioactivity respectively.

## Excretion

**Study title:** Absorption and Excretion of [<sup>14</sup>C]PD-0332991 Following Oral Administration to Rats

### Key Study Findings:

Fecal excretion and biliary excretion were the predominate routes of elimination [<sup>14</sup>C]PD-0332991 following oral administration in rats. Urinary excretion appeared to be a minor elimination route following oral administration.

**Study no:** 8273768

**Volume #, and page #:** electronic submission, page 1-57

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** October 9, 2012

**GLP compliance:** no

**QA report:** yes ( ) no (x )

**Drug, lot #, radiolabel, and % purity:** [<sup>14</sup>C]PD-0332991  
Lot No.: CFQ41521  
Purity: 97.6% (radiopurity)

**Formulation/vehicle:** a sterile, isotonic, ready to administer solution, no other details provided

**Methods:** Each rat received a single 50 mg/kg (approximately 100 µCi/kg) oral dose of [<sup>14</sup>C]PD-0332991. Rats were assigned to three groups for this study. At designated times following dosing, blood, urine, feces, and bile were collected (see table below) and analyzed for total radioactivity by liquid scintillation counting (LSC).

Group	Number of Animals		Dose Route	Target Dose Level (mg/kg)	Target Dose Volume (mL/kg)	Samples Collected
	Male	Female				
1	4	4	Oral	50	10	Blood
2	2	2	Oral	50	10	Urine, Feces, and Carcass
3	1	1	Oral	50	10	Bile, Urine, Feces, and Carcass

BDC Bile duct-cannulated.

Note: The dose was approximately 100  $\mu$ Ci/kg.

### Dosing:

Species/strain: Sprague Dawley rats

#animals in the study: 7/sex

Weight: 189 to 338 g

Age: 6 to 11 weeks of age

Doses in administered units: 50 mg/kg (approximately 100  $\mu$ Ci/kg)

Route, form, volume, and infusion rate: oral at dose volume of 10 mL/kg

**Results:** The following figures were excerpted from the Applicant's submission.

### Excretion and Mass Balance

Figure 30. Concentrations of radioactivity in plasma at specified times after a single oral administration of [ $^{14}$ C]PD-0332991 to male and female rats (Group 1, 50 mg/kg)

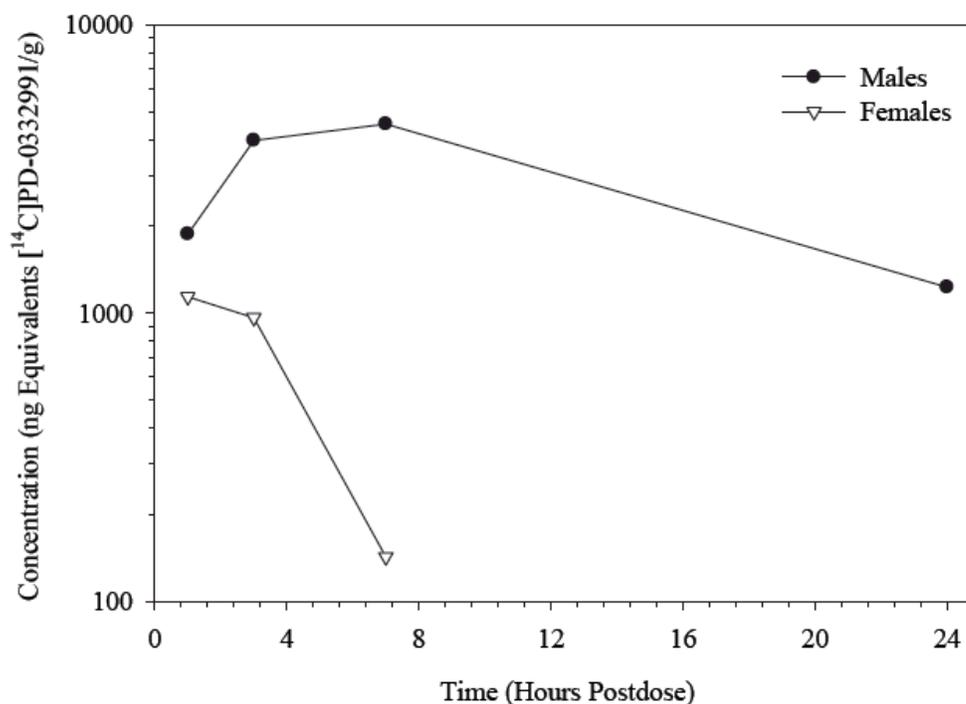
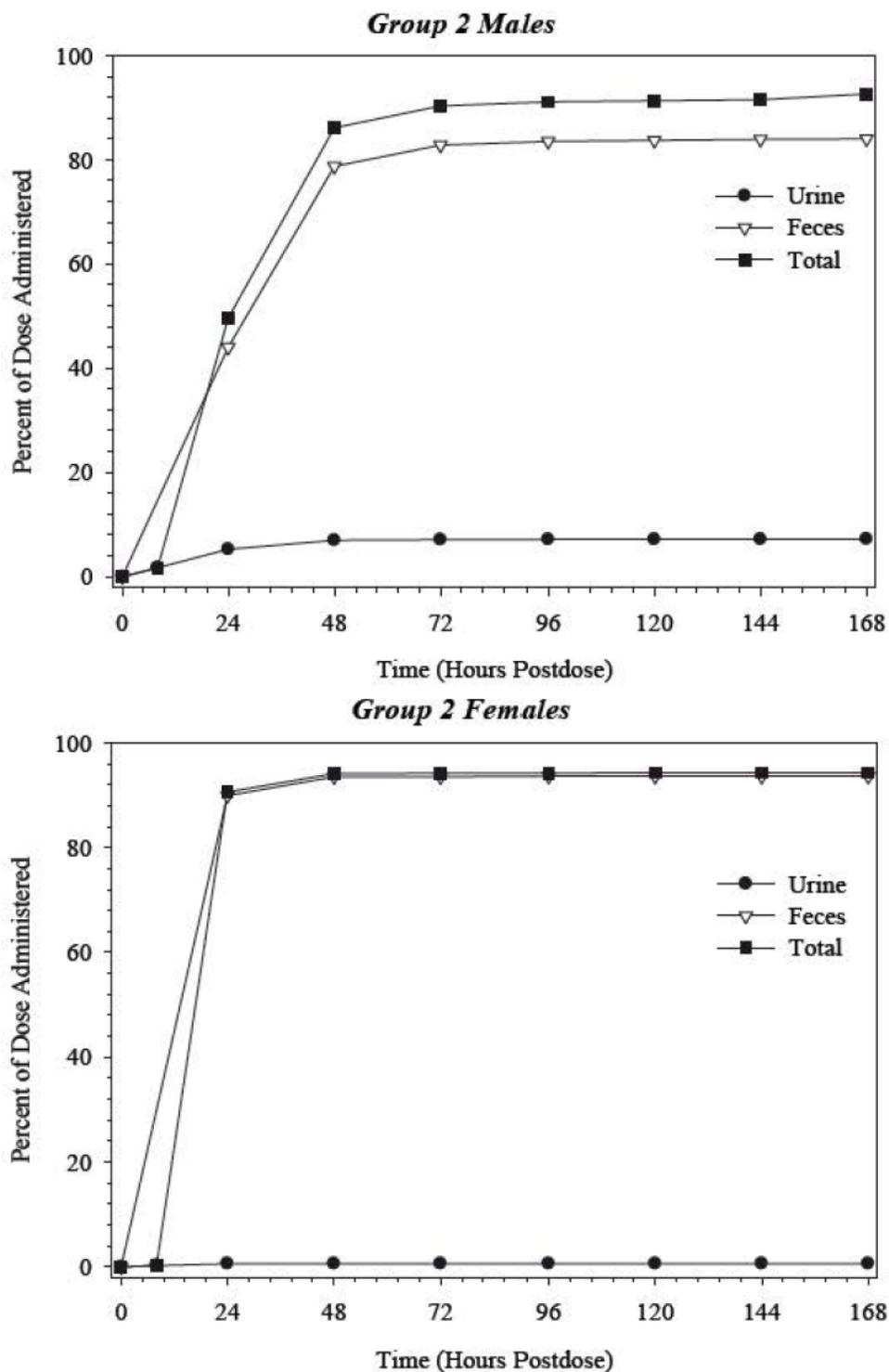
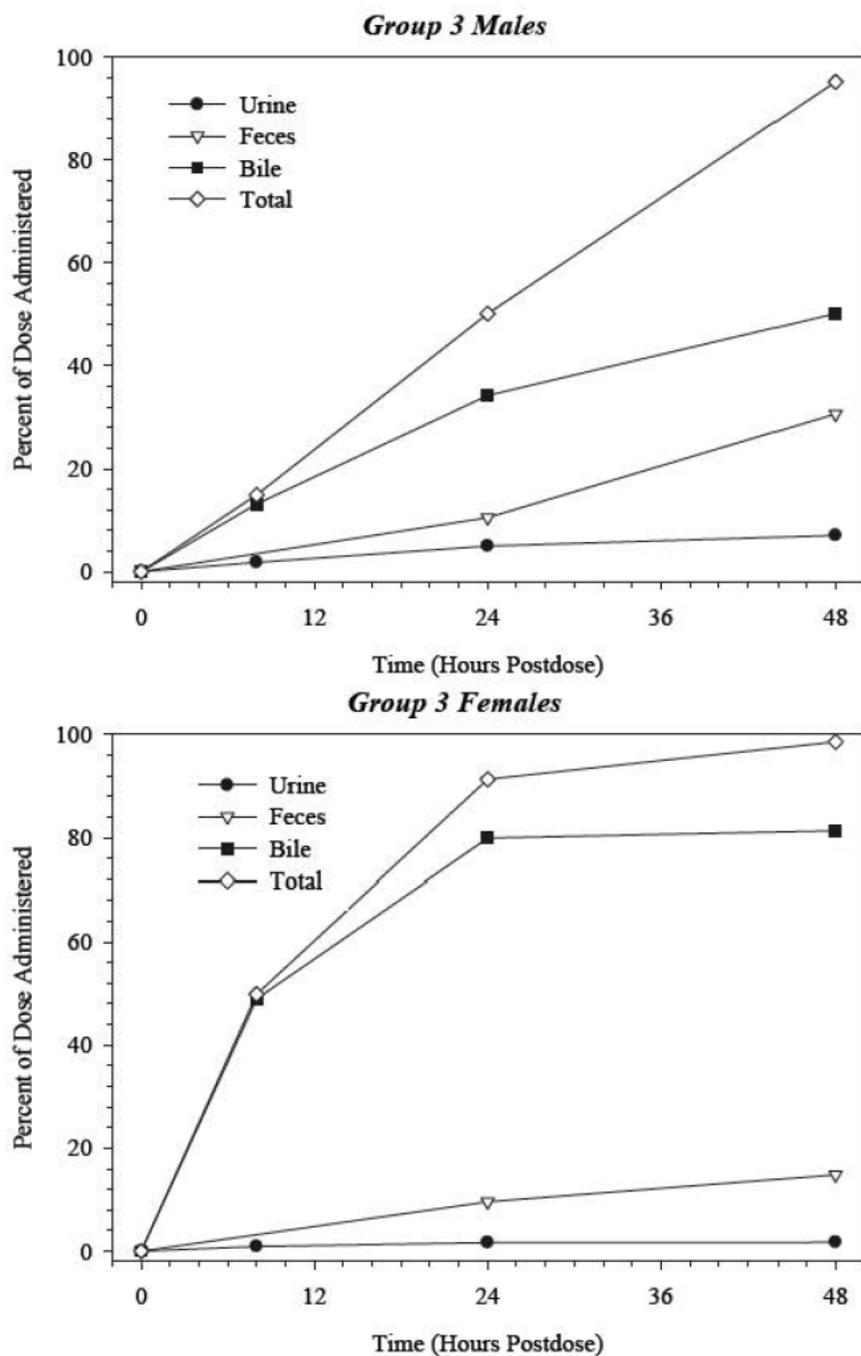


Figure 31. Cumulative percent of radioactive dose in urine and feces at specified intervals after a single oral administration of [<sup>14</sup>C]PD-0332991 to male or female rats (Group 2, 50 mg/kg)



Note: Total includes urine, feces, cage rinse, cage wash, and cage wipe.

Figure 32. Cumulative percent of radioactive dose in urine, feces, and bile at specified intervals after a single oral administration of [<sup>14</sup>C]PD-0332991 to male or female rats (Group 3, 50 mg/kg)



Note: Total includes urine, feces, bile, cage rinse, cage wash, cage wipe, bile cannula, and jacket rinse.

**Summary:** The exposure of total radioactivity was higher in males with plasma  $C_{max}$  approximately 4-fold higher when compared with female rats. The bulk of the radioactivity was excreted in feces within the first 72 or 24 hours and accounted for 82.8% and 89.9% of the dose for male and female rats, respectively. Urine excretion over these same intervals accounted for only 7.11% and 0.603% of the dose for male and female rats, respectively. Through 48 hours postdose, the last collection interval, the amount of the dosed radioactivity recovered in bile was 50.1% for males and 81.3% for females. Fecal recoveries of radioactivity over this same 48 hour interval were 30.6% for males and 14.8% for females.

Note: The high percentage of radioactivity in feces from intact rats and in bile from bile duct-cannulated (BDC) rats suggested that hepatic clearance and biliary excretion played an important role in the elimination of [ $^{14}C$ ]PD-0332991-derived radioactivity.

**Study title:** Absorption and Excretion of [ $^{14}C$ ]PD-0332991  
Following Oral Administration to Dogs

**Key Study Findings:**

Following oral administration of [ $^{14}C$ ]PD-0332991 to male and female dogs, the predominant excretion route of radioactivity occurred via feces. Excretion of radioactivity was essentially complete by 96 hours postdose for both males and females. Urinary excretion appeared to be a minor route of elimination.

**Study no:** 8276000

**Volume #, and page #:** electronic submission, page 1-51

**Conducting laboratory and location:**



**Date of study initiation:** December 5, 2012

**GLP compliance:** no

**QA report:** yes ( ) no (x)

**Drug, lot #, radiolabel, and % purity:** [ $^{14}C$ ]PD-0332991  
Lot No.: CFQ41521  
Purity: 97.6% (radiopurity)

**Formulation/vehicle:** a sterile, isotonic, ready to administer solution, no other details provided

**Methods:** Each dog received a single  $1.97 \pm 0.00577$  mg/kg ( $19.6 \pm 0.0577$   $\mu$ Ci/kg) oral dose of [ $^{14}C$ ]PD-0332991. Blood was collected at specific times through 24 hours postdose. Excretion profiles of [ $^{14}C$ ]PD-0332991-derived radioactivity were determined for urine and feces through 192 hours postdose. Excreta and plasma were analyzed for total radioactivity by liquid scintillation counting (LSC).

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

**Dosing:**

Species/strain: beagle dogs

#animals in the study: 2/sex

Weight: 8.6 to 10.0 kg

Age: 9 to 11 months

Doses in administered units: 2 mg/kg (approximately 20  $\mu$ Ci/kg)

Route, form, volume, and infusion rate: oral at dose volume of 2 mL/kg

**Results:** The following figures are excerpted from the Applicant's submission.

Excretion and mass balance

Figure 33. Concentrations of radioactivity in plasma at specified times after a single oral dose of [14C]PD-0332991 to male dogs (Group 1, 2 mg/kg)

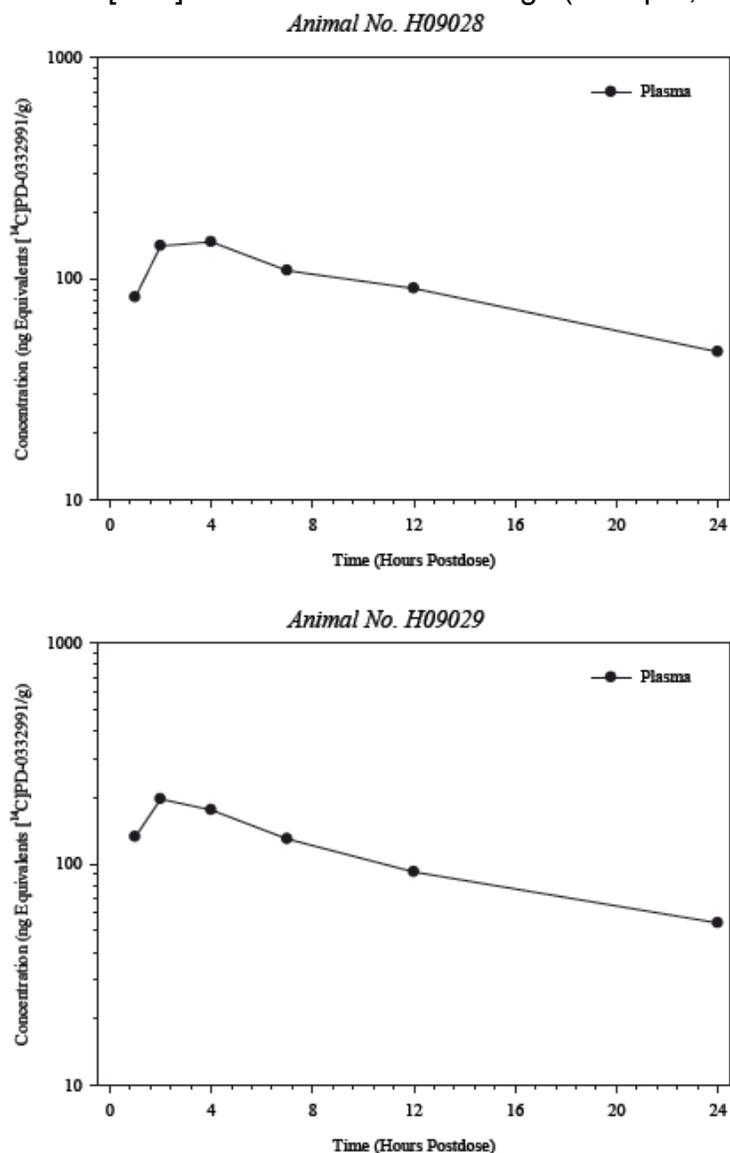
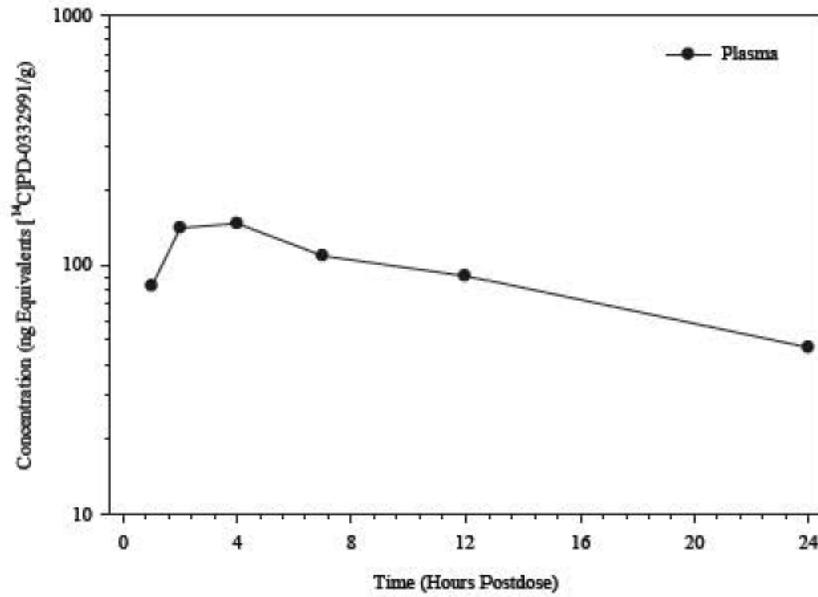


Figure 34. Concentrations of radioactivity in plasma at specified times after a single oral dose of [14C]PD-0332991 to female dogs (Group 1, 2 mg/kg)

*Animal No. H09028*



*Animal No. H09029*

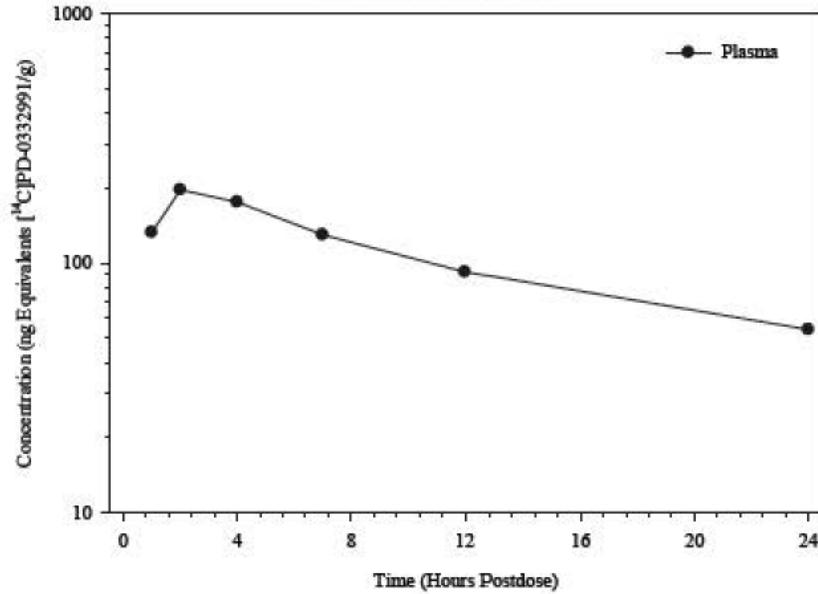
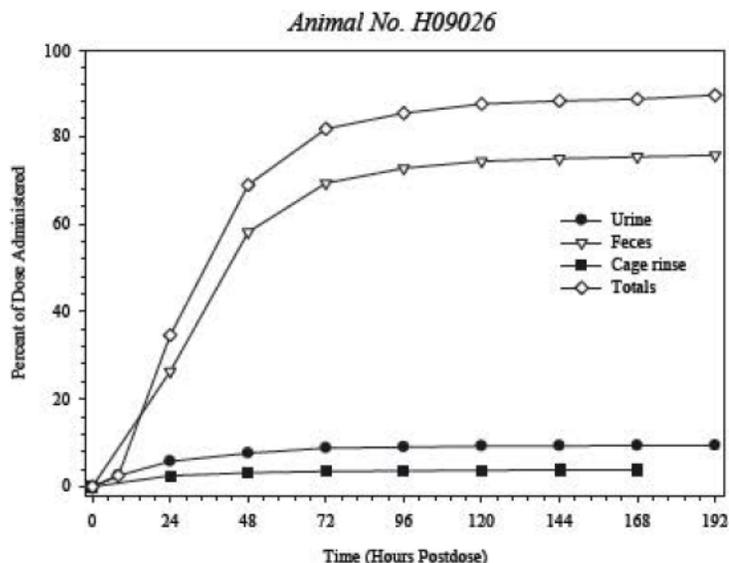
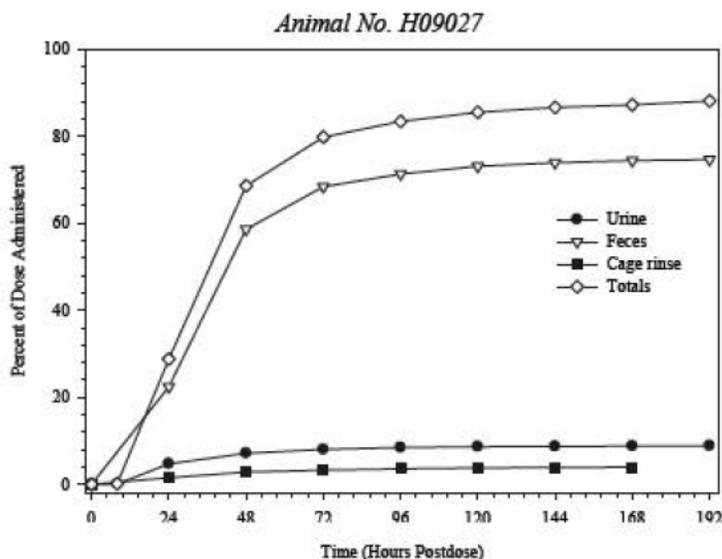


Figure 35. Cumulative percent of radioactive dose in urine, feces, and cage wash at specified intervals after a single oral dose of [14C]PD-0332991 to male dogs (Group 1, 2 mg/kg)

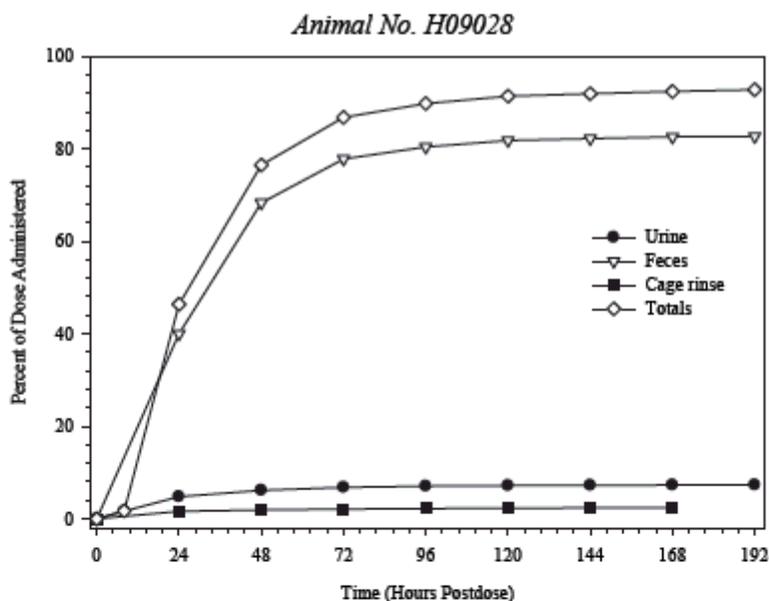


Note: Total includes urine, feces, cage rinse, cage wash, cage wipe, and cage debris.

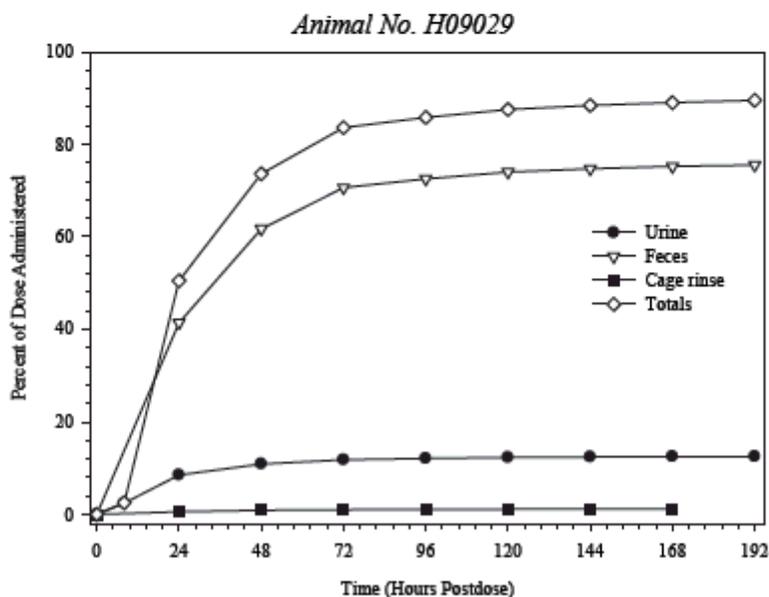


Note: Total includes urine, feces, cage rinse, cage wash, cage wipe, and cage debris.

Figure 36. Cumulative percent of radioactive dose in urine, feces and cage wash at specified intervals after a single oral dose of [ $^{14}\text{C}$ ]PD-0332991 to female dogs (Group 1, 2 mg/kg)



Note: Total includes urine, feces, cage rinse, cage wash, cage wipe, and cage debris.



Note: Total includes urine, feces, cage rinse, cage wash, cage wipe, and cage debris.

**Summary:** [ $^{14}\text{C}$ ]PD-0332991-derived radioactivity was rapidly absorbed after a single oral dose to male and female dogs. Fecal excretion of [ $^{14}\text{C}$ ]PD-0332991-derived radioactivity within the first 96 hours accounted for averages of 72.0 and 76.5% of the administered dose for males and females, respectively. The average urinary excretion over this same interval accounted for only 8.75% for males and 9.58% for females. No sex-dependent differences in excretion of [ $^{14}\text{C}$ ]PD-0332991-derived radioactivity was evident.

## Pharmacokinetic drug interactions

**Study title:** Effect of PD-0332991 on human drug metabolizing enzymes in vitro

### Key Study Finding:

PD-0332991 did not demonstrate pharmacokinetic drug interactions with compounds for which CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and/or CYP2D6 mediated metabolism constitutes the primary mechanism of clearance. PD-0332991 demonstrated time dependent inhibition of CYP3A.

**Study no:** 181141

**Volume #, and page #:** electronic submission, page 1-24

**Conducting laboratory and location:** not provided

**Date of study initiation:** not provided

**GLP compliance:** no

**QA report:** yes ( ) no (x )

**Drug, lot #, radiolabel, and % purity:** PD-0332991-0054  
Lot KZ00002685/A4  
Purity: not provided

**Methods:** PD-0332991 was examined for effects on several drug metabolizing enzyme activities in pooled human liver microsomes. PD-0332991 was examined for time dependent inhibition effects on several drug metabolizing enzyme activities in pooled human liver microsomes. Standard marker activity substrates were incubated with pooled human liver microsomes (HL-102) in the presence of NADPH (1.3 mM; Sigma) in 100 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4 containing 3.3 mM MgCl<sub>2</sub> at 37°C open to air. Microsomal protein concentrations, substrate concentrations, incubation times, and reaction termination solvents for each activity are summarized in the following table (copied from the Applicant's submission).

### Summary of Incubation Conditions: IC50 and Ki Determinations

Marker Substrate Activity	Enzyme	Substrate Concentration (μM)	Microsomal Protein Concentration (mg/mL)	Incubation Time (min)	Termination Solvent
Phenacetin <i>O</i> -Deethylase	CYP1A2	20.4 μM	0.03	15	5/92/3
Bupropion Hydroxylase	CYP2B6	94.5 μM	0.03	10	5/92/3
Amodiaquine <i>N</i> -Deethylase	CYP2C8	1.90 μM	0.025	10	5/92/3
Paclitaxel 6α-Hydroxylase	CYP2C8	6.00 μM	0.03	20	5/92/3
Diclofenac 4'-Hydroxylase	CYP2C9	2.89 μM	0.03	10	5/92/3
S-Mephenytoin 4'-Hydroxylase	CYP2C19	52.6 μM	0.1	40	5/92/3
Dextromethorphan <i>O</i> -Demethylase	CYP2D6	1.44 μM	0.03	10	5/92/3
Felodipine Oxidase	CYP3A	1.24 μM	0.01	10	50/47/3
Midazolam 1'-Hydroxylase	CYP3A	2.30 μM	0.01	4	92/5/3
Testosterone 6β-Hydroxylase	CYP3A	88.2 μM	0.01	10	5/92/3

Termination solvent ratio = Acetonitrile/Water/Formic Acid

Filtered terminated incubation mixtures were analyzed by HPLC-MS/MS using a Micromass Ultima tandem quadrupole mass spectrometer fitted with an electrospray interface.

**Results:** The following tables and figure are copied from the Applicant's submission.

Table 32. Summary of IC<sub>50</sub> data for PD-0332991 in human liver microsomes

Marker Substrate Activity	Enzyme	% of control at	IC <sub>50</sub> (μM)	
		[I] = 30 μM	Mean	± SE
Phenacetin O-Deethylase	CYP1A2	120	>30	
Bupropion Hydroxylase	CYP2B6	100	>30	
Coumarin 7-Hydroxylase	CYP2A6	99	>30	
Amodiaquine N-Deethylase	CYP2C8	93	>30	
Diclofenac 4'-Hydroxylase	CYP2C9	75	>30	
S-Mephenytoin 4'-Hydroxylase	CYP2C19	87	>30	
Dextromethorphan O-Demethylase	CYP2D6	90	>30	
Felodipine Oxidase	CYP3A	70	>30	
Midazolam 1'-Hydroxylase	CYP3A	87	>30	
Testosterone 6β-Hydroxylase	CYP3A	72	>30	

Table 33. Summary of Single Concentration-Time dependent inhibition (SC-TDI) data for PD-0332991 in pooled human liver microsomes (percent decrease in activity with 30 minute preincubation)

Marker Substrate Activity	Enzyme	Preincubation	Result
		Conc. (μM)	(Percent Decrease)
Phenacetin O-Deethylase	CYP1A2	100	-0.34
Bupropion Hydroxylase	CYP2B6	100	-0.17
Paclitaxel 6α-Hydroxylase	CYP2C8	100	-12
Diclofenac 4'-Hydroxylase	CYP2C9	100	5.8
S-Mephenytoin 4'-Hydroxylase	CYP2C19	100	-1.2
Dextromethorphan O-Demethylase	CYP2D6	100	-20
Midazolam 1'-Hydroxylase	CYP3A	100	91
Testosterone 6β-Hydroxylase	CYP3A	100	67

*Summary:* Palbociclib demonstrated little or no reversible and/or time-dependent inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6 enzyme activities (IC<sub>50</sub> >30 μM). PD-0332991 demonstrated time dependent inhibition of CYP3A.

*Note:* The relevance of this in vitro finding was confirmed in a human DDI study. Coadministration of midazolam with multiple doses of IBRANCE increased the midazolam AUC by 61%, in healthy subjects, compared with administration of midazolam alone.

**Study title:** In vitro investigation of the potential for PF-00080665 to induce cytochrome P450 (CYP1A2, CYP2B6, CYP2C8 AND CYP3A4) in cultured cryopreserved human hepatocytes

Note: PF-00080665 was also designated as PD-0332991

**Key Study Finding:**

- PF-00080665, at concentrations up to 3  $\mu$ M, did not cause induction of CYP1A2, CYP2B6, CYP2C8 and CYP3A4 activity in cultured human hepatocytes

**Study no:** 165849

**Volume #, and page #:** electronic submission, page 1-19

**Conducting laboratory and location:**



**Date of study initiation:** not provided

**GLP compliance:** no

**QA report:** yes ( ) no (x)

**Drug, lot #, radiolabel, and % purity:**

PF-00080665

Batch # PF-00080665-73-006

Purity: not provided

**Formulation/vehicle:** DMSO, 0.1% v/v

**Methods:** Cultured human hepatocytes were incubated for 24 hours, then treated once daily for three consecutive days with dimethyl sulfoxide (DMSO, 0.1% v/v, vehicle control), positive controls, and several concentrations of test compound (0.3 to 30  $\mu$ M) in triplicate. Enzyme activities were measured with LC/MS/MS methods and mRNA levels were determined by measuring absorbance at 260 and 280 nm on a NanoDrop 8000 Spectrophotometer (Thermo Scientific).

**Results:** Palbociclib did not cause induction of CYP1A2, CYP2B6, CYP2C8, or CYP3A4 mRNA expression and/or enzyme activity at concentrations up to 3  $\mu$ M of palbociclib, which was the highest concentration that provided >80% cell viability.

Note: 3  $\mu$ M was higher than 50 times the steady-state unbound palbociclib  $C_{max}$  (1.9  $\mu$ M) achieved with the clinical Phase 3 dose (125 mg QD).

**Tables and figures to include comparative TK summary**

Table 34. Pharmacokinetic parameters of palbociclib in male rats, dogs, and monkeys after single IV or oral administration

Species (Strain)	Dose (mg/kg)	Route	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	t <sub>1/2</sub> (h)	CL (mL/min/kg)	V <sub>ss</sub> (L/kg)	AUC <sub>inf</sub> (ng·h/mL)	F <sup>c</sup> (%)
Rat (S-D)	1	IV <sup>a</sup>	--	--	2.2 (0.34)	38.0 (3.79)	5.65 (0.736)	442 (46.7)	--
	5	IV <sup>a</sup>	--	--	2.6 (0.19)	37.4 (1.58)	7.07 (0.317)	2230 (103)	--
	5	Oral <sup>b</sup>	178 (47.4)	3.5 (1.9)	2.1 (0.12)	--	--	1200 (393)	56.1
	20	Oral <sup>b</sup>	1110 (61.8)	5.0 (1.2)	2.8 (0.36)	--	--	10800 (651)	--
	50	Oral <sup>b</sup>	1660 (245)	4.5 (1.9)	4.9 (1.4)	--	--	23000 (6740)	--
	200	Oral <sup>b</sup>	2240 (166)	30 (0)	--	--	--	76800 <sup>d</sup> (8900)	--
Dog (Beagle)	1	IV <sup>a</sup>	--	--	11 (0.29)	7.22 (0.853)	6.22 (0.789)	2330 (258)	--
	20	Oral <sup>b</sup>	664 (24.7)	8.7 (3.1)	21 (5.7)	--	--	17400 <sup>d</sup> (6900)	36.9 (12.4)
Monkey (Cynomolgus)	0.5	IV <sup>a</sup>	--	--	4.7 (1.4)	13.4 (0.896)	5.05 (1.01)	624 (42.8)	--
	2.66	Oral <sup>b</sup>	86.2 (31.0)	2.7 (1.2)	5.3 (0.89)	--	--	768 (150)	23.1 (3.6)

Note: Data are mean (standard deviation); n = 3.

-- = Data not applicable or available; AUC<sub>inf</sub> = Area under concentration-time curve from time zero to infinity postdose; AUC<sub>t</sub> = Area under concentration-time curve from time zero to 24 hours postdose for intravenous dose and 48 hours postdose for oral dose; CL = Systemic plasma clearance; C<sub>max</sub> = Peak plasma concentration; D5W = 5% dextrose in water; DMA = Dimethylacetamide; F = Bioavailability; IV = Intravenous; n = Number of animals; SD = Standard deviation; S-D = Sprague-Dawley; t<sub>1/2</sub> = Apparent terminal elimination half-life; T<sub>max</sub> = Time to reach C<sub>max</sub>; V<sub>ss</sub> = Apparent volume of distribution at steady state.

a. IV vehicle/formulation solution = 5% DMA/25% PEG/70% D5W.

b. Oral vehicle/formulation = 95%, 0.5% methylcellulose/5%, PEG 200 as a suspension for rat and monkey studies and as a capsule for the dog study.

c. F (%) =  $\frac{[AUC_{inf}(\text{Oral}) \times \text{Dose}(\text{IV})]}{[AUC_{inf}(\text{IV}) \times \text{Dose}(\text{Oral})]} \times 100$ .

d. Value represents AUC<sub>t</sub>.

Table 35. Protein binding

<b>Study System:</b>	In Vitro				
<b>Test system:</b>	Plasma				
<b>Method:</b>	Equilibrium dialysis assay				
Species	Fraction Unbound (fu: Mean) <sup>a</sup>				Mean fu
	500 ng/mL (1.12 μM)	1000 ng/mL (2.24 μM)	2500 ng/mL (5.59 μM)	5000 ng/mL (11.2 μM)	
Mouse	0.148	0.188	0.134	0.167	0.159
Rat	0.122	0.122	0.127	0.127	0.125
Rabbit	0.0733 <sup>b</sup>	--	--	--	0.0733
Dog	0.405	0.453	0.409	0.383	0.413
Human	0.137	0.152	0.138	0.161	0.147
Human Serum Albumin	0.735	0.619	0.475	0.657	0.622
α1-Acid Glycoprotein	0.626	0.583	0.652	0.724	0.646

Notes: The unbound fraction of drug (fu) was calculated using the following equation:  $f_u = (C_u/C_{pp})$ , where  $C_u$  is the unbound palbociclib free base concentration after dialysis, and  $C_{pp}$  is the post-dialysis plasma concentration. Calculated molecular weight of palbociclib = 447.5 g/mol.

-- = Data not available or not applicable.

a. Data are mean from n = 3, except for rabbit which is a mean from n = 12.

b. 1 μM.

<b>Type of Study:</b>	In Vitro Red Blood Cell Distribution
<b>Study System:</b>	Whole blood
<b>Palbociclib Concentration:</b>	5.6 $\mu$ M (2500 ng/mL)
<b>Duration of Incubation (h):</b>	2
<b>Method of Analysis:</b>	LC-MS/MS
<b>Analyte:</b>	Palbociclib

Species	$C_b^a$ ( $\mu$ g/mL)	$C_b^a$ ( $\mu$ g/mL)	$C_b/C_p$	$K_p$
Mouse	2295 $\pm$ 337	2037 $\pm$ 30.6	1.13	1.36
Rat	2460 $\pm$ 159	2540 $\pm$ 10.0	0.97	0.94
Dog	2413 $\pm$ 11.5	2413 $\pm$ 30.6	1.00	1.00
Monkey	1466 $\pm$ 76.9	1423 $\pm$ 143	1.04	1.09
Human	2460 $\pm$ 100	1507 $\pm$ 47.3	1.63	2.44

Note: Calculated molecular weight of palbociclib = 447.5 g/mol.

$C_b$  = Concentration in whole blood;  $C_p$  = Concentration in plasma;  $K_p$  = Partition coefficient; SD = Standard deviation.

a. Data are mean  $\pm$  SD (n = 3).

Table 36. Pharmacokinetics: metabolism in vitro

Metabolite Label	m/z	Presence of Metabolites		
		Hepatocytes		
		Rat	Dog	Human
Palbociclib (Parent)	448	Yes	Yes	Yes
M23e	464	Yes	No	No
M23a	464	Yes	No	Yes
M22	624	Yes	No	Yes
M32	422	Yes	Yes	Yes
M23c	464	Yes	No	Yes
M23d	464	Yes	No	No
M28	446	No	No	Yes
M14	450	Yes	No	Yes
M17	462	Yes	No	Yes
M23f	464	Yes	No	Yes
M11	528	Yes	No	Yes
M12	490	Yes	No	No

m/z = Mass to charge ratio; LC/MS = Liquid chromatography/mass spectrometry; M = Metabolite.

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

Reports from single dose rat and dog studies were submitted to this NDA and were previously reviewed under IND 69,324 (by Dr. Wei Chen) and the following summary information was copied from the pharmacology/toxicology review of IND 69,324 completed on 03/31/2004

#### Acute Oral Toxicity Study of PD332991 in rats

Single dose of PD332991 was administered orally to rats (3/sex/group) at doses at 125, 250, 500, 1000, or 2000mg/kg, clinical signs and body weight changes were observed for 14 days following dose administration. Gross pathology was performed at necropsy on Day 15.

- Single oral dose of PD332991-54 resulted in lethality at  $\geq 1000$  mg/kg.
- Clinical signs for toxicity of PD332991-54 were observed at  $\geq 500$  mg/kg.
- GI is the main target organ.

#### Oral escalating dose toxicity study of PD332991-2B in dogs

Single dose of PD332991 gelatin capsule was orally administered to dogs (1/sex) with the doses and administration days described in the following table

Day	1	2	3	7	8	9	22*
Dose (mg/kg)	1	3	10	30	100	60	30

\*female only

- Repeated administration of PD332991-2B resulted in lethality in the male dog at day 18 following the dosing of 60 mg/kg;
- Clinical signs for the toxicity were observed at  $\geq 30$  mg/kg;
- Gastrointestinal tract effects and bone marrow suppression were the main toxicities.

### 6.2 Repeat-Dose Toxicity

Two-week oral dose range-finding studies and 3-week repeat-dose toxicity studies were conducted in rats and dogs, and these study reports were originally submitted under IND 69,324 to support a Phase 1 trial with PD332991. These study reports were previously reviewed by Wei Chen and the following summary information was copied from the pharmacology/toxicology review of IND 69,324 completed on 03/31/2004.

#### 2-week Oral Dose Range-finding Study of PD332991-54 in Rats

PD332991 was administered once daily to rats (main: 5/sex/group, PK: 6/sex/group) orally for 14 days at doses up to 600 mg/kg in males and up to 300 mg/kg in females.

- Mortality was observed at  $\geq 300$  mg/kg dose groups in male.
- Daily oral administration of PD332991 to rats induced bone marrow and GI toxicity at  $\geq 300$  mg/kg; no toxicity effect were seen in female at  $\leq 100$  mg/kg.
- GI toxicity and bone marrow toxicity were more pronounced in male rats.

- The exposure was significant greater in males than females at 300 mg/kg.
- The differences in toxicity observed between male and female probably reflected the differences in systemic PD332991 exposure.

#### Three-week oral toxicity study of PD332991-54 in rats

PD332991 was administered by oral gavage once daily to rats (main: 15/sex/group, recovery: 5/sex/group, PK: 6/sex/group) for 3 weeks at doses up to 200 mg/kg in males and up to 400 mg/kg in females.

- Mortality was observed at 200 mg/kg in male and 400 mg/kg in female.
- Pancytic bone marrow and lymphoid depletion, and decreases in circulating blood cells were the primary toxicity.
- Testicular degeneration was dose-dependent.
- Pulmonary changes including necrosis and mucosa atrophy in the trachea occurred at all doses.
- Observed toxicities generally reversed after the recovery period.
- GI toxicity and bone marrow toxicity were more pronounced in male rats.
- Micronucleus formation occurred in males at dose  $\geq$  100 mg/kg, indicating that PD 332991-54 has clastogenic potential.

#### Two-week Oral Dose Range-finding Study of P D332991-54 in Dogs

PD332991 was administered once daily to dogs (main: 2/sex/group, PK: 2/sex/group) orally for 14 days at doses up to 20.

- Mortality was observed at  $\geq$  10 mg/kg dose group.
- Administration of PD332991-54 to dogs resulted in bone marrow suppression at all doses.
- Toxic effects were observed in GI tract.

#### Three-week Oral Toxicity Study of PD332991-54 in dogs

PD332991 was administered by oral gavage once daily to dogs (main: 15/sex/group, recovery: 5/sex/group, PK: 6/sex/group) for 3 weeks at doses up to 2 mg/kg.

- Oral administration of PD 332991-54 to dogs for 3 weeks resulted in bone marrow and lymphoid depletion , and testicular degeneration at  $\geq$  0.6 mg/kg.
- Dose- and time-dependent decrease in hematology parameters occurred at all doses.
- Changes in bone marrow, lymphoid tissue, and hematology parameters were reversible.

**Study title:** A 15-week toxicity study of PD-0332991 by oral gavage administration in rats with a 4-Week recovery period

Study no.: 20026125  
Study report location: Electronic submission, M4. pages 1-1144  
Conducting laboratory and location:  (b) (4)

Date of study initiation: April 16, 2012  
GLP compliance: yes  
QA statement: yes ( X ) no ( )  
Drug, lot #, and % purity: PD-0332991  
Batch No: GR06024  
% purity: 98.7%

**Key Study Findings**

- No treatment-related mortalities were observed with daily oral administration of PD-0332991 to male rats at doses up to 100 mg/kg/day and to females at doses up to 200 mg/kg/day;
- Decreased body weights and reduced food consumption were observed in males at 100 mg/kg;
- The dose- and time-dependent decreases in counts of red cell mass parameters and leukocytes were observed in males and females, with greater magnitude in male rats compared to female rats;
- Increases in AST, ALT, ALK, GGT and urea nitrogen and decreases in albumin and A/G ratio were observed in male rats at 100 mg/kg/day;
- Treatment related changes involved in the adrenal gland, bone marrow, kidney, lung, lymph nodes, pancreas, spleen, testes, epididymides, and thymus in male rats, involved in the bone marrow, kidney, lung, lymph nodes, spleen in female rats;
- The treatment related changes were mostly reversible after a 4-week recovery period.
- The presence of glucose in urine was noted in males at 100 mg/kg at the end of the dosing period and after a 4-week recovery period;
- MTD was 30 mg/kg in male rats considering that the severity of kidney toxicity observed at 30 mg/kg was limited and reversible; MTD was 200 mg/kg (HD) in female rats with reversible toxicities in the hematopoietic lymphoid system.

## Methods

Doses: Male: 10, 30, 100 mg/kg/day  
 Female: 50, 100, 200 mg/kg/day  
 Note: Doses were selected based on findings from a 3-week oral dose study  
 Frequency of dosing: Daily x 21, 28 days/cycle for total of 4 cycles. Dosing occurred on the following days: Days 1 to 21, 29 to 49, 57 to 77, and 85 to 105  
 Route of administration: Oral gavage  
 Dose volume: 10 mL/kg  
 Formulation/Vehicle: 0.5% (w/v) Methylcellulose in Reverse Osmosis Deionized (RODI) Water  
 Species/Strain: Sprague Dawley Crl:CD(SD) rats  
 Number/Sex /Group: Main:10/sex/group  
 Recovery: 5/sex/group (control and HD only)  
 Age: approximately 8 weeks old  
 Weight: Males: 226 to 279 g; females: 173 to 216 g  
 Satellite groups: Yes, 6/sex/group (3/sex for the control group) for TK study  
 Unique study design: none  
 Deviation from study protocol: Yes, but no major impact on the study results

## Observations and Results

**OBSERVATIONS AND TIMES:**

<u>Mortality</u>	daily
<u>Clinical examinations</u>	Daily for mortality/moribundity and cage side observations
<u>Detailed physical examinations</u>	Weekly
<u>Body weights</u>	Weekly, on the day of randomization (Day -3 [females]/Day -2 [males]) and on Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 99, 105, 112, 119, 126, and 133.
<u>Food consumption</u>	weekly
<u>Ophthalmoscopy</u>	Once prior to in-life initiation (Day -3 [females]/Day -2 [males]) and once during the last week of the treatment period (Day 103 [females]/Day 104 [males]).
<u>Clinical Pathology:</u>	Days 21, 28, 48, and Days 106, 134
<u>Gross pathology:</u>	All animals at death or at scheduled sacrifice on Day 106, or Day 134
<u>Organ weights:</u>	All animals at death or at scheduled sacrifice on Day 106, or Day 134

<u>Histopathology:</u>	All animals at death or at scheduled sacrifice on Days 103, or Day 134
<u>Toxicokinetics:</u>	At 1, 2, 12, 24 hours posting dosing on Days 1, 49, and 105

**RESULTS:****Mortality:**

One female (No.3134) at 100 mg/kg was euthanized moribund on day 65

Note: A gavage accident was the cause of death.

One 100 mg/kg/day toxicokinetic male that was euthanized moribund on Day 79, demonstrated hind paw swelling, impaired mobility of the hindlimbs, breathing abnormalities (i.e., shallow, rapid, and labored), decreased activity, yellow extremities, and 13% body weight loss in a week. The findings were consistent with moribundity in the 27-week rat toxicity study (8282224).

**Clinical Signs****Main*****unscheduled death***

Rapid and shallow breathing and a hunched posture were observed prior to euthanasia.

***Terminal sacrifice:***

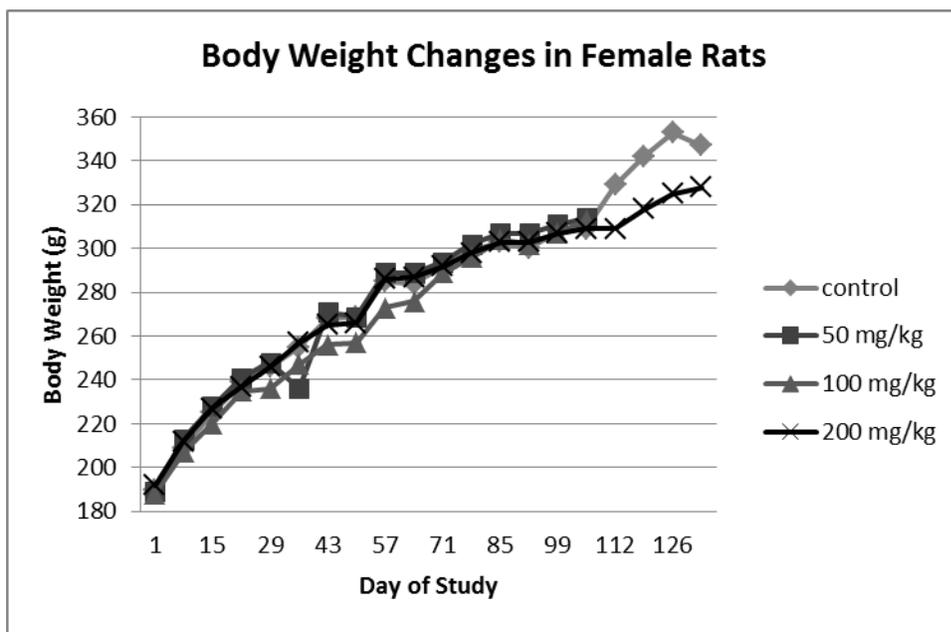
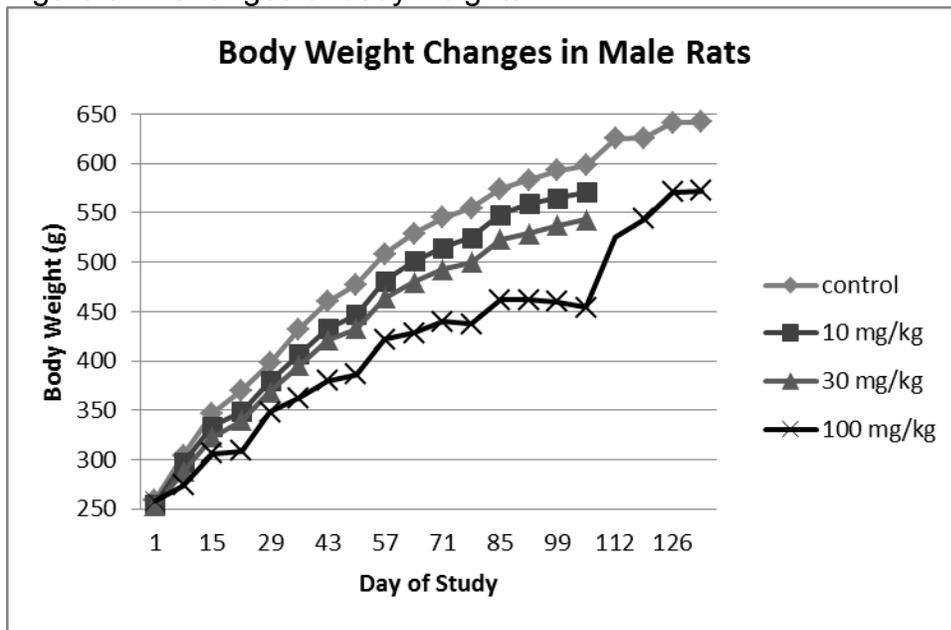
Dosing period: A dose-related increase in the incidence of struggling during dosing was observed in the test article-treated groups, with the greatest incidence of struggling noted in 100 mg/kg/day males and 200 mg/kg/day females. In addition, females appeared to be more affected than males.

Additional clinical signs in males at 100 mg/kg included swelling of the forepaws and urogenital area in a few males, yellow extremities in one male on Day 78, and sporadic observations of thin appearance and rough coat.

**Recovery:** unremarkable

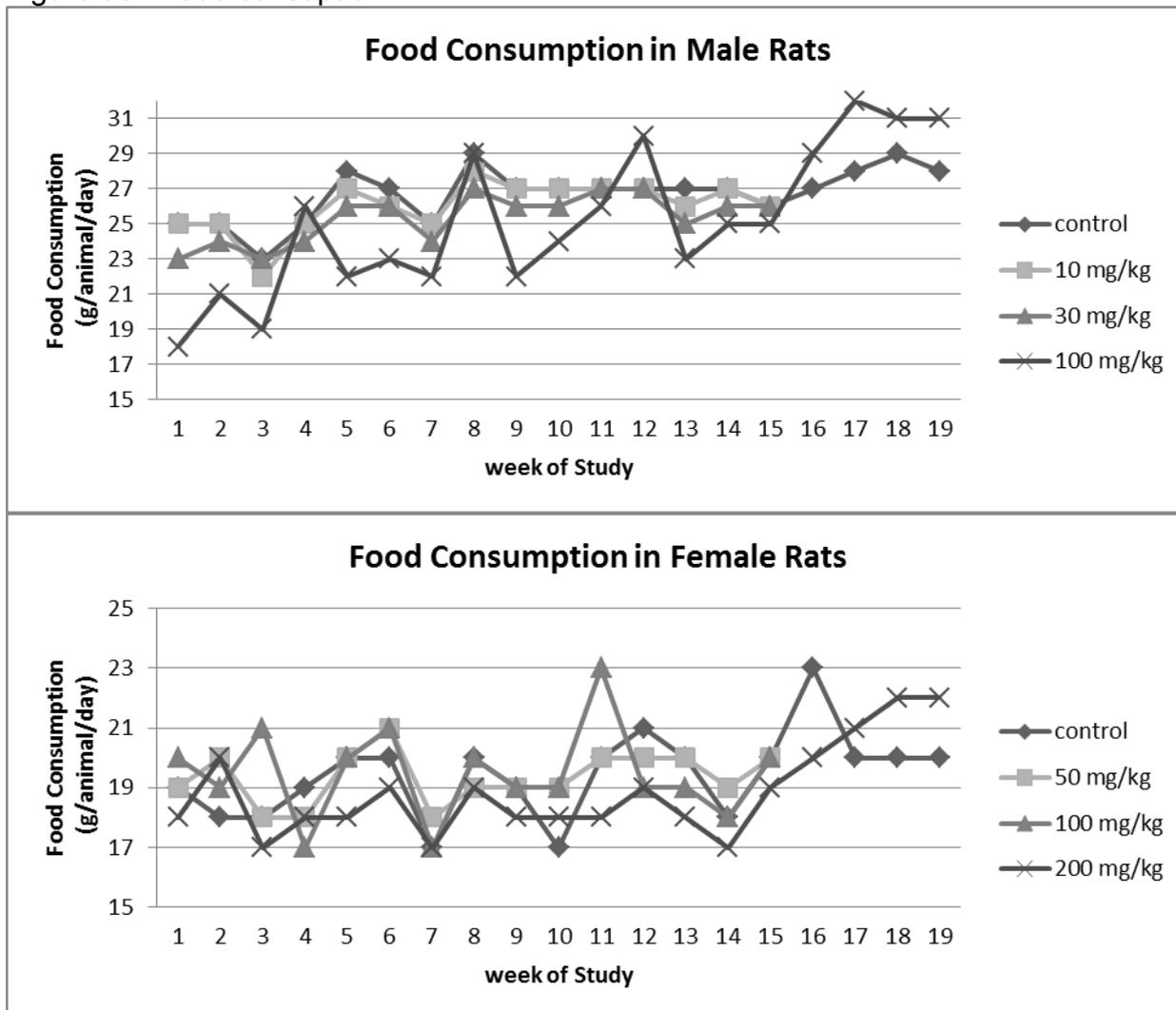
Body Weights

Figure 37. Changes of body weights



**Summary:** Treatment related, dose dependent lower body weights were observed in males compared to the controls (↓up to 24%) during the study. There were no body weight changes in test article-treated females. The change of body weights in males were recovered after a 4-week of recovery period.

Food Consumption  
 Figure 38. Food consumption



**Summary:** Test article-related decreases in food consumption were noted in 100 mg/kg/day males compared to the controls ( ↓ up to 28%) during the dosing phase of the study. There were no changes in food consumption in females. The observed reduced food consumption recovered after a 1-week off dose period for each cycle.

Ophthalmoscopy: unremarkable

## Hematology:

Table 37. Changes of hematologic parameters

Gender	Percent Change From Vehicle Control					
	Male			Female		
	Doses (mg/kg)	10	30	100	50	100
Day 21						
Erythrocytes	-6	-11	-17	-5	-5	-5
Hemoglobin			-10			
Hematocrit			-11		-5	
MCH		9	9			
MCV	4	10	8			
Reticulocytes	-19	-7			-10	-8
Platelets		25	13	26	15	20
MPV		6	14	5	6	5
Leukocytes		-32	-42	15	-5	-10
Lymphocytes		29	42	15	-4	-7
Monocytes	40	10	-73	33	22	14
Segd neutrophils	-12	-47	-34	7	-14	-27
Eosinophils	-35	-74	-78		-28	-39
Basophils	-14	-38	-55		-5	-13
LUC	-8	-44	-49	32	6	
Day 28						
Erythrocytes	-4	-10	-18	-7	-5	-5
Hemoglobin			-7			
Hematocrit			-5			
MCH		12	15			
MCV	4-	11	17			
Reticulocytes	-5	14	73	8	12	23
Platelets		8	10	14	-13	9
MPV	12	11	22			
Leukocytes	20		-40	13		-6
Lymphocytes	14		40	16		
Monocytes		+10	-44	6	-11	-14
Segd neutrophils	55	31	-34		-5	-29
Eosinophils	23	-10	-71			-30
Basophils	7	11	-48	11		7
LUC	-7	-25	-21	37	17	
Day 49			Day 48			
Erythrocytes	-8	-19	-31	-5	-7	-9
Hemoglobin		-5	-12			
Hematocrit			-10			
MCH	5	16	27			7
MCV	7	18	30			-8

Reticulocytes	-15	<u>16</u>	-11	-11	-7	-7
Platelets	<u>25</u>	<u>42</u>	<u>34</u>	15	8	<u>18</u>
MPV	<u>5</u>	<u>13</u>	<u>21</u>		<u>10</u>	<u>11</u>
Leukocytes	-5	-25	<u>-59</u>	10	-14	-13
Lymphocytes		<u>25</u>	<u>59</u>	12	-9	-12
Monocytes	16	10	<u>-88</u>	20	-10	-8
Segd neutrophils	-24	<u>-29</u>	<u>-47</u>		-34	-19
Eosinophils	-66	<u>-82</u>	<u>-94</u>	8	<u>-48</u>	<u>-48</u>
Basophils		-27	<u>-61</u>	14	-4	-10
LUC	20	13	<u>-66</u>		-15	-14
Day 106						
Erythrocytes	<u>-10</u>	<u>-21</u>	<u>-30</u>	-5	<u>-7</u>	<u>-10</u>
Hemoglobin			-14			
Hematocrit			<u>-10</u>			
MCH	<u>9</u>	<u>20</u>	<u>44</u>			<u>9</u>
MCV	<u>9</u>	<u>21</u>	<u>51</u>	<u>5</u>	<u>5</u>	<u>11</u>
Reticulocytes	-4	-14	-21	14	6	4
Platelets	13	<u>28</u>	<u>21</u>	10	13	<u>22</u>
MPV		<u>7</u>	<u>21</u>			6
APTT		<u>-8</u>	<u>-12</u>			
PT			<u>16</u>		<u>5</u>	<u>6</u>
Leukocytes		<u>-37</u>	<u>-70</u>	27		
Lymphocytes		<u>36</u>	<u>74</u>	37	10	5
Monocytes	10	<u>-46</u>	<u>-85</u>	40	15	9
Segd neutrophils	-19	-34	<u>-45</u>	-24	-20	-23
Eosinophils	-44	<u>-72</u>	<u>-83</u>		-30	<u>-49</u>
Basophils	-27	<u>-58</u>	<u>-69</u>	9		-27
LUC	-12	<u>-52</u>	<u>-79</u>	43		
Day 134 - Recovery						
Erythrocytes	-	-	<u>-15</u>			-4
Hemoglobin	-	-				
Hematocrit	-	-	<u>4</u>			
MCH	-	-	<u>20</u>			5
MCV	-	-	<u>23</u>			4
Reticulocytes	-	-	-5			16
Platelets	-	-	10			14
MPV	-	-	0			
APTT	-	-	4			
Leukocytes			-4			42
PT			<u>5</u>			
Lymphocytes			-7			48
Monocytes			18			13

Segd neutrophils			10			28
Eosinophils			-33			-5
Basophils			0			
LUC			-20			26

Blank cells: unremarkable

Numbers underlined: P < 0.05

*Summary:* The decreases in red cell mass parameters (red blood cell count, hemoglobin, hematocrit, and reticulocytes) and the decreases in the counts of leukocytes including neutrophils, monocytes, Basophils, eosinophils and LUC were observed in both males and females. These changes were generally dose- and time-dependent. The magnitude of changes was greater in male rats compared to female rats. In addition, non-dose-related increases in platelet counts were observed in males and females. None of the above described changes showed any recovery on Day 28 after a 1-week off dose period, but fully recovered or had recovery trends after a 4-week recovery period following 4 cycles of treatment.

#### Clinical Chemistry:

Table. 38 Change of clinical chemistry

Gender	Percent Change From Vehicle Control							
	Male				Female			
	106		134		106		134	
<b>Doses (mg/kg)</b>	10	30	100	100	50	100	200	200
AST	-20		<u>82</u>	-9	-7	-11	-23	9
ALT	10	55	<u>106</u>	-8	-26	-16	-33	26
ALK Phos'tase	6	7	42	12		23	-9	22
GGT, serum (IU/L)*	0*	0*	<u>1.77</u>	0*	0*	0*	0*	0*
Albumin			<u>-13</u>					
A/G ratio			<u>-18</u>					
Urea nitrogen			36	6	-10	-10	-20	

Blank cells: unremarkable

Numbers with underlines: P < 0.05

\*-actual values

*Summary:* Increases in AST, ALT, ALK, GGT and urea nitrogen and decreases in albumin and A/G ratio were observed in male rats at 100 mg/kg/day on Day 106. All clinical chemistry changes recovered after a 4-week recovery period.

Note: These changes were suggestive of hepatocellular damage and/or cholestasis; however, there were no microscopic correlates.

Urinalysis: The presence of glucose and casts in urine were noted in 3/10 and 4/10 male rats 100 mg/kg/day on Day 106, and day 134 (after 4-week recovery period) respectively.

## Gross Pathology

Table 39. Summary of gross pathology

## Main (Day 106)

Gender	Male				Female			
Dose (mg/kg)	0	10	30	100	0	50	100	200
No. animals examined	10	10	10	10	10	10	10	10
Thymus Small			2	10				
Testes Small Soft				6 6	- -	- -	- -	- -
Prostate Discoloration, pale or area pale	1			7	-	-	-	-
Epididymides Small				1	-	-	-	-
Kidney Discoloration, pale, tinged yellow				5				
Liver Discoloration, pale			1	5				
Lung Discoloration, pale focus or area				5				
Lymph node, mesenteric Discoloration, pale, tinged yellow				4				
Carcass Discoloration, pale Adipose tissue decreased				7 1				

## Recovery (Day 134)

Gender	Male		Female	
Dose (mg/kg)	0	100	0	200
No. animals examined	5	5	5	5
Testes Small		1	-	-

Blank cells: unremarkable

**Summary:** Gross findings related to treatment were observed primarily in the thymus, testes, epididymides, prostate, kidney, liver, lung, mesenteric lymph node, and carcass. The observed changes recovered after a 4-week recovery period.

**Note:** Microscopically, the decrease in size of the thymus was associated with decreased lymphoid cellularity. Smaller and/or softer testes and smaller epididymides were correlated with decreased spermatogenesis within the seminiferous tubules and hypospermia in the epididymides, respectively. The pale discoloration within the kidney was associated microscopically with vacuolar change and hypertrophy of tubular epithelium. Pale discoloration of the liver, prostate gland, and carcasses at 100 mg/kg/day was probably correlated with decreased red blood cell parameters.

## Organ Weights:

Table 40. Summary of organ weights (Day 106)

Study	Percentage deviation from control (n=10)							
	Absolute Organ Weight				Organ Weight/Body Weight			
	Main			Recovery	Main			Recovery
Dose Group (mg/kg)	10	30	100	100	10	30	100	100
No. Animal/group	10	10	10	5	10	10	10	5
Male								
Adrenal gland	-9		33		-5	11	<u>91</u>	
Spleen		<u>-16</u>	<u>-34</u>	-9		-7	<u>-5</u>	
Testes		-7	<u>-40</u>	<u>-29</u>	5		-14	<u>-21</u>
Epididymides		-7	<u>-37</u>	<u>-19</u>	4		-10	-10
Prostate	-12	-12	<u>-30</u>		-9			
Thymus	-20	<u>-53</u>	<u>-80</u>	<u>45</u>	-17	<u>-49</u>	<u>-72</u>	<u>56</u>
Female								
Dose Group (mg/kg)	50	100	200	200	50	100	200	200
No. Animal/group	10	10	10	5	10	10	10	5
Spleen		-6	-3	<u>15</u>	-5	-10	-7	<u>23</u>
Thymus		-12	-19	9		-16	-23	15

Blank cells: unremarkable

Values underlined: P &lt; 0.05

**Summary:** Treatment related organ weight changes were noted in male rats in the adrenal gland (↑), spleen (↓), testes (↓), epididymides (↓), prostate (↓), and thymus (↓). PD-0332991-related organ weight changes were noted in female rats in the spleen (↓), and thymus (↓). By the end of the recovery phase, the changes in the testes and epididymides did not recover, while other observed changes were fully reversed.

## Histopathology

Table 41. Summary of histopathology (15-week rat study)

Main (Day 106)

Gender	Male				Female			
	0	10	30	100	0	50	100	200
No. animals examined	10	10	10	10	10	10	10	10
Adrenal gland Hypertrophy, cortical -minimal				7				
Bone marrow, femur Hypocellularity -minimal -mild -moderate Vacuolated cells -minimal		4 5	6 4	2 8 5				
Bone marrow, sternum Hypocellularity -minimal -mild -moderate Vacuolated cells -minimal	3 2	5 5	3 4 2 7			1	2	1

Kidney Degeneration/regeneration, Tubular -minimal -mild Vacuolar change,tubular-minimal -mild	2	1	5	8 1 3 3				
Lung Vacuolated alveolar macrophages -minimal -mild -moderate				2 4 4			1	1
Lymph node, mesenteric Vacuolated macrophages, Sinusoidal -minimal -mild Decreased cellularity, lymphoid -minimal -mild Inflammation, chronic/active -minimal -moderate			3	4 6 4 4 1 1				
Pancreas, islets cells Vacuolar change -minimal -mild				7 1				
Spleen Decreased cellularity, lymphoid -minimal -mild Decreased red cells -minimal -mild		2	2	4 2 2 4	2	6 1	4	4 1
Testes Degeneration of seminiferous Tubules -minimal -mild -moderate			1	4 3 3	- - -	- - -	- - -	- - -
Epididymides Hypospermia (uni- or bilateral) -minimal -mild -moderate Cellular debris, intraductal (uni- And bilateral) -minimal -mild			1	4 2 2	- - -	- - -	- - -	- - -
Thymus Decreased cellularity, lymphoid			2	9 1	- -	- -	- -	- -

-minimal			5				
-mild			4	1			
-moderate				8			

**Recovery (Day 134)**

Gender	Male		Female	
Dose (mg/kg)	0	100	0	200
No. animals examined	5	5	5	5
Bone marrow, femur Hypocellularity -minimal				1
Bone marrow, femur Hypocellularity -minimal				1
Lung Vacuolated alveolar macrophages -minimal -mild		1		1
Lymph node, mesenteric Vacuolated alveolar Macrophages -minimal -mild		1		1
Pancreas Vacuolar change, islet cell -minimal		1		
Testes Degeneration of seminiferous tubules -minimal		5	-	-
Epididymides Hypospermia -minimal		2	-	-
Cellular debris, intraductal -minimal		2	-	-
-mild		2	-	-

Blank cells: unremarkable

*Summary:* Treatment related changes involved in male rats in the adrenal gland, bone marrow, kidney, lung, lymph nodes, pancreas, spleen, testes, epididymides, and thymus; treatment related changes in female rats involved the bone marrow, lung, lymph nodes, and spleen. The reversibility of the changes was observed at the end of a 4-week recovery period.

Toxicokinetics:

Table 42. Summary of toxicokinetic parameters in rats (PD-0332991)

Study day	Dose level	Gender	C <sub>max</sub> (ng/mL)	Dose	AUC <sub>0-24</sub> (ng.h/mL)	Dose	t <sub>max</sub> (h)
	(mg/kg/dose)			normalized C <sub>max</sub>		Normalized AUC <sub>0-24</sub>	
1	10	M	270	27	2920	292	4
	50	F	265	5	1400	28	2
	30	M	1270	42	18000	600	4
	100	F	356	4	3280	33	3
	100	M	2110	21	35300	353	9
	200	F	704	4	6450	32	2
49	10	M	385	39	3600	360	4
	50	F	349	7	1430	29	1
	30	M	1140	38	15100	503	3
	100	F	268	3	1850	19	1
	100	M	2270	22	43700	437	6
	200	F	639	3	5640	28	1
105	10	M	367	37	3770	377	4
	50	F	247	5	1710	34	2
	30	M	1260	42	18300	610	3
	100	F	308	3	2820	28	3
	100	M	2260	23	41600	416	3
	200	F	586	3	7050	35	4

## Conclusion:

- On Days 1, 49 and 105 plasma exposures (C<sub>max</sub> and mean AUC<sub>0-24</sub>) were generally increased more than proportionally to the dose increases from 10 mg/kg to 30 mg/kg, but increased less than proportionally to the dose increases from 30 mg/kg to 100 mg/kg in male rats; plasma exposures (C<sub>max</sub> and mean AUC<sub>0-24</sub>) were generally increased proportionally to the dose increases at the tested ranges (50 mg/kg to 200 mg/kg) in female rats.
- Higher exposure for the males was observed on all occasions compared to the females;
- No apparent accumulation was observed following repeated dosing;
- T<sub>max</sub> ranged from 4 to 9 hours in male rats, and 1 to 4 hours in female rats.

**Study title:** 27-week oral gavage chronic toxicity and toxicokinetic study with PD-0332991 in rats with a 12-week recovery phase

Study no.: 8282224  
Applicant Reference Number 13LJ036  
Study report location: Electronic submission, M4. pages 1-1990  
Conducting laboratory and location:  (b) (4)  
Date of study initiation: May 21, 2013  
GLP compliance: yes  
QA statement: yes ( X ) no ( )  
Drug, lot #, and % purity:  
PD-0332991  
Batch No: E010014102  
% purity: 98.9%

**Key Study Findings**

- No treatment related mortalities occurred in males rats at doses up to 30 mg/kg, or in female rats up to 300 mg/kg (HD);
- The cause of the moribund condition was attributed to degeneration and/or inflammation in one or more of the feet;
- Decreased body weights and reduced food consumption were observed in males at 100 mg/kg and in females at 300 mg/kg;
- Treatment related toxicities involved in the hematolymphoid system, teeth, liver, kidney, pancreas, eyes, adrenal gland, lung, glandular and nonglandular stomach, skin, heart, aorta, stifle joint, foot, and male reproductive systems;
- Kidney and eye lesion did not recovery after a 12-week recovery period;
- NOAEL was not determined due to the observed liver and renal toxicities in males at 10 mg/kg (LD), and observed lens degeneration and changes in the pancreas with decreased insulin levels at 50 mg/kg (LD) in females;
- MTD is 30 mg/kg in males and 300 mg/kg in females.

## Methods

Doses: Male: 10, 30, 100 mg/kg/day  
Female: 50, 100, 300 mg/kg/day  
\* Doses were selected based on 15-week oral dose study.

Frequency of dosing: Daily x 21, 28 days/cycle for total of 27 weeks.

Route of administration: Oral gavage

Dose volume: 10 mL/kg

Formulation /Vehicle: 0.5% (w/v) methylcellulose (4000 cps) in reverse osmosis water

Species/Strain: Sprague Dawley rats

Number/Sex /Group: 20/sex/group including 5/sex/group for recovery

Age: 6 to 7 weeks old

Weight: Males: 197 to 249 g; females: 158 to 210 g

Satellite groups: Yes, 4/sex/group (3/sex for the control group) for TK study

Unique study design: immunohistochemistry evaluations of pancreas in males at treatment group;  
Transmission Electron Microscopy of pancreas on Animal Nos. B68365, B68366, B68367, B68380, B68381, B68382 (Group 1 males), B68436, B68437, B68442, B68448, B68450, B68451, and B68454 (Group 4 males);  
Gene expression analysis of liver tissue for Animal Nos. B68365, B68366, B68367, B68380, B68381, B68382 (Group 1 males), B68436, B68437, B68442, B68448, B68450, B68451, and B68454 (Group 4 males).

Deviation from study protocol: Yes, but no major impact on the study results

## Observations and Results

**OBSERVATIONS AND TIMES:**

<u>Mortality</u>	daily
<u>Clinical examinations</u>	Twice daily for mortality, abnormalities, and signs of pain or distress.
<u>Detailed physical examinations</u>	weekly
<u>Body weights</u>	Weekly, twice weekly for Group 4 beginning on the first day of the third dosing cycle (Day 57 of the dosing phase),
<u>Food consumption</u>	Weekly
<u>Ophthalmoscopy</u>	Once during the predose phase On Day 188 of the dosing phase On Day 83 of the recovery phase
<u>Clinical Pathology:</u>	On Days 22, 29, 106, 113, and 190 of the dosing phase and on Days 50 and 85 of the recovery phase.
<u>Gross pathology:</u>	All animals at death or at scheduled sacrifice.
<u>Organ weights:</u>	All animals at death or at scheduled sacrifice.
<u>Histopathology:</u>	All males at death or at scheduled Sacrifice; For females, the eyes, thymus, and mesenteric lymph node were examined for all groups, other tissue for control and high dose only.
<u>Toxicokinetics:</u>	1, 2, and 4 hours postdose on Day 1 and Weeks 15 and 27 for control. 1, 2, 4, 7, 12, and 24 hours postdose on Day 1 and Weeks 15 and 27 for treatment groups.

**RESULTS:**

Mortality: 8 animals at 100 mg/kg (7 males, 1 female)

The causes of deaths:

- 5 males (B68438, B68441, B68445, B68448, and B68453): moribund on Day 43 or 44  
Degeneration and/or inflammation in one or more of the feet with microscopic findings of myxomatous degeneration, vacuolated macrophage infiltrates, and neutrophilic inflammation.
- 1 male (B68440): moribund on Day 180  
Inflammation and related microscopic findings of inflammation of the prostate (moderate) and mesenteric lymph node (marked).
- 1 male (B68447): found dead on Day 164, a mesenteric thrombus

1 female (B68518): an aortic rupture

### Clinical Signs

#### Main

##### *Unscheduled euthanasia*

Hunched posture, swollen feet, discolored (white) teeth, thin appearance, hypoactivity, irregular or audible respiration, yellow skin, and rough haircoat.

##### *Terminal euthanasia*

#### Males

< 100 mg/kg: unremarkable

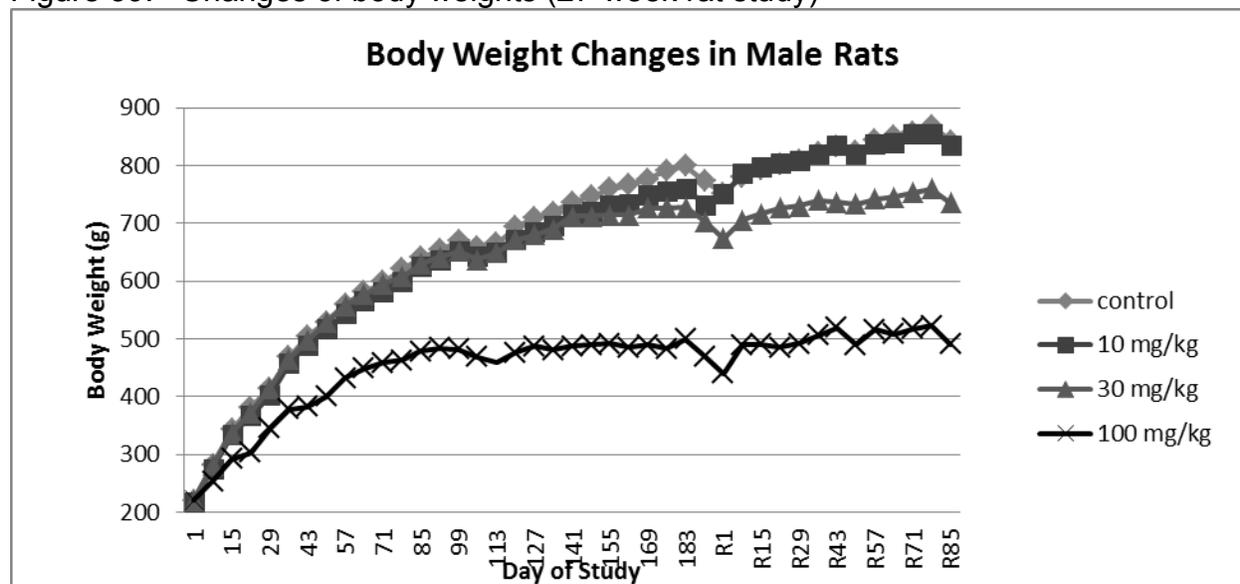
100 mg/kg: rigid stance; small testes; swollen feet, legs, abdomen, penis, or perioral area; white incisor teeth; thin appearance; hypoactivity; lateral recumbence; nonformed feces; clear or red oral discharge; pale eyes, feet, ears, tail, or oral mucosa; audible or irregular respiration; cold to touch (entire body or hind feet); discolored (yellow) skin on the ears, entire body, feet, nose, or tail; discolored (red) skin on the feet, nose, penis, or tail; discolored (red) haircoat on the entire head, nose, perioral; and rough haircoat.

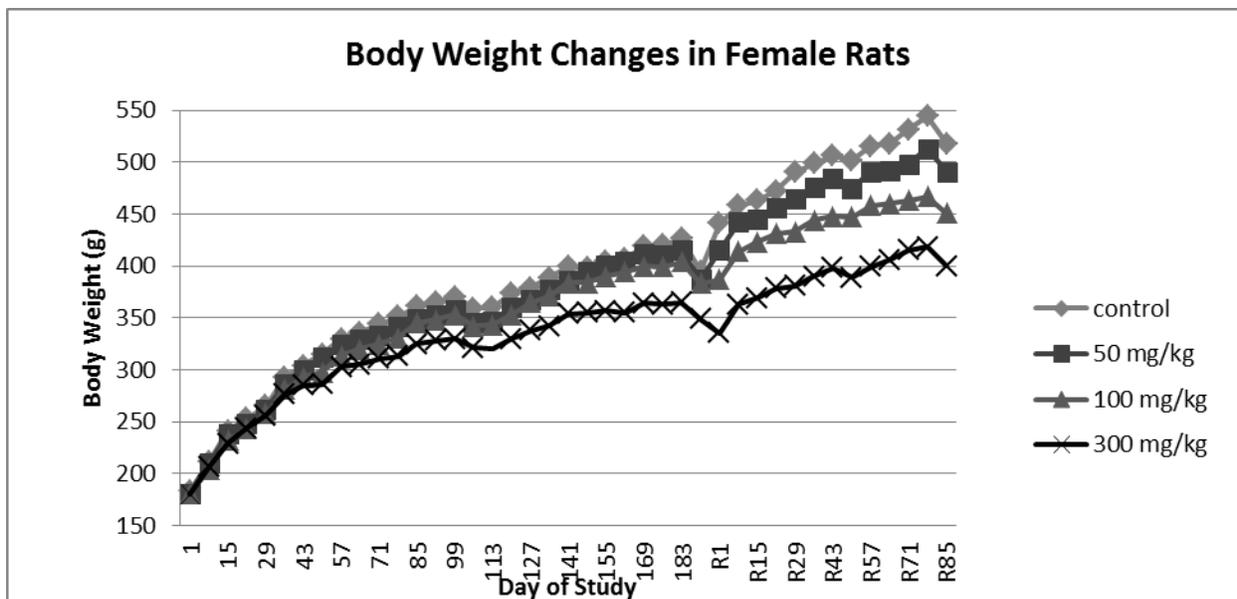
Females: unremarkable

Recovery: unremarkable, except for thin appearance, and rough haircoat in a few recovery males.

### Body Weights

Figure 39. Changes of body weights (27-week rat study)

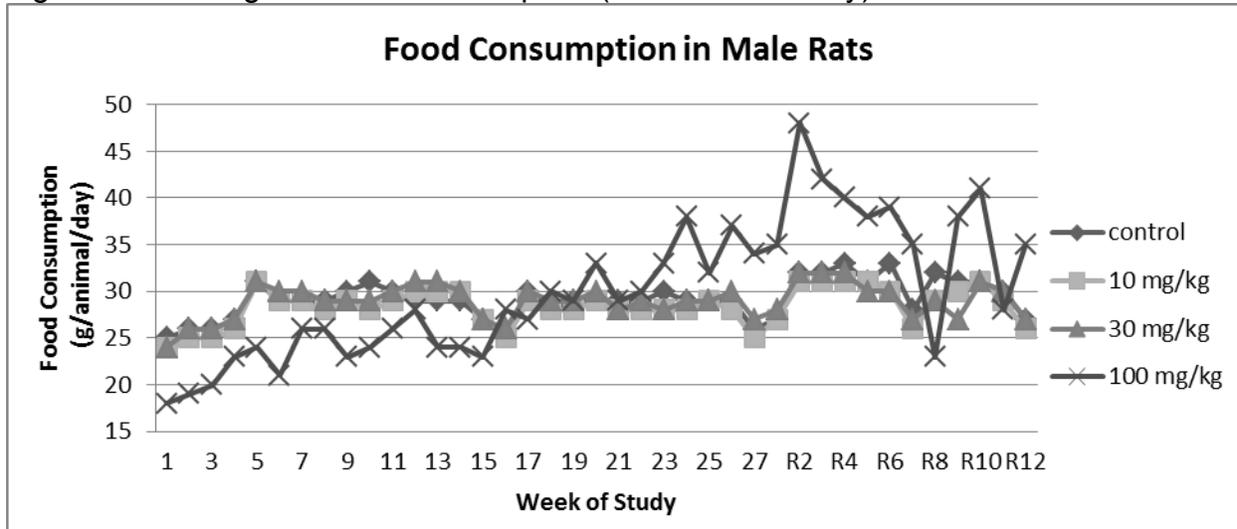


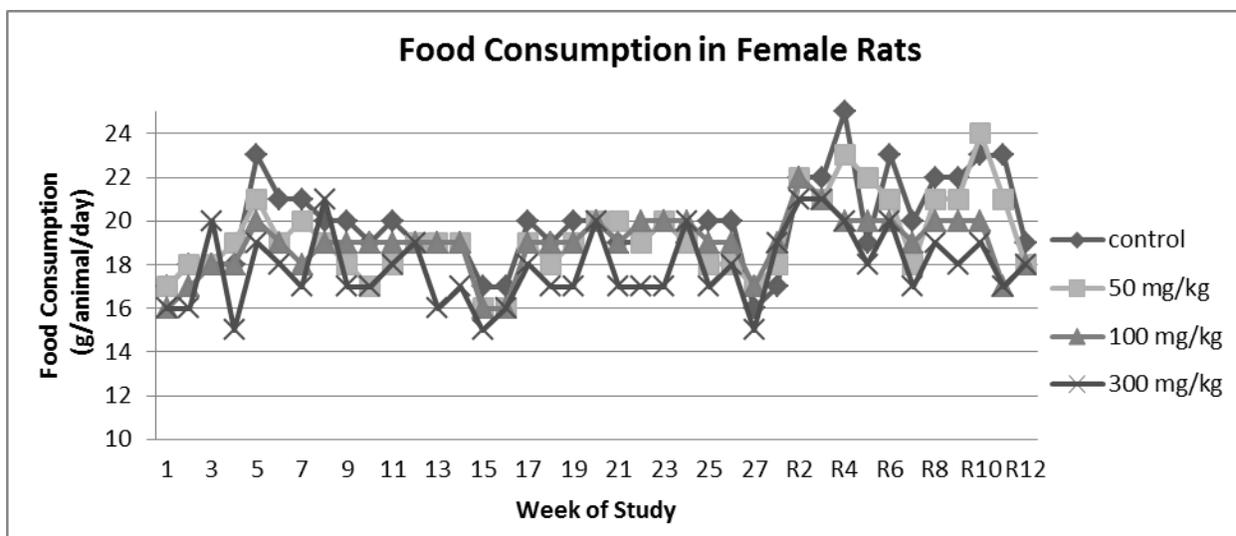


*Summary:* Dose dependent decreased body weights were observed in males ( ↓ up to 38%) and females ( ↓ up to 14%) compared to the controls. There were recovery trends of decreased body weights during the recovery period.

Food Consumption

Figure 40. Changes of food consumption (27-week rat study)





Note: Males given 100 mg/kg/day were fed the meal form of the diet from the first day of the third dosing cycle (Day 57 of the dosing phase) continuing throughout the remainder of the dosing phase.

Summary: Lower food consumption was noted during most weekly intervals for males given 100 mg/kg/day (the nadir at Week 1, ↓ 28%), and for females given 300 mg/kg/day (the nadir at Week 5, ↓ 17%). During the recovery phase, food consumption in males given 100 mg/kg/day remained generally higher than control; food consumption in females was generally remained comparable to controls through the recovery phase.

Ophthalmoscopy:

Slit Lamp Biomicroscopy Observations:

Table 43. Summary of ophthalmoscopy results (27-week rat study)

Male

Gender	Main				Recovery			
	0	10	30	100	0	10	30	100
Dose (mg/kg)								
No. animals examined	20	20	20	20	5	5	5	5
Lens								
Anterior cortical cataract, eyes			2	5				
Complete cataract, eyes				1				3
Incomplete cataract, eyes, diffuse				3				
Fundus								
Degraded view, eyes				9				3
Lens								
Complete cataract, eyes				1				
Incomplete cataract, eyes,				2				
Diffuse incomplete cataract, eyes, focal				1				

Female: unremarkable

Indirect Ophthalmoscopy Observations: The sole abnormality was present in nine males given 100 mg/kg/day.

Note: The sole abnormality identified on indirect ophthalmoscopy was degraded view of the fundus attributable to the presence of advanced stages of cataract.

#### Hematology:

##### *Unscheduled euthanasias*

Hematology measurements were only available from four males euthanized on Day 43 or 44 and from one male euthanized on Day 180 of the dosing phase. Notable findings included lower red cell mass (i.e., red blood cell count, hemoglobin, and hematocrit), decreased reticulocytes, and lower white blood cell count with a generalized decrease in all leukocytes.

##### *Terminal euthanasias*

Table 44. Summary of hematology results in terminal euthanasia (27-week rat study)

Gender	Percent Change From Vehicle Control					
	Male			Female		
	Doses (mg/kg)	10	30	100	50	100
Day 22						
RBC	-4	-13	-20	-4	-4	-9
HGB			-11	-4		
HCT			-14	-4		-5
MCH	6	13	11	2	2	6
MCV	5	13	8			4
Reticulocytes	-12	-24	-21		5	12
Platelets				26	15	20
MPV			15			
WBC	-10	-29	-44			-17
NEUT	-36	-42	-14		-30	-32
Lymphocytes		29	-49			
Monocytes	46	50	-57			
Eosinophils	-43	-61	-100			-50
Basophils	-25	-50	-50		-5	-13
LUC			-50			
Day 29						
RBC	-5	-12	-23		-3	-7
HGB			-9			
HCT			-8			
MCH	7	12	17		3	6
MCV	6	13	19		3	6
Reticulocytes	16	22	94	7	6	23
Platelets		12	37		12	11
MPV		8	17			

Gender	Percent Change From Vehicle Control					
	Male			Female		
	Doses (mg/kg)	10	30	100	50	100
WBC			<u>-43</u>			
NEUT			<u>-18</u>	29		25
Lymphocytes			<u>-49</u>			
Monocytes	<u>40</u>	26	<u>-23</u>			
Eosinophils			<u>-75</u>			
Basophils				11		7
Erythrocytes	<u>-7</u>	<u>-5</u>	<u>-5</u>			
Day 106						
RBC	<u>-14</u>	<u>-25</u>	<u>-37</u>	<u>-6</u>	<u>-11</u>	<u>-18</u>
HGB		<u>-6</u>	<u>-10</u>		<u>-4</u>	<u>-4</u>
HCT	<u>-4</u>	<u>-6</u>	<u>-12</u>		<u>-5</u>	<u>-8</u>
MCH	<u>13</u>	<u>25</u>	<u>43</u>	<u>4</u>	<u>7</u>	<u>13</u>
MCV	<u>11</u>	<u>24</u>	<u>42</u>	<u>3</u>	<u>6</u>	<u>12</u>
Reticulocytes	<u>-19</u>	<u>-24</u>	<u>-23</u>	-10	<u>-26</u>	<u>-27</u>
Platelets	<u>13</u>	<u>14</u>	<u>43</u>	6	<u>13</u>	<u>25</u>
MPV		<u>6</u>	<u>20</u>			
WBC	-12	<u>-38</u>	<u>-59</u>			-7
NEUT		<u>-26</u>			<u>-32</u>	-19
Lymphocytes	-11	<u>-39</u>	<u>-66</u>			
Monocytes		<u>-40</u>	<u>-77</u>			
Eosinophils	<u>-50</u>	<u>-81</u>	<u>-94</u>	<u>-27</u>	<u>-45</u>	<u>-73</u>
Basophils	<u>-33</u>	<u>-50</u>	<u>-67</u>			-50
LUC		<u>-43</u>	<u>-71</u>			
Day 113						
RBC	<u>-10</u>	<u>-20</u>	<u>-34</u>	<u>-4</u>	<u>-11</u>	<u>-14</u>
HGB						<u>-4</u>
HCT						<u>-4</u>
MCH	<u>11</u>	<u>23</u>	<u>43</u>	<u>4</u>	<u>8</u>	<u>13</u>
MCV	<u>11</u>	<u>24</u>	<u>45</u>	<u>4</u>	<u>7</u>	<u>12</u>
Reticulocytes		<u>30</u>	<u>86</u>		9	<u>34</u>
Platelets	<u>16</u>	<u>19</u>	<u>62</u>		11	<u>22</u>
MPV		<u>5</u>	<u>16</u>			
WBC			<u>-45</u>			
NEUT						
Lymphocytes			<u>-51</u>			
Monocytes	10	<u>-46</u>	<u>-85</u>			
Eosinophils						
Basophils	<u>-27</u>	<u>-58</u>	<u>-69</u>	9		<u>-27</u>
Day 190						

Gender	Percent Change From Vehicle Control					
	Male			Female		
Doses (mg/kg)	10	30	100	50	100	300
RBC	<u>-14</u>	<u>-25</u>	<u>-28</u>	<u>-7</u>	<u>-11</u>	<u>-19</u>
HGB		<u>-4</u>				<u>-7</u>
HCT					<u>-4</u>	<u>-8</u>
MCH	<u>13</u>	<u>23</u>	<u>41</u>	<u>5</u>	<u>9</u>	<u>14</u>
MCV	<u>12</u>	<u>26</u>	<u>37</u>	<u>4</u>	<u>7</u>	<u>13</u>
Reticulocytes			-20		-12	<u>-22</u>
Platelets	<u>18</u>	<u>11</u>	12			
MPV		9	16			
WBC	<u>-23</u>	<u>-50</u>	<u>-58</u>			<u>-27</u>
NEUT	<u>-38</u>	<u>-59</u>	<u>-43</u>	-15	-29	-26
Lymphocytes		<u>-43</u>	<u>-65</u>			<u>-41</u>
Monocytes		<u>-74</u>	<u>-74</u>			13
Eosinophils	<u>-40</u>	<u>-60</u>	<u>-40</u>		<u>-44</u>	<u>-67</u>
Basophils					<u>-33</u>	<u>-33</u>
LUC	-33	<u>-67</u>	<u>-78</u>			
Recovery 50						
RBC	-8	-10				
HGB		11				
MCH	<u>10</u>	<u>11</u>	<u>13</u>			
MCV	<u>8</u>	<u>10</u>	<u>9</u>			
Reticulocytes						-13
Platelets				4	17	<u>18</u>
NEUT		19	134	-30	-26	-29
Recovery 85						
RBC	-7	-6				
HGB			6			
MCH	<u>7</u>	<u>7</u>	4			
MCV	<u>6</u>	<u>8</u>				
Reticulocytes						-15
Platelets	13	18	9		19	15
NEUT	20	21	<u>138</u>	-34	-15	-38
Monocytes						<u>-37</u>

Blank cells: unremarkable

Numbers underlined: P < 0.05

*Summary:* The decreases in red cell mass parameters (red blood cell count, hemoglobin, hematocrit), reticulocytes, counts of leukocytes, lymphocytes, monocytes, eosinophil, and LUC were observed in both males and females with greater magnitude of changes in males. The increase in platelet counts was observed in both males and females. The observed changes fully recovered or had recovery trends after a 12-week recovery period.

## Clinical Chemistry:

*Unscheduled euthanasias*

Clinical chemistry measurements were only available from four males euthanized on Day 43 or 44 and from one male euthanized on Day 180 of the dosing phase. Notable findings included lower total protein, albumin, albumin:globulin ratio, cholesterol, and calcium; higher glucose, and gamma glutamyltransferase activity.

*Terminal euthanasias*

Table 45. Summary of clinical chemistry in terminal euthanasia (27-week rat study)

Gender	Percent Change From Vehicle Control					
	Male			Female		
Doses (mg/kg)	10	30	100	50	100	300
Day190						
GLU			<u>37</u>			
UN			<u>131</u>			
AST			<u>103</u>			
ALT			202			
ALP			63			
Recovery 85						
UN			<u>167</u>			
AST	34	23	43			
ALT		61	70			
ALP			111			

Blank cells: unremarkable

Numbers underlined: P < 0.05

*Summary:* Higher glucose and urea nitrogen, higher enzyme activities for aspartate and alanine aminotransferase and alkaline phosphatase were observed in males at 100 mg/kg. Urea nitrogen and ALP did not reverse and remained higher than control at the end of the recovery phase. Increased AST, ALP and ALP may indicate gluconeogenesis, which would correlate with the increased glucose and decreased insulin levels.

Urinalysis: unremarkable.

## Insulin and C-Peptide Analysis:

Table 46. Summary of insulin and c-peptide analysis (27-week rat study)

Gender	Percent Change From Vehicle Control					
	Male			Female		
Doses (mg/kg)	10	30	100	50	100	300
Day 85						
Insulin		-25	<u>-51</u>	-14	-39	-37
C-Peptide		-34	<u>-59</u>			
Day 190						
Insulin		-32	<u>-60</u>	97	-20	19
C-Peptide		-18	<u>-61</u>	52	-13	-9

*Summary:* Lower mean insulin and lower mean C-peptide were observed in male rats at  $\geq 30$  mg/kg/day and in females rats at  $\geq 100$  mg/kg compared to controls.

Note:

- 1) The Applicant stated that there was lower mean insulin in recovery male rats given 100 mg/kg/day compared to control, but no data were provided.
- 2) In most animals, insulin values correlated with microscopic findings of lens degeneration, pancreatic islet cell vacuolation, ameloblast degeneration and/or renal tubuloepithelial cell vacuolation.

## Gross Pathology

*Unscheduled euthanasia*

Table 47. summary of gross pathology in unscheduled euthanasia (27-week rat study)

Sex	Male	Female
Dose (mg/kg)	100	100
Number of Animals	7	1
Foot		unremarkable
Swollen	5	
Tooth, Left Upper Incisor		
Discolored, white	2	
Tooth, Right Upper Incisor		
Discolored, white	2	
Spleen		
Small	1	
Thymus		
Not identified	2	
Lymph Node, Inguinal		
Discolored, yellow	2	
Lymph Node, Mesenteric		
Discolored, yellow	1	
Adrenal		
Large	1	
Lung		
Discolored, dark red, tan	1	
Duodenum		
Large	2	
Jejunum		
Large	2	
Cecum		
Large	2	
Epididymis		
Small	1	
Prostate		
Small	1	
Seminal Vesicle		
Small	1	
Testis		
Small and/or soft	4	
Adipose, Other		
Gelatinous	1	
Skin/Subcutis		
Discolored yellow and/or gelatinous	6	

*Terminal Euthanasia*

Table 48. Summary of gross pathology in terminal euthanasia (27-week rat study)

Male

Gender	Main				Recovery			
Dose (mg/kg)	0	10	30	100	0	10	30	100
No. animals examined	15	15	15	8	5	5	5	5
Adrenal Discolored				1				
Cecum Large				3				2
Duodenum Large				4				
Abnormal contents, gas								2
Epididymis Discolored				1				
Small				1				1
Eye Discolored								2
Ileum Large								2
Abnormal contents, gas								2
Jejunum Large				3				2
Abnormal contents, gas								2
Kidney Rough surface				1				
Lung Discolored				1				
Lymph Node, Inguinal								
Not identified				1				
Lymph Node, Mesenteric								
Small				1				
Spleen small				1				
Stomach Discolored	1			3				
Raised area			1	1				
Seminal Vesicle Small				2				
Thymus Not identified			6	4				1
Testes Small and/or discolored				5				1
Tooth								
Left Lower Incisor Discolored				2				
Left Upper Incisor Discolored				4				
Right Upper Incisor Discolored				2				

Blank cells: unremarkable

*Summary:* Macroscopic changes in male rats involved in the adrenal, kidney, lung, incisor tooth, spleen, thymus, lymph nodes, GI tract, epididymis, seminal vesicle, and testis. The changes were reversible after a 12-week recovery period.

Female: a small thymus observed in one female given 300 mg/kg/day.

## Organ Weights:

Main (Day 106)

Table 49. Summary of organ weight (27-week rat Study)

Study	Percentage deviation from control (n=10)											
	Absolute Organ Weight						Organ Weight/body Weight					
	Main			Recovery			Main			Recovery		
Dose Group (mg/kg)	10	30	100	10	30	100	10	30	100	10	30	100
No./group	15	15	8	5	5	5	15	15	8	5	5	5
Male												
Adrenal Gland	-5		13					10	<u>96</u>			
Spleen	-13	<u>-17</u>	<u>-44</u>				-8	-8	-9			
Thymus	-15	<u>-41</u>	<u>-33</u>	-22			-9	-36		-21	13	-18
Testes		-9	<u>-40</u>						<u>-27</u>		13	30
Epididymis		-9	<u>-37</u>				6	5			13	31
Female												
Unremarkable												

Blank cells: unremarkable

Values underlined: P &lt; 0.05

*Summary:* Test article-related organ weight changes were observed in the adrenal, spleen, thymus, testis, and epididymis in males. By the end of the recovery phase, the observed changes were fully reversed or had recovery trends.

## Histopathology

Table 50. Summary of histopathology (27-week rat Study)

*Unscheduled Euthanasia*

Sex		Male	Female
	Dose (mg/kg)	100	100
	Number of Animals	7 (# actually examined)	1
Pancreas			unremarkable
Vacuolar change, islet cell	-Minimal	2	
	-Mild	4	
	-Moderate	1	
Eye			
Degeneration, lens, bilateral	-Minimal	1	
	-Moderate	1	
Tooth, Right Upper Incisor			
Degeneration/necrosis, ameloblasts	-Minimal	3	
	-Mild	3	
	-Moderate	1	
Infiltrate, mononuclear cell, pigmented	-Minimal	4	
	-Mild	3	
Inflammation, neutrophilic	-Minimal	3	
	-Mild	2	
Tooth, Right Lower Incisor			
Degeneration/necrosis, ameloblasts	-Mild	1(2)	
Infiltrate, mononuclear cell, pigmented	-Minimal	1(2)	
Tooth, Left Upper Incisor			
Degeneration/necrosis, Ameloblasts	-Minimal	1(2)	
	-Moderate	1(2)	
Infiltrate, mononuclear cell, pigmented	-Minimal	1(2)	
	-Mild	1(2)	
Kidney			
Degeneration/regeneration, tubular	-Minimal	2	
	-Mild	3	
Vacuolar change, tubular	-Minimal	1	
	-Mild	5	
Dilatation, tubule(s)	-Mild	1	
Marrow, Femur			
Hypocellular	-Minimal	1	
	-Moderate	5	
Megakaryocytes, increased	-Minimal	3	
	-Mild	1	
Vacuolated cells	-Minimal	5	
Marrow, Sternum			
Hypocellular	-Minimal	2	
	-Mild	4	
Megakaryocytes, increased	-Mild	6	
Vacuolated cells	-Minimal	5	

Sex		Male	Female
	Dose (mg/kg)	100	100
	Number of Animals	7 (# actually examined)	1
Testis			
Degeneration, seminiferous tubule	-Minimal	1	
	-Mild	4	
	-Moderate	1	
	-Severe	1	
Epididymis			
Debris, cellular, lumen	-Minimal	4	
	-Mild	1	
Hypospermia	-Minimal	2	
	-Severe	1	
Prostate			
Decreased secretion	-Marked	1	
Degeneration/necrosis, individual cell	-Minimal	1	
Seminal Vesicle			
Decreased secretion	-Marked	1	
Spleen			
Lymphocytes, decreased	-Minimal	3	
	-Mild	3	
	-Moderate	1	
Erythrocytes, decreased	-Minimal	3	
	-Moderate	1	
Thymus			
Lymphocytes, decreased	-Moderate	1	
	-Marked	6	
Atrophy, adipose	-Mild	5	
	-Moderate	2	
Lymph Node, Mesenteric			
Lymphocytes, decreased	-Minimal	1	
	-Mild	2	
	-Moderate	2	
	-Marked	2	
Infiltrate, macrophages, increased	-Mild	2	
	-Moderate	3	
	-Marked	1	
Inflammation, neutrophilic	-Minimal	3	
	-Mild	2	
Erythrocytes, sinus	-Minimal	2	
	-Mild	1	
Lymph Node, Inguinal			
Lymphocytes, decreased	-Minimal	2(6)	
	-Mild	2(6)	
	-Moderate	1(6)	
GALT/Peyer's Patch			

Sex		Male	Female
Dose (mg/kg)		100	100
Number of Animals		7 (# actually examined)	1
Lymphocytes, decreased	-Minimal	3(6)	
	-Mild	2(6)	
Femur			
Trabeculae, decreased	-Mild	1	
	-Moderate	2	
Adrenal, Cortex			
Hypertrophy	-Mild	3	
	-Moderate	4	
Liver			
Hypertrophy/vacuolation, hepatocellular	-Minimal	3	
	-Mild	2	
Heart			
Hyperplasia, mesenchymal cell	-Minimal	1	
	-Mild	3	
Inflammation, neutrophilic	-Minimal	2	
	-Mild	1	
Infiltrate, macrophages, vacuolated	-Minimal	4	
Aorta			
Inflammation, neutrophilic	-Minimal	2	
Lung			
Infiltrate, macrophages, alveolus	-Mild	4	
	-Moderate	3	
Foot			
Degeneration, myxomatous	-Mild	1(5)	
	-Moderate	2(5)	
	-Marked	2(5)	
Infiltrate, macrophages, vacuolated	-Minimal	5(5)	
Inflammation, neutrophilic	-Minimal	1(5)	
	-Mild	2(5)	
Hemorrhage	-Minimal	4(5)	
Joint, Stifle			
Degeneration, myxomatous	-Minimal	1	
	-Mild	2	
Inflammation, neutrophilic	-Minimal	1	
	-Mild	1	

*Terminal Euthanasia*

## Male

Study	Main				Recovery			
	0	10	30	100	0	10	30	100
Dose (mg/kg)	0	10	30	100	0	10	30	100
No. animals examined	15	15	15	8	5	5	5	5
Pancreas Vacuolar change, islet cell								
-minimal			3	3				
-mild				3				
-moderate				1				
Eye								
Degeneration, lens, bilateral								
-minimal		1	1	1				
-mild			2	3				
-moderate				3				2
-marked								1
Degeneration, lens, unilateral								
-minimal							1	
Tooth, Right Upper Incisor								
Degeneration/necrosis,								
Ameloblasts								
-minimal				3				
-mild				3				
Infiltrate, mononuclear cell,								
Pigmented								
-minimal				6				3
-mild				2				2
Tooth, Right Lower Incisor								
Degeneration/necrosis,								
Ameloblasts								
-minimal				1				
-mild				1				
Infiltrate, mononuclear cell,								
Pigmented								
-minimal				4			1	3
-mild				1				
Tooth, Left Upper Incisor								
Degeneration/necrosis,								
Ameloblasts								
-minimal				2				
-mild				2				
Infiltrate, mononuclear cell,								
Pigmented								
-minimal				2				
-mild				2				
Tooth, Left lower Incisor								
Degeneration/necrosis,								
Ameloblasts								
-mild				1				
Kidney								
Vacuolar change, tubular								
-minimal				2				

Study	Main				Recovery			
	0	10	30	100	0	10	30	100
Dose (mg/kg)								
No. animals examined	15	15	15	8	5	5	5	5
Dilatation, tubule(s) Nephropathy, chronic progressive	-mild		1	3				2
	-moderate			1				1
	-minimal			4				
	-minimal	2	7	7	5	2	2	4
	-mild	1	0	2	3	1		1
	-moderate						1	1
Marrow, Femur Hypocellular	-minimal			4	3			
	-mild			6	5			
	Megakaryocytes, increased				1			
	-minimal				2			
	-mild							
Marrow, Sternum Hypocellular	-minimal			7	2			
	-mild			3	6			
	Megakaryocytes, increased				3			
	-minimal				3			
	-mild							
Testis Degeneration, seminiferous Tubule	-minimal			1				1
	-mild			1	3			
	-moderate				2			2
	-marked			1	2			
Epididymis Debris, cellular, lumen-	-minimal			1	4			3
	-mild			2	2			1
	-moderate				2			
	Hypospermia				1			
	-minimal				3			
	-mild				1			1
	-moderate				2			
	-severe							
Seminal Vesicle Decreased secretion	-moderate				1			
	-marked				1			
Lymph Node, Mesenteric Small								
					1			
Spleen Lymphocytes, decreased								
	-minimal				2			
	-mild				2			

Study	Main				Recovery			
	0	10	30	100	0	10	30	100
Dose (mg/kg)	0	10	30	100	0	10	30	100
No. animals examined	15	15	15	8	5	5	5	5
Erythrocytes, decreased -mild Hematopoiesis, extramedullary, Increased -mild				1				1
Thymus Lymphocytes, decreased -minimal -mild -moderate -marked  Atrophy, adipose -minimal -mild -moderate	2	9 1	3 4 6 1	4 3 5 1 3 2		1 1	1	1 2  1 1
Lymph Node, Mesenteric Lymphocytes, decreased -minimal -mild -moderate Infiltrate, macrophages, Increased -minimal -mild -moderate Inflammation, neutrophilic -mild	1		1	2 3 2 2 2 4 1		1		1  2 1
Lymph Node, Inguinal Lymphocytes, decreased -minimal -mild -moderate			7	1 1 2				
GALT/Peyer's Patch Lymphocytes, decreased -minimal				2				
Femur Trabeculae, decreased-mild -moderate -marked		6	4 7	2 6		2	1 1	1 2
Adrenal, Cortex Hypertrophy -minimal -mild -moderate	2	7 1	7 5	4 4				
Liver Hypertrophy/vacuolation, Hepatocellular -minimal -mild	3	8 3	7 6	4 3				

Study	Main				Recovery			
	0	10	30	100	0	10	30	100
Dose (mg/kg)								
No. animals examined	15	15	15	8	5	5	5	5
Lung Infiltrate, macrophages, alveolus								
-minimal	3	2	4	4	1	1		
-mild				2				1
-moderate				1				
Stomach, Glandular Decreased mucus, glands								
-mild			1	5				
-moderate								3
Stomach, Nonglandular Degeneration, epithelial cell								
-minimal			2					1
-mild			1	2				1
Hyperplasia/hyperkeratosis								
-minimal			2					1
-mild			1	3				
-moderate								1
Inflammation, neutrophilic								
-minimal								1
-mild			1	1				
Erosion/ulcer-minimal				1				
-mild			1					
Skin/Subcutis Atrophy, adipose								
-minimal				2				
-mild				1				
-moderate				3				

Blank cells: unremarkable

*Summary:* Treatment related changes involved in male rats in the pancreas, eye, tooth, kidney, hematolymphoid system, reproductive system, thymus, adrenal cortex, skin, lung, and stomach. Changes in eye and kidney did not recover. All other changes reversed or had recovery trends at the end of a 12-week recovery period.

Female

Study	Main				Recovery			
	0	50	100	300	0	50	100	300
Dose (mg/kg)	0	50	100	300	0	50	100	300
No. animals examined	15	15	14	15	5	5	5	5
Pancreas								
Vacuolar change, islet cell -minimal		1						
Fibrosis/hemorrhage, islet -minimal		1	2					
Eye								
Degeneration, lens, bilateral -minimal		1	1					
Degeneration, lens, unilateral -minimal			1					
Thymus								
Lymphocytes, decreased -minimal -mild	3	2	3	6			1	
Lymph Node, Mesenteric								
Infiltrate, macrophages, Increased -minimal -mild -moderate		4	3 2	12 3			3	4
No. animals examined	15	0	0	15	0	0	0	0
Kidney								
Nephropathy, chronic progressive -minimal	3	-	-	2	-	-	-	-

Blank cells: unremarkable

“-“ not examined

*Summary:* Treatment related changes were observed in the pancreas, eyes, thymus and lymph node. The treatment-related changes recovered or partially recovered after a 12-week recovery period.

Others:

## Immunohistochemistry

Pancreases from control and high dose (100 mg/kg/day) males from the dosing and recovery phases were prepared by IHC for morphometric analysis. Tissue sections of pancreas were stained for anti-glucagon (vector red) and anti-insulin (DAB), and imaged using a Perkin Elmer Vectra automated imaging system equipped with a multispectral camera. Images of each islet within a pancreas section were acquired in an automated fashion and analyzed with Perkin Elmer's InForm software. The relative area and numbers of alpha and beta cells were calculated as well as the average cell size for each tissue section.

The following summary was provided by the Applicant:

- Reduction in the relative percentage of beta cell area in animals given 100 mg/kg/day PD-0332991;
- Reduction in the percentage and total number of beta cells in animals given 100 mg/kg/day PD-0332991;
- Higher average alpha cell size in animals given 100 mg/kg/day PD-0332991.

#### Transmission Electron Microscopy

The following summary was provided by the Applicant.

- Unscheduled necropsy (1 male at 100 mg/kg): Pancreatic islets in Animal No. B68448 given 100 mg/kg/day and necropsied at an unscheduled interval had Beta cell granules larger than those present in controls. Because only one animal necropsied at an unscheduled interval was submitted for examination, the relationship of this finding to PD-0332991 was not clear.
- Terminal necropsy (3 males at 100 mg/kg/day): 2 animals had vacuoles in their Beta cells that were correlate of the light microscopic finding.
- Recovery animals (3 males at 100 mg/kg/day): unremarkable.

#### Gene Expression

Four genes associated with gluconeogenesis (PGC1a, PEPCK, G6PC, and FOXO1) and four housekeeping genes (ACTB, GAPDH, B2M, and HPRT1) were evaluated by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) using standard methods.

The following summary was provided by the Applicant:

Gene expression analysis for both FOXO1 and PGC1 $\alpha$  in rat livers could not be interpreted as a number of samples resulted in an average Ct value greater than 35, which is the threshold for confident interpretation of changes in expression. The gene expression data suggest that chronic inhibition of CDK4/6 over 6 months did not induce expression of two genes associated with gluconeogenesis, specifically G6PC and PEPCK, when evaluated at the end of the dosing phase (Day 190 of the dosing phase). However, given the poor quality of RNA isolated from the formalin fixed paraffin embedded samples, other experimental approaches would be needed to more definitively determine if chronic CDK4/6 inhibition induced genes associated with gluconeogenesis.

Toxicokinetics:

Table 51. Summary of Toxicokinetic Parameters in rats (PD-0332991) (27-week rat study)

Study day	Dose level (mg/kg/dose)	Gender	C <sub>max</sub> (ng/mL)	Dose Normalized C <sub>max</sub>	AUC <sub>0-24</sub> (ng.h/mL)	Dose Normalized AUC <sub>0-24</sub>	t <sub>max</sub> (h)
1	10	M	278	28	3080	308	6
		F	-	-	-	-	-
	30	M	739	25	10100	337	7
		F	-	-	-	-	-
	50	M	-	-	-	-	-
		F	193	4	1700	34	5
	100	M	1740	17	32200	322	11
		F	287	3	2390	24	2
300	M	-	-	-	-	-	
	F	556	2	6890	23	7	
103	10	M	514	51	5570	557	4
		F	-	-	-	-	-
	30	M	1490	50	21700	723	4
		F	-	-	-	-	-
	50	M	-	-	-	-	-
		F	396	8	2310	46	1
	100	M	2060	21	35000	350	5
		F	407	4	3580	36	4
300	M	-	-	-	-	-	
	F	552	2	5360	19	2	
187	10	M	573	57	6000	600	4
		F	-	-	-	-	-
	30	M	1490	50	21300	710	5
		F	-	-	-	-	-
	50	M	-	-	-	-	-
		F	447	9	2650	53	1
	100	M	1970	20	29300	293	5
		F	1200	12	4450	45	1
300	M	-	-	-	-	-	
	F	1010	3	12800	43	2	

## Conclusion:

- On Days 1, 103, 187 plasma exposures (C<sub>max</sub> and mean AUC<sub>0-24</sub>) were increased in proportion to the dose increases from 10 mg/kg to 30 mg/kg, but increased less than proportionally to the dose increases from 30 mg/kg to 100 mg/kg in male rats; plasma exposures (C<sub>max</sub> and mean AUC<sub>0-24</sub>) were generally increased less than proportionally to the dose increases at the tested ranges (50 mg/kg to 300 mg/kg) in female rats.
- Higher exposure for the males was observed at 100 mg/kg compared to females at 300 mg/kg;

- No apparent accumulation was observed following repeated dosing;
- $T_{max}$  ranged from 4 to 11 hours in male rats, and 1 to 7 hours in female rats.

**Study title:** A 15-week toxicity study of PD-0332991 by oral gavage administration in dogs with a 4-week recovery period

Study no.: 20026126

Study report location: M4.2.3.2, page 1-1150

Conducting laboratory and location:

(b) (4)

Date of study initiation: April 17, 2012

GLP compliance: yes

QA statement: yes ( X ) no ( )

Drug, batch #, and % purity: PD-0332991

Batch#: GR06024

Purity: 98.7%

**Key study findings:**

- Once daily oral administration of PD-0332991 did not cause severe toxicity in dogs at doses up to 2.0 mg/kg/day;
- Target organ effects were observed in the thymus, bone marrow, gut-associated lymphoid tissue, testes, and epididymides;
- All PD-0332991 effects observed on Day 106 had partially or completely reversed by the end of the recovery period, with the exception of effects on the testes and epididymides.

**METHODS:**

<b>Doses:</b>	0.2, 0.6, 2 mg/kg* * Doses for this study were based on findings from a 3-week oral dose study.
<b>Frequency of dosing:</b>	Once daily for 3 weeks followed by a 1-week off dose period repeated up to 4 times followed by a 4-week recovery period
<b>Route of administration</b>	Oral gavage.
<b>Dose volume</b>	1 mL/kg
<b>Formulation/Vehicle</b>	0.5% (w/v) Methylcellulose in Reverse Osmosis Deionized (RODI) Water
<b>Species/strain:</b>	Beagle dog
<b>Age</b>	approximately 13 months old
<b>Weight</b>	Male: 7.1 - 9.4 kg; Female: 6.0 - 8.4 kg
<b>Number/sex/group</b>	3/sex/group (main); 2/sex/group (recovery, control and HD groups)
<b>Satellite groups</b>	none
<b>Unique study design</b>	none
<b>Deviation from study protocol</b>	none

**OBSERVATIONS AND TIMES:**

<b>Mortality</b>	Once daily																																									
<b>Cage Side Observations</b>	Once daily																																									
<b>Detailed physical examinations</b>	Weekly, beginning week -1, and at termination																																									
<b>Body weights</b>	on the day of randomization (Day -1) and on Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, 92, 99, 105, 112, 119, 126, and 133																																									
<b>Food consumption</b>	daily, beginning Day -7, throughout the dosing and recovery phases																																									
<b>Ophthalmoscopy</b>	Day -7, and Day 105																																									
<b>EKG</b>	once prior to in-life initiation (Day -6/Day -1 for repeats), predose and postdose during the last week of dosing (4 to 5 hours postdose [at approximate $T_{max}$ ] on Day 100), and prior to recovery termination (Day 128).																																									
<b>Hematology and coagulation</b>	Week -1, on Days 21, 28, 46, 106, and 134																																									
<b>Serum chemistry</b>	Week -1, on Days 21, 28, 46, 106, and 134																																									
<b>Urinalysis</b>	on Days 106, and 134																																									
<b>Gross pathology:</b>	All animals at necropsy, on Day 106 or Day 134																																									
<b>Organ weights:</b>	All animals at necropsy, on Day 106 or Day 134																																									
<b>Histopathology:</b>	All animals at necropsy, on Day 106 or Day 134 Adequate Battery: yes (x), no ( ) Peer review: yes (x), no ( )																																									
<b>Toxicokinetics:</b>	<table border="1"> <thead> <tr> <th rowspan="2">Group No.</th> <th colspan="6">Sample Collection Time Points (Time Postdose) on Days 1, 49, and 105</th> </tr> <tr> <th>1 hr</th> <th>2 hr</th> <th>4 hr</th> <th>7 hr</th> <th>12 hr</th> <th>24 hr</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> </tr> <tr> <td>2</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> </tr> <tr> <td>3</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> </tr> <tr> <td>4</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> </tr> </tbody> </table> <p>X = sample collected.</p>	Group No.	Sample Collection Time Points (Time Postdose) on Days 1, 49, and 105						1 hr	2 hr	4 hr	7 hr	12 hr	24 hr	1	X	X	X	X	X	X	2	X	X	X	X	X	X	3	X	X	X	X	X	X	4	X	X	X	X	X	X
Group No.	Sample Collection Time Points (Time Postdose) on Days 1, 49, and 105																																									
	1 hr	2 hr	4 hr	7 hr	12 hr	24 hr																																				
1	X	X	X	X	X	X																																				
2	X	X	X	X	X	X																																				
3	X	X	X	X	X	X																																				
4	X	X	X	X	X	X																																				

**RESULTS:**

Mortality: none

Clinical signs: A slight dose-related increase in the incidence of soft stools and red/swollen pinna(e) was noted at 2.0 mg/kg/day compared to the controls during the dosing phase of the study.

Bodyweight: Slight test article-related decreases in body weight compared to the control group were noted during the fourth dosing cycle at 2.0 mg/kg/day in males (↓ up to 4%) and females (↓ up to 5%). Body weight gain in test article-treated groups was comparable to the controls during the recovery phase.

Food consumption: unremarkable

Ophthalmoscopy: unremarkable

EKG: unremarkable

Hematology:

Table 52. Summary of hematology (15-week dog study)

Gender	Percent Change From Vehicle Control					
	Male			Female		
	Doses (mg/kg)	0.2	0.6	2	0.2	0.6
Day 21						
Erythrocytes		-4	<u>-12</u>	-7		-10
Hemoglobin			-10			-9
Hematocrit			-9	-7		-10
Reticulocytes	14	-17	-15	-20	-38	-28
Platelets		-5	-11		-13	<u>-38</u>
Fibrinogen			62			13
Leukocytes	-18	-29	<u>-47</u>	<u>-35</u>	<u>-44</u>	<u>-73</u>
Lymphocytes	-5	-28	-28	-37	-40	<u>-52</u>
Monocytes	-8	-18	-55	-40	-37	<u>-81</u>
Segd neutrophils	-27	-29	<u>-57</u>	-36	-46	<u>-82</u>
Eosinophils	-24	-57	-76	15	-64	<u>-82</u>
Basophils	34	-14	-32	-38	-38	<u>-77</u>
LUC	19	-5	-29	-44	-58	-50
Day 28						
Erythrocytes	-7	-10	<u>-18</u>			
Hemoglobin	-4	-7	<u>-14</u>			
Hematocrit		-7	<u>-14</u>			
Reticulocytes	18		34		-17	
Platelets				14	-21	<u>-36</u>
Fibrinogen		23	27			15
Leukocytes		-13		-21	-10	<u>-46</u>
Lymphocytes		-10	28	27	14	18
Monocytes			40	-27		-26
Segd neutrophils		-10	-9	-18	-8	<u>-62</u>
Eosinophils	-20	-65	-69		-55	<u>-78</u>
Basophils	-9	-42		-31		-22
LUC	-17	-33	10	-31	-31	-33
Day 46						
Erythrocytes	-8	-10	<u>-18</u>			-7
Hemoglobin	-5	-7	<u>-15</u>	-7		-5
Hematocrit		<u>-6</u>	<u>-12</u>			
Reticulocytes		-23	23		-44	-27
Platelets	15	7	15			<u>-33</u>
Fibrinogen			40		8	19
Leukocytes	-22	-33	<u>-44</u>	-31	-31	<u>-65</u>

Gender	Percent Change From Vehicle Control					
	Male			Female		
	Doses (mg/kg)	0.2	0.6	2	0.2	0.6
Lymphocytes	-9	-21	-22	-30	-36	<u>-52</u>
Monocytes	-28	-45	<u>-38</u>	-45	-30	<u>-74</u>
Segd neutrophils	-29	-35	<u>-51</u>	-33	-27	<u>-72</u>
Eosinophils	-36	-66	<u>-83</u>	71	-41	<u>-68</u>
Basophils	7	<u>-56</u>	<u>-64</u>	-38	-46	<u>-68</u>
LUC	-7	-63	-11	-33	-33	-40
Day 106						
Erythrocytes	-9	-12	<u>-26</u>		-10	-13
Hemoglobin		<u>-5</u>	<u>-17</u>		-6	
Hematocrit		-5	<u>-15</u>			<u>10</u>
MCH	<u>6</u>	<u>8</u>	<u>12</u>			<u>9</u>
MCV			<u>15</u>	<u>5</u>	<u>5</u>	<u>18</u>
Reticulocytes	-11	-43	-45	-12	-37	-47
Platelets			-7			<u>-31</u>
Fibrinogen	-31	-27	-12	-16	12	24
PT			<u>16</u>	-4	-4	-7
Leukocytes	-35	<u>-47</u>	<u>-60</u>	-27	-40	<u>-61</u>
Lymphocytes	-22	<u>-35</u>	<u>-42</u>	-37	-38	<u>-47</u>
Monocytes	-34	<u>-43</u>	<u>-63</u>	-19	-35	<u>-59</u>
Segd neutrophils	<u>-45</u>	<u>-54</u>	<u>-69</u>	-22	-41	<u>-70</u>
Eosinophils		-40	<u>-70</u>	-12	-60	<u>-79</u>
Basophils	-15	<u>-60</u>	<u>-78</u>	-7	-43	<u>-63</u>
LUC	-28	-49	-39	15	4	
Day 134						
Erythrocytes	-	-	-18			-9
Hemoglobin	-	-	<u>-11</u>			-4
Hematocrit	-	-	<u>4</u>			6
MCH	-	-	<u>8</u>			5
MCV	-	-	12			7
Reticulocytes	-	-	-15			-6
Platelets	-	-				-34
Leukocytes			31			-34
Lymphocytes			-7			-46
Monocytes			106			-9
Segd neutrophils			42			-28
Eosinophils			-33			-5
Basophils			44			-29
LUC			233			-37

Blank cells: unremarkable

Values underlined: P < 0.05

**Summary:** Dose-related decreases in red blood cell parameters, decreases in leukocytes including neutrophil counts, lymphocyte counts, monocyte counts and eosinophil counts, and decreases in platelets were observed at the end of each dosing period in both sexes. The magnitudes of decreases were similar on day 21, 46 and 106 (ends of dosing periods). The observed changes recovered at least partially on Day 28, following a 1-week off dose period. After a 4-week recovery period, all findings had recovered in males and females.

Clinical Chemistry: unremarkable

Urinalysis: unremarkable

### Gross Pathology

Table 53. Summary of gross pathology (15-week dog study)

Main (Day 106)

Gender	Male				Female			
	0	0.2	0.6	2	0	0.2	0.6	2
Dose (mg/kg)	0	0.2	0.6	2	0	0.2	0.6	2
No. animals examined	3	3	3	3	3	3	3	3
Thymus Small								2
Testes Small			1	1	-	-	-	-
Epididymides Small				1	-	-	-	-

Recovery (Day 134)

Gender	Male		Female	
	0	2	0	2
Dose (mg/kg)	0	2	0	2
No. animals examined	2	2	2	2
Testes Small		1	-	-
Epididymides Small		1	-	-

Blank cells: unremarkable

**Summary:** Gross findings related to treatment were observed primarily in the thymus, testes, and epididymides. The observed changes in the testes and epididymides did not recover after a 4-week recovery period.

**Note:** Small thymus correlated microscopically with decreased lymphoid cellularity and reduced thymic weights. Small testes and epididymides microscopically correlated with seminiferous tubule degeneration in the testes and hypospermia in the epididymis.

## Organ Weights:

Table 54. Summary of organ weight (15-week dog Study)

Main (Day 106)

Study	Percentage deviation from control (n=10)							
	Absolute Organ Weight				Absolute Organ Weight			
	Main		Recovery		Main		Recovery	
Dose Group (mg/kg)	0.2	0.6	2	2	0.2	0.6	2	2
No. Animal/group	3	3	3	2	3	3	3	2
Male								
Testes	24	-24	-29	-37	18	-27	-30	-38
Thymus	-19	<u>-25</u>	<u>-49</u>		-19	-26	-47	
Female								
Thymus	23	-40	-75		14	-44	-75	

Blank cells: unremarkable

Values underlined: P &lt; 0.05

*Summary:* PD-0332991-related organ weight decreases were noted in the testes, and thymus in males and females. The changes in the testes appeared to progress even after cessation of the treatment.

**Histopathology**

Main (Day 106)

Table 55. Summary of histopathology (15-week dog study)

Gender	Male				Female			
Dose (mg/kg)	0	0.2	0.6	2	0	0.2	0.6	2
No. animals examined	3	3	3	3	3	3	3	3
Bone marrow, femur Hypocellularity, Hematopoietic -minimal				2				
Bone marrow, sternum Hypocellularity, Hematopoietic -minimal				2				1
Gut-Associated Lymphoid Tissue Decreased Cellularity, Lymphoid -minimal								1
Testes Degeneration of seminiferous Tubules -minimal -mild -moderate	2	2	3	3	-	-	-	-
Epididymides Hypospermia -minimal -mild -severe			1		-	-	-	-
Cellular debris, intraductal -minimal	1		1	2	-	-	-	-

Gender	Male				Female			
Dose (mg/kg)	0	0.2	0.6	2	0	0.2	0.6	2
No. animals examined	3	3	3	3	3	3	3	3
-mild			1		-	-	-	-
Thymus								
Decreased cellularity, lymphoid								
-minimal	2	1	1		1			
-mild				2				1
-moderate				1				2

Recovery (Day 134)

Gender	Male		Female	
Dose (mg/kg)	0	100	0	200
No. animals examined	2	2	2	2
Testes				
Degeneration of seminiferous tubules				
-mild		1	-	-
-moderate		1	-	-
Epididymides				
Hypospermia				
-mild		1	-	-
-moderate		1	-	-
Cellular debris, intratubular		2	-	-

Blank cells: unremarkable

*Summary:* Treatment related changes involved in the testes, epididymides in males, and bone marrow, lymph nodes, and thymus in males and females. The changes in the testes and epididymides did not recover by the end of a 4-week recovery period.

Toxicokinetics:

Table 56. Summary of toxicokinetic parameters in rats (PD-0332991) (15-week dog)

Study day	Dose level (mg/kg/dose)	Gender	C <sub>max</sub> (ng/mL)	Dose Normalized C <sub>max</sub>	AUC <sub>0-24</sub> (ng.h/mL)	Dose Normalized AUC <sub>0-24</sub>	T <sub>max</sub> (h)
1	0.2	M	3.6	18	59	295	6
		F	5.5	27.5	68	340	3
	0.6	M	17.7	29.5	282	470	3
		F	22.5	37.5	357	595	3
	2	M	55.4	27.7	866	433	11
		F	69.9	35	1080	540	5
49	0.2	M	7.7	38.5	136	680	4
		F	9.1	45.5	135	675	4
	0.6	M	23.7	39.5	419	698	5
		F	21.4	35.7	375	625	4
	2	M	112	56	2040	1020	6
		F	94.6	47.3	1690	845	5
105	0.2	M	9.6	48	153	765	5
		F	8.2	41	129	645	4
	0.6	M	29.5	49.2	521	868	5
		F	32.4	54	543	905	4
	2	M	98.7	49.4	1660	830	6
		F	101	50.5	1650	825	4

## Conclusion:

- On Days 1, 49, 105 plasma exposures (C<sub>max</sub> and mean AUC<sub>0-24</sub>) were generally increased proportionally to the dose increases from 0.2 mg/kg to 2 mg/kg;
- There were no apparent gender difference;
- The exposure of PD-0332991 on Days 49 and 105 increased by 2-fold when compared to Day 1, indicating accumulation s following repeated dosing;
- The exposure of PD-0332991 on Days 49 and 105 generally remained similar, indicating no apparent accumulation with repeated cycles;
- T<sub>max</sub> ranged from 6 to 11 hours in male rats, and 3 to 5 hours in female rats.

**Note:** The report for the following study was reviewed for the purpose of comparing the incidence, severity and dose-relationship of findings to those in the 15-week repeat-dose toxicity study in dogs. No new toxicities were identified in the 39-week study compared to the 15-week study, therefore only summary of the study and results is presented below.

**Study title:** 39-week oral gavage chronic toxicity and toxicokinetic study with PD-0332991 in dogs with a 12-week recovery phase

**Methods:** Male and female beagle dogs (6/sex/group) were given vehicle control article or PD-0332991 at a dose level of 0.2, 0.6, or 3.0 mg/kg/day via oral gavage with a dosing regimen consisting of 3-week daily dosing cycles with 1 week off repeated 10 times (for a total dosing phase of 39 weeks). The reversibility or persistence of any effects was assessed after a 12-week recovery. Standard toxicology procedure was followed for assessment of toxicity. Blood samples were collected for toxicokinetic evaluations.

**Results:** No unscheduled deaths occurred, and no test article-related clinical observations, ophthalmic observations or effects on body weights, food consumption, or ECGs were noted during the dosing or recovery phases. Effects on hematopoiesis including decreases in leukocyte and absolute reticulocyte counts were noted during 3 weeks of dosing in each cycle followed by partial or complete recovery during the week without dosing. PD-0332991-related decreases in red blood cell and platelet counts and increases in mean corpuscular volume, mean corpuscular hemoglobin, red cell distribution width, and mean platelet volume exhibited little or no evidence of reversibility in the week without dosing. All PD-0332991-related hematology effects exhibited reversibility during the 12-week recovery phase. Bone marrow hypocellularity (hematopoietic) and/or decreased lymphoid cellularity were present in the gut-associated lymphoid tissue (GALT), mesenteric lymph node, spleen, and/or thymus of males given 3.0 mg/kg/day and females given >0.6 mg/kg/day. In the male reproductive system, degeneration of the seminiferous tubules was present in the testes of males in all dosed groups. Following the 12-week recovery phase, PD-0332991-related microscopic findings were limited to the testes and epididymides, where mild degeneration of the seminiferous tubules (testis) with or without intratubular cellular debris (epididymis) was present in one male given 0.6 mg/kg/day and one male given 3.0 mg/kg/day.

**Histopathology inventory**

<b>Study</b>	20026125	8282224	20026126
<b>Species</b>	<b>Rat</b>	<b>Rat</b>	<b>Dog</b>
Adrenals	x*	x*	x*
Aorta	x	x	x
Bone Marrow smear	x	x	x
Bone (femur)	x	x	x
Brain	x*	x*	x*
Cecum	x	x	x
Cervix	x	x	x
Colon	x	x	x
Duodenum	x	x	x
Epididymis	x*	x*	x
Esophagus	x	x	x
Eye	x	x	x
Fallopian tube			
Gall bladder		x	x
Gross lesions	x	x	x
GALT	x	x	x
Harderian gland	x	x	
Head (including lower jaw and teeth)	x		
Heart	x*	x*	x*
Ileum	x	x	x
Injection site			
Jejunum	x	x	x
Joint (knee)			
Kidneys	x*	x*	x*
Lachrymal gland			
Larynx	x	x	x
Liver	x*	x*	x*
Lungs	x	x	x
Lymph nodes, cervical			
Lymph nodes, inguinofemoral	x		
Lymph nodes mandibular			
Lymph nodes, mesenteric	x	x	x
Lymph nodes, popliteal			x

Mammary Gland	X	X	X
Nasal cavity			
Optic nerves	X	X	X
Ovaries	X*	X*	X
oviducts		X*	X
Pancreas	X	X	X
Parathyroid	X	X	X
Peripheral nerve			
Peyer's patches			
Pharynx			
Pituitary	X	X	X
Prostate	X	X*	X
Rectum	X		
Salivary gland	X	X	X
Sciatic nerve	X	X	X
Seminal vesicles	X		
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord	X	X	X
Spleen	X*	X*	X*
Sternum	X	X	X
Stomach	X	X	X
Teeth		X	
Testes	X*	X*	X*
Thymus	X*	X*	X*
Thyroid	X	X	X
Tongue	X	X	X
Trachea	X	X	X
Ureters	X	X	X
Urinary bladder	X	X	X
Uterus	X	X	X
Vagina	X	X	X
Zymbal gland			

X, histopathology performed

\*, organ weight obtained

## 7 Genetic Toxicology

### In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

**Study title:** Bacterial mutagenicity assay of PD 332991-54

**Key findings:** Under the study conditions, PD332991-54 was not mutagenic to test strains TA98, TA100, TA1535, TA1517 and TA102 (*S. typhimurium*) or WP2uvrA pKM101(*E. coli*) with or without metabolic activation.

**Study no.:** 1012574

**Volume #, and page #:** electronic submission, page 1-42

**Conducting laboratory and location:** Pfizer Global Rresearch & Development  
Ann Arbor Laboratories, Ann Arbor, Michigan

**Date of study initiation:** May 15, 2003

**GLP compliance:** yes

**QA reports:** yes ( x ) no ( )

**Drug, lot #, and % purity:** PD 332991-54  
Lot # LCD-4245  
Purity 74.15 % (active moiety)

**Methods:** plate incorporation

**Strains:** TA98, TA 100, TA1535, TA97a, and TA102

**Concentrations used in definitive study:**

In the absence of S9:

TA-98 and TA-1537: 31.25, 62.5, 125, 250, and 500 µg /plate  
TA-100 and TA-1535: 100: 200, 400, 800, and 1600 µg/plate;  
WP2uvrA pKM101: 312.5, 625, 1250, 2500, and 5000 µg/plate

In the presence of S9:

TA-98, TA-100, TA-1535, and TA-1537: 100, 200, 400, 800, and 1600 µg/plate  
WP2uvrA pKM101: 312.5, 625, 1250, 2500, and 5000 µg/plate

Note: In the absence of metabolic activation, cytotoxicity was observed at 500 µg/plate in TA-1537 and TA-98, ≥800 µg/plate in TA-1535, and 1600 µg/plate in TA-100.

Cytotoxicity was not observed in WP2uvrA pKM101. In the presence of metabolic activation, cytotoxicity was observed at 1600 µg/plate in TA-98, TA-100, TA-1535, and TA-1537, and was not observed in WP2uvrA pKM101.

**Basis of concentration selection:** bacteriotoxicity or ICH S2 criteria for the highest concentration of 5000 µg/plate

**Metabolic activation system:** Liver S9 mix from male rats, Aroclor 1254-pretreated

**Negative controls:** Deionized water, 0.1 µl/plate

**Positive controls:**

Tester Strain	Nonactivation Assays (µg/plate)	Activation Assays (µg/plate)
TA98	2-nitrofluorene (1)	Benzo(a)pyrene (2.5)
TA100	sodium azide (75)	2-aminoanthracene (2)
TA1535	sodium azide (1)	2-aminoanthracene (2)
TA1537	9-aminoacridine (75)	2-aminoanthracene (2)
WP2uvrA pKM101	4-nitroquinoline-N-oxide (1)	2-aminoanthracene (20)

**Incubation and sampling times:** 72 hours at 37 ± 2°C

**Results**Study validity:

No. of replicates: 3

Counting method:

An automated colony counter (Synbiosis ProtoCOL Colony Counter 3.06)

Criteria for positive results: Criteria for a positive mutagenic response required mean revertants per plate to increase with increasing dose and exceed twice (TA-98, TA-100, and WP2uvrA pKM101) or 3 times (TA-1535 and TA-1537) the corresponding vehicle.

Control selection of bacteria tester strains was adequate based upon Guideline For Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses. Drug concentration selection was adequate based upon observed bacteriotoxicity or ICH S2 criteria.

Study outcome:

Table 57. Mean revertant counts in the absence of metabolic activation

Test article	Conc. (µg/plate)	Bacteria strain and mean of revertant colonies per plate				
		TA98	TA100	TA1535	TA1537	WP2uvrApKM101
control		31	89.7	13.7	7	143.3
PD 332991- 54	31.25	32.3	-	-	6.7	-
	62.5	24	-	-	6.7	-
	100	-	94.7	14	-	-
	125	32	-	-	9	-
	200	-	83	13.7	-	-
	250	31.7	-	-	6	-
	312.5	-	-	-	-	150.3
	400	-	89.3	9	-	-
	500	12	-	-	3.3	-
	625	-	-	-	-	138.3
	800	-	81.0	3	-	-
	1250	-	-	-	-	142.0
	1600	-	50.7	0	-	-
	2500	-	-	-	-	139.3
5000	-	-	-	-	141.3	
Positive control		240.7	884	497	434	2263

## Mean revertant counts in the present of metabolic activation

Test article	Conc. (µg/plate)	Bacteria strain and mean of revertant colonies per plate				
		TA98	TA100	TA1535	TA1537	WP2uvrApKM101
control		26.7	95.3	11.7	9.3	160.7
PD 332991- 54	100	25	104.7	-	11	-
	200	31	104.7	-	11	-
	312.5	-	-	14	-	158
	400	33.3	108	-	8	-
	625	-	-	13.7	-	167
	800	18	77.7	-	5.3	-
	1250	-	-	-	-	158.7
	1600	3.3	22.3	9	1.0	-
	2500	-	-	-	-	160.3
	5000	-	-	-	-	109
Positive control		150	2136.7	441	442.7	1273.7

"-"= not applicable

- The mean mutant numbers of negative control plates lay within the range of acceptable negative control values
- The positive controls induced a response equal to twice (or more) the negative control incidence, which is formally the criterion for a mutagenic activity of a compound
- Treatment with the test item did not increase the number of revertants above the corresponding negative control values

**Comments and conclusions:** The study was valid under the conditions of this study. PD 332991-54 is not mutagenic to test strains TA98, TA100, TA1535, TA1537 and WP2uvrA pKM101 in the Ames reverse-mutation assay.

### ***In Vitro Assays in Mammalian Cells***

**Study title:** Chromosomal aberrations in cultured human peripheral blood lymphocytes  
With PD 332991-0054

**Key findings:** PD 332991-0054 was negative in the human blood peripheral lymphocyte assay with or without S9 activation, under the conditions of this assay.

**Study no.:** 25093-0-449OECD

**Applicant study no.:** (b) (4) 3122

**Volume #, and page #:** electronic submission, page 1-90

**Conducting laboratory:** (b) (4)

**Date of study initiation:** May 6, 2003

**GLP compliance:** Yes

**QA reports:** Yes

**Drug, lot #, and % purity:** PD 332991-0054,  
Lot# LCD-4245  
Purity 74.15 % (active moiety)

**Vehicle:** DMSO**Methods**

Strains/species/cell line: Human peripheral blood lymphocytes

Concentrations used in definitive study:

Initial trials: 9.86 to 1200 µg/mL

Confirmatory trial:

without metabolic activation- 0.311 to 50.0 µg/mL.

with metabolic activation- 3.13 to 100 µg/mL.

Basis of concentration selection:

The concentrations selected for analysis of chromosomal aberrations was based on the level of toxicity noted for the incubations. The high concentration was selected so that at least 50% reduction in the mitotic index relative to the solvent control was noted. If the aberration results are negative and there is no significant reduction (approximately  $\geq 50\%$ ) in mitotic index, the assay must include the highest applicable dose (5 mg/mL or 10 mM, whichever is lower) or a dose exceeding the solubility limit in culture medium.

Negative controls: DMSO

Positive controls:

-S9: Mitomycin C (1 µg/mL)

+S9: Cyclophosphamide (25 µg/mL)

Incubation and sampling times:

Initial trials: 3 hours

Confirmatory trial:

without metabolic activation- 22 hrs

with metabolic activation: 3 hours

Cells were harvested ~22 hours after initiation and used to prepare microscope slides.

**Results**

Study validity:

No. of replicates: 2

Data analysis: The mitotic index (MI) was determined using 1000 cells and was expressed as a percentage of the negative control. Aberrations were quantitated using 200 cells with good morphology at each concentration of PD 332991-54. In the MMC and CP positive controls, 100 cells were used to quantitate aberrations. The percent polyploidy and endoreduplication were evaluated using 100 metaphase cells

Acceptable Controls: The vehicle control cultures must contain less than approximately 5% cells with aberrations. The positive control result must be significantly higher ( $p \leq 0.01$ ) than the vehicle controls.

Control Selection was adequate based upon Guideline For Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses.

Drug concentration selection was adequate based upon observed bacteriotoxicity or ICH S2 criteria.

Study outcome:

No significant increase in cells with chromosomal aberrations was noted in the analyzed cultures.

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

**Study title:** Three-week oral toxicity study of PD332991-54 in rats

Micronuclei formation was evaluated as part of a 3-week GLP repeat-dose toxicity study conducted in rats. PD332991 was administered by oral gavage once daily to rats (main: 5/sex/group, recovery: 5/sex/group, PK: 6/sex/group) for 3 weeks at doses up to 200 mg/kg in males and up to 400 mg/kg in females. Micronucleus assessment was performed from bone marrow collected at necropsy. The study results were reviewed and the following table was copied from the pharmacology/toxicology review of IND 69324.

Table 58. Micronucleus formation in vivo

Deviation from Control						
Sex	Male			Female		
Dose (mg/kg)	100	200	20 mg/kg cp	200	400	20 mg/kg cp
Polychromatic Erythrocytes(%)	NC	NC	-52%**	NC	NC	-68%**
Micronucleated Polychromatic Erythrocytes (%)	112%*	165%*	504%**	NC	NC	644%**

cp: Cyclophosphamide; "\*" p < 0.05; "\*\*" p < 0.01

**Summary:** Micronucleus formation occurred in males, indicating that PD 332991-54 has clastogenic potential.

**Other Genetic Toxicology Studies: studies on the mechanism of genetic toxicity**

**Study title:** Exploratory kinetochore in vitro micronucleus analysis in CHO-WBL Cells

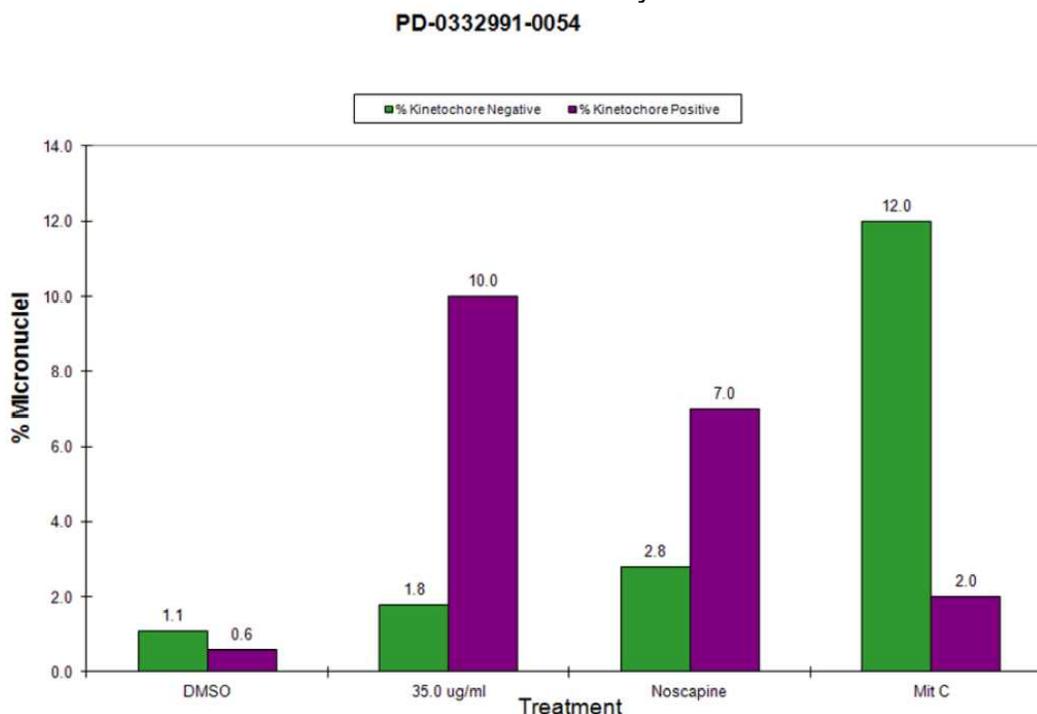
**Study no.:** PD-0332991-0054

**Methods:** CHO-WBL cells were used to further evaluate the cells for the presence or absence of kinetochore signals in the micronuclei. Slides were prepared from a 24-hour treatment at concentrations of 0.7, 2.5, 8.8, 18, and 35 µg/mL, as well as the vehicle control, Mitomycin-C as the clastogenic control and Noscaphine as the aneugenic staining control. Slides were evaluated under oil using an epi-fluorescence microscope, equipped with a Plan-Neofluor 100X objective. For both the vehicle control and the treated cultures, one thousand binucleated cells were evaluated for the presence or

absence of a kinetochore signal in micronuclei. For the positive controls, five hundred binucleated cells were evaluated.

**Results:** the following figure is copied from the Applicant's submission

Figure 41. Percentage of micronuclei with and without kinetochore signals per total number of cells analyzed



**Summary:** PD-0332991-0054 induced significant increases in micronuclei with kinetochore signals in CHO-WBL cells in the absence of metabolic activation. The results from this exploratory kinetochore in vitro micronucleus assay indicate that PD-0332991-0054 induces micronuclei through aneugenic chromosomal events.

#### Genetic toxicology summary:

PD332991 was shown to be non-mutagenic in the bacterial mutagenicity assay in the presence and absence of mammalian metabolic activation in several strains of *Salmonella typhimurium* (*S. typhimurium*) and in *Escherichia coli* (*E. coli*) WP2uvrA pKM101, and non-clastogenic in the chromosomal aberration assay within a biologically relevant concentration range of PD332991 using cultured human peripheral blood lymphocytes in the presence and absence of metabolic activation. PD332991 was positive for 1) micronucleus formation in vitro (The Applicant stated that the conducted in vitro micronucleus assay was positive, but did not submit the study report with the application. The Applicant submitted a study report for the kinetochore assay showing that PD-0332991-0054 induced increases in micronuclei with kinetochore signals in CHO-WBL cells in the absence of metabolic activation); 2) micronucleus formation in male and female rats following oral administration (part of 3-week toxicology study in rats, study no. RR745-03731).

## 8 Carcinogenicity

No study reports were submitted with this application. Carcinogenicity studies were not required to support an application for this indication.

## 8 Reproductive and Developmental Toxicology

### Fertility and Early Embryonic Development

**Study title:** A fertility and early embryonic development study of PD-0332991 by oral (gavage) administration in female rats

Study no.: 20037853

Study report location:

(b) (4)

Conducting laboratory and location:

Date of study initiation: March 19, 2013

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity: PD-0332991,  
lot# E010013487 and GR06533,  
99.79%

### Key Study Findings

- Doses up to 300 mg/kg did not result in toxicities in females;
- At doses up to 300 mg/kg, there were no treatment-related effects on the estrous cycling, number of pregnancies or copulation rates, the percentage of preimplantation loss, viable and nonviable embryos and the percentage of postimplantation loss.

## Methods

Doses: 0, 30, 100, and 300 mg/kg  
 \* Doses were selected based on previous general toxicity studies and a range-finding study in pregnant rats

Frequency of dosing: Once daily  
 Dose volume: 10 mL/kg

Route of administration: oral gavage  
 Formulation/Vehicle: 0.5% (w/v) Methylcellulose in Reverse Osmosis Deionized Water

Species/Strain: Crl:CD(SD) female rats  
 Number/Sex/Group: 20/group  
 Satellite groups: TK, N=3 control, N=5 treated groups  
 Study design: PD-0332991 was administered beginning 15 days before cohabitation, during cohabitation and continuing until Gestation Day 7 (GD 7). All rats assigned to the main study were euthanized on GD 14 and all rats assigned to the toxicokinetic study were euthanized on Day 15 of study.

Deviation from study protocol: none

## Observations and Results

**OBSERVATIONS AND TIMES:**

<u>Mortality</u>	at least twice daily (AM and PM)
<u>Clinical observations</u>	daily before each dose was administered during the precohabitation and cohabitation periods, daily beginning on GD 0 through the day of euthanasia. Postdose observations were conducted between 1 and 2 hours after dosing and at the end of the normal working day
<u>Body weights</u>	at least twice weekly during the dose period and on GDs 0, 3, 7, 10 and 14.
<u>Food consumption</u>	Weekly during the precohabitation period and on GDs 0, 3, 7, 10, and 14 (food left value). Food consumption was not measured during the cohabitation period when two rats occupied the same solid bottom cage.
<u>Estrous Cycle Evaluations - Main Study</u>	Vaginal lavage samples were collected for 14 consecutive days before initiation of dose administration, for 14 consecutive days beginning with the day after the first dose administration and then until spermatozoa were observed in a smear of the vaginal contents and/or a copulatory

	plug was observed in situ during the cohabitation period.
<u>Gross pathology:</u>	All animals at necropsy (on Day 14/15) for a gross necropsy of the thoracic, abdominal, and pelvic viscera
<u>Organ weights:</u>	All animals at death or at scheduled sacrifice on Day 106, or Day 134
<u>Histopathology:</u>	All animals at necropsy (on Day 14/15), cervix, gross lesions, ovaries, and uterus
<u>Toxicokinetics:</u>	at 1, 2, 4, 7, 12, and 24 hrs following the 14th dose (Day 14)

## Results

**Mortality:** none

**Clinical Signs:** unremarkable

**Body Weight:** unremarkable

**Food consumption:** unremarkable

**Estrous Cycling, Mating and Fertility:** unremarkable

**Necropsy:** unremarkable

**Ovarian and Uterine Examinations:** unremarkable

### Toxicokinetics:

Table 59. Summary PD-0332991 toxicokinetic parameters in female Sprague-Dawley Rat Plasma Following 30 mg/kg, 100 mg/kg, and 300 mg/kg Oral Gavage Administration of PD-0332991 on Day 14

Dose level (mg/kg/dose)	C <sub>max</sub> (ng/mL)	Dose Normalized C <sub>max</sub>	AUC <sub>0-24</sub> (ng.h/mL)	Dose Normalized AUC <sub>0-24</sub>	T <sub>max</sub> (h)
10	93	9	740	74	4.2
100	306	3	3040	30	7.0
300	627	2	8010	27	6.0

### Summary:

- Systemic exposure increased less than proportional to dose increase;
- Mean time-to-peak concentrations (T<sub>max</sub>) were 4.2 to 7.0 hours postdose

**Embryonic Fetal Development**

**Study title:** An Embryo-Fetal Development Study of PD-0332991 by Oral (Gavage) in Rats

Study no.: 20039574  
Pfizer Reference No. 13GR062

Study report location: Module 4  
Conducting laboratory and location:

(b) (4)

Date of study initiation: March 24, 2013  
GLP compliance: Yes  
QA statement: yes ( x ) no ( )  
Drug, lot #, and % purity: PD-0332991  
Lot #: E010013487  
Purity: 100%

**Key Study Findings**

- No maternal mortality was observed at dose up to 300 mg/kg (HD);
- Reductions in the average maternal body weight gain was observed at 300 mg/kg at intervals of GD 6 to 18 and GD 6 to 21 compared to controls;
- Reductions in maternal food consumption occurred at 300 mg/kg for the dosing interval of GD 6 to 18;
- Fetal body weights were reduced in the 300 mg/kg dose group compared to controls;
- An increased incidence in cervical ribs (skeletal variation) was observed at  $\geq 100$  mg/kg;
- There were no maternal gross observations, no PD-0332991-related effects on the average gravid uterine weight and ovarian or uterine parameters or embryo-fetal survival at any dose.

## Methods

Doses: 30, 100, 300 mg/kg/day  
 Dose justification: Dose selection was based on results from previous general toxicity studies and a dose range-finding study in pregnant rats  
 Frequency of dosing: once daily  
 Dose volume: 10 mL/kg  
 Route of administration: oral gavage  
 Formulation/Vehicle: 0.5% (w/v) Methylcellulose in R.O. water  
 Species/Strain: female CrI:CD(SD) Sprague Dawley rats  
 Number/Sex/Group: 20 animals/group  
 Study design: daily dosing from gestation days (GD) 6-17  
 Necropsy/cesarean on gestation day 22  
 \*the day of confirmation of mating was considered GD0  
 Deviation from study protocol: none

## Observations and Results

**Observations and times:**

<u>Mortality</u>	at least twice daily (AM and PM)
<u>Clinical observations</u>	The rats were observed for general appearance twice during the acclimation period, on GD 0, before each dose was administered, and once daily during the postdose period. Postdose observations were recorded between 1 and 2 hours after dosing and at the end of the normal working day.
<u>Body weights</u>	once during the acclimation period, on GD 0, and daily during the dose and postdose periods
<u>Food consumption</u>	on GDs 0, 6, 9, 12, 15, 18 and 21
<u>Mating Performance</u>	daily during the cohabitation period
<u>Terminal Procedures:</u>	On GD 21, see the details at the table below (copied from the Applicant's submission)

Group No.	No. of Female Rats	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology
			Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights		
1	3 <sup>a</sup>	GD 18	Pregnancy Status	-	-	-	-	-
2	5 <sup>a</sup>						-	-
3	5 <sup>a</sup>						-	-
4	5 <sup>a</sup>						-	-
Unscheduled Deaths <sup>a</sup>			-	-	-	-	-	
1	20	GD 21	X	X	X <sup>b</sup>	X <sup>c</sup>	-	-
2	20						-	-
3	20						-	-
4	20						-	-
Unscheduled Death <sup>d</sup>			X	X	X <sup>b</sup>	-	-	

GD = Gestation Day; X = Procedure conducted; - = Not applicable.  
<sup>a</sup> Rats assigned to the toxicokinetic study.  
<sup>b</sup> See Section 9.13.5 (Tissue Collection and Preservation) for tissues that were retained.  
<sup>c</sup> The gravid uterus was weighed for all rats that survived to scheduled euthanasia.  
<sup>d</sup> See Section 9.13.2 (Unscheduled Deaths – Main Study).

<u>Gross pathology:</u>	All animals on GD 21 for examination of the thoracic, abdominal and pelvic viscera																																										
<u>Histopathology:</u>	<table border="1"> <thead> <tr> <th>Tissue</th> <th>Collected</th> <th>Weighed</th> </tr> </thead> <tbody> <tr> <td>Cervix</td> <td>X</td> <td>-</td> </tr> <tr> <td>Esophagus</td> <td>X</td> <td>-</td> </tr> <tr> <td>Gravid Uterus</td> <td>-</td> <td>X</td> </tr> <tr> <td>Gross lesions/masses</td> <td>X</td> <td>-</td> </tr> <tr> <td>Heart</td> <td>X</td> <td>-</td> </tr> <tr> <td>Kidneys</td> <td>X</td> <td>-</td> </tr> <tr> <td>Liver</td> <td>X</td> <td>-</td> </tr> <tr> <td>Lungs</td> <td>X</td> <td>-</td> </tr> <tr> <td>Ovaries</td> <td>X</td> <td>-</td> </tr> <tr> <td>Spleen</td> <td>X</td> <td>-</td> </tr> <tr> <td>Stomach</td> <td>X</td> <td>-</td> </tr> <tr> <td>Trachea</td> <td>X</td> <td>-</td> </tr> <tr> <td>Uterus</td> <td>X</td> <td>-</td> </tr> </tbody> </table>	Tissue	Collected	Weighed	Cervix	X	-	Esophagus	X	-	Gravid Uterus	-	X	Gross lesions/masses	X	-	Heart	X	-	Kidneys	X	-	Liver	X	-	Lungs	X	-	Ovaries	X	-	Spleen	X	-	Stomach	X	-	Trachea	X	-	Uterus	X	-
Tissue	Collected	Weighed																																									
Cervix	X	-																																									
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Spleen	X	-																																									
Stomach	X	-																																									
Trachea	X	-																																									
Uterus	X	-																																									
<u>Toxicokinetics:</u>	1, 2, 4, 7 and 12 hour postdose on GD 17 and at euthanasia (24 hour timepoint) for treatment groups. at approximately 1, 2 and 4 hours postdose.for controls																																										

Parameters and endpoints evaluated:

Reproductive parameters: The gravid uterus was weighed. The ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, placentae (size, color, and shape), live and dead fetuses and early and late resorptions.

Fetal examination: Each fetus (live or dead) was sexed, weighed, and examined for abnormalities, external malformations/variations, visceral/skeletal malformations/variations).

Statistical evaluations: Proportional data (e.g., clinical observations, mortality, fetal alterations, placentae appeared normal, etc.) were analyzed using the Variance Test for Homogeneity of the Binomial Distribution. Continuous data (e.g., body weights, body weight changes, food consumption and litter averages for percent nonviable conceptuses) were analyzed using Bartlett's Test of Homogeneity of Variances and the Analysis of Variance<sup>9</sup>, when appropriate [i.e., Bartlett's Test was not significant ( $p > 0.001$ )]. If the Analysis of Variance was significant ( $p \leq 0.05$ ), Dunnett's Test was used to identify the statistical significance of the individual groups using two-tailed probability criteria. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant ( $p \leq 0.001$ )], the Kruskal-Wallis Test<sup>11</sup> was used ( $\leq 75\%$  ties). In cases where the Kruskal-Wallis Test was statistically significant ( $p \leq 0.05$ ), Dunn's Method of Multiple Comparisons<sup>12</sup> was used to identify the statistical significance of the individual groups using two-tailed probability criteria. If there were greater than 75% ties, Fisher's Exact Test was used to analyze the data using two-tailed probability criteria. Count data (e.g., number of corpora lutea, implantations, live fetuses, etc.) were evaluated using the procedures described above for the Kruskal-Wallis Test and Dunn's Method of Multiple Comparisons.

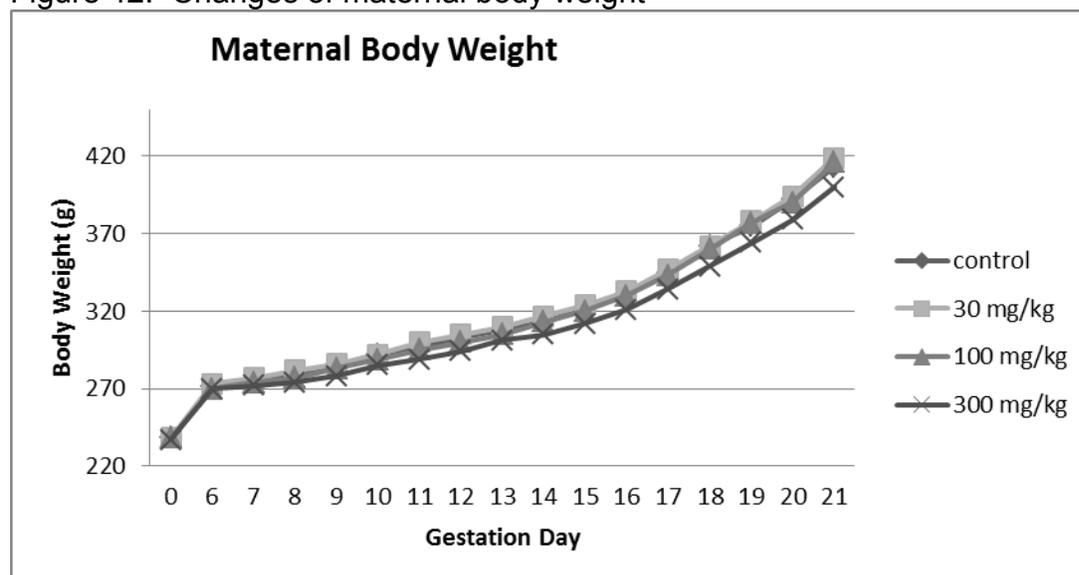
## Results

### In-life observations:

Mortality: none

Clinical signs: unremarkable

Body weight:  
Figure 42. Changes of maternal body weight



**Summary:** Overall, the average maternal body weight gain was significantly reduced ( $p \leq 0.01$ ) for the intervals of GD 6 to 18 and GD 6 to 21 compared to controls (86% and 92% of controls, respectively).

**Note:** The corrected maternal body weight gain (Gestation Day 21 body weight minus the gravid uterine weight) for the intervals of GD 6 to 21 in the 300 mg/kg dose group was 78% of controls, which was consistent with the test article-related decrease in average maternal body weight gain.

**Food consumption:**

$\leq 100$  mg/kg: unremarkable

300 mg/kg: Within the dose period, food consumption was reduced at each tabulated interval between GD 6 through 18, but the reductions were only statistically significant ( $p \leq 0.05$  or  $p \leq 0.01$ ) for the dosing intervals of GD 9 to 12 and GD 15 to 18.

**Toxicokinetics:**

Table 60. A summary of the estimated TK parameters

Dose (mg/kg)	$C_{max}$ (ng/mL)	Dose normalized $C_{max}$	AUC <sub>0-24</sub> (ng.hr/mL)	Dose Normalized AUC <sub>0-24</sub>	$T_{max}$
30	111	3.7	836	28	2.6
100	278	2.8	2960	30	4.2
300	456	1.5	7310	24	11

**Summary:**

- PD-0332991 exposure increased in a dose proportional manner for AUC<sub>(0-24)</sub> and in a less than dose proportional manner for  $C_{max}$ ;
- $T_{max}$  increased with dose increases.

Terminal and necropsy evaluations:**Dams:***Necropsy evaluation:* unremarkable*Uterine and Ovarian parameters*Changes of uterine weight

Table 61. Changes in gravid uterus

Groups (mg/kg/day)	(control)	30	100	300
Gravid Uterus (mean, g)	107	107	107	103
% deviation from control				-5

*Summary:* mean gravid uterine weights were reduced at HD (95% of controls).Fertility parameters: unremarkable**Embryo-fetal data:***Changes of mean fetal body weight*

Table 62. Changes in mean fetal body weight

Index	Fetal weight (g)				Percentage deviation from control			
	0	30	100	300	0	30	100	300
Group (mg/kg)								
Male	5.83	5.86	5.87	5.58*				-4*
Female	5.57	5.46	5.51	5.23**				-6**
Male+ female	5.70	5.66	5.69	5.42**				-5**

Blank: unremarkable; \*  $P \leq 0.05$ ; \*\*  $p \leq 0.01$ *Summary:* Mean fetal body weights in both males and females were decreased at 300 mg/kg in males, and decreases in all treatment groups in females compared to control groups. This change was statistically significant at 300 mg/kg in both males and females.

*Fetal morphological data:*

Table 63. Fetal morphological changes

Index	Fetus				Litter			
	0	30	100	300	0	30	100	300
Group (mg/kg)	0	30	100	300	0	30	100	300
External malformation								
Number examined	266	266	282	282	19	19	20	20
Live	266	266	282	282	19	19	20	20
dead	0	0	0	0	0	0	0	0
unremarkable								
Soft tissue malformation								
Number examined	129	128	137	135	19	19	20	20
Live	129	128	137	135	19	19	20	20
dead	0	0	0	0	0	0	0	0
unremarkable								
Skeletal variations								
Number examined	138	138	145	147	19	19	20	20
Live	138	138	145	147	19	19	20	20
dead	0	0	0	0	0	0	0	0
Cervical vertebrae: Cervical rib present at 7th cervical vertebra								
-N	3	2	9	17	3	2	7	13
-%	2.2	1.4	6.2	11.6**	15.8	10.5	35	65**

\*\*p&lt;0.01

*Summary:* Fetal examination revealed PD-0332991-related increases in incidences of cervical ribs at  $\geq 100$  mg/kg in the litter and fetal, with statistical significance at 300 mg/kg.

Note: The Applicant explained that this skeletal variation is not considered to be an adverse effect of PD-0332991 because: 1) the ossification sites were small in nature; and 2) these types of ossification sites are known to resorb into the vertebral process, and are therefore of no consequence postnatally. The reviewer agrees that this observation was not clinically meaningful.

**Conclusion:** PD-0332991 was orally administered to pregnant Sprague Dawley rats once daily from Gestation Day 6 through 17 at 0, 30, 100, and 300 mg/kg. The maternal and developmental NOAEL was 100 mg/kg/day ( $C_{max}$  of 278 ng/mL;  $AUC_{0-t}$  of 2960 ng•hr/mL), based on decreased maternal body weight gain, maternal food intake and fetal body weights at 300 mg/kg/day.

**Study title:** An embryo-fetal development study of PD-0332991 by oral (gavage) in rabbits

Study no.: 20039575

Pfizer Reference No. 13GR063

Study report location: Module 4

Conducting laboratory and location:

(b) (4)

Date of study initiation: March 25, 2013

GLP compliance: yes

QA statement: yes ( x ) no ( )

Drug, lot #, and % purity: PD-0332991

Lot #: E010013487

Purity: 99%

### Key Study Findings

- There were no PD-0332991-related clinical signs at any dose level;
- Maternal effects were observed at 20 mg/kg, including a reduction in maternal body weight gain and reductions in food consumption;
- Fetal skeletal examination revealed a low incidence of small phalanges on the forepaws at 20 mg/kg;
- The maternal NOAEL for PD-0332991 is 10 mg/kg ( $C_{max}$  of 1280 ng/mL;  $AUC_{0-t}$  of 7460 ng•hr/mL);
- The developmental NOAEL for PD-0332991 was 10 mg/kg.

### Methods

Doses: 2, 10, 20 mg/kg/day

Dose justification: Dose levels were based on the results of a dose range-finding embryo-fetal development study.

Note: Dose range finding study results: At 100 mg/kg/day, 2 rabbits were euthanized on GD 11 due to declining clinical condition. The remaining 4 rabbits at 100 mg/kg/day were found dead between GD 9 and 11. At 30 mg/kg/day, on GD 21, one rabbit was euthanized due to declining clinical condition and another one due to abortion.

Frequency of dosing: once daily

Dose volume: 10 mL/kg

Route of administration: oral gavage

Formulation/Vehicle: 0.5% (w/v) Methylcellulose in Reverse Osmosis Deionized Water (R.O. deionized water)

Species/Strain: Pregnant New Zealand White [Hra:(NZW)SPF] female rabbits

2.8 kg to 3.7 kg and 6 months of age  
 Number/Sex/Group: 20 animals/group  
 Satellite groups: 5 animals/group (3 animals for control group)  
 Study design: daily dosing from gestation days (GD) 7-19\*,  
 Necropsy/cesarean on gestation day 29  
 (TK animals on DG 20), see the table below for the  
 details.  
 \*The day mating occurred was considered to  
 be GD 0

Experimental Design

Group No.	Test Material	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Main Study Rabbits (Assigned Rabbit Numbers)	Number of Toxicokinetic Rabbits (Assigned Rabbit Numbers) <sup>a</sup>
1	Control Article	0	0	10	20 (3701-3720)	3 (3781-3783)
2	PD-0332991	2	0.2	10	20 (3721-3739, 9891 <sup>b</sup> )	5 (3784-3788)
3	PD-0332991	10	1.0	10	20 (3741-3753, 5960 <sup>c</sup> , 3755-3760)	5 (3789-3793)
4	PD-0332991	20	2.0	10	20 (3761-3780)	5 (3794-3798)

<sup>a</sup> Toxicokinetic animals were used for toxicokinetic evaluation only.

<sup>b</sup> Prior to dose administration on GD 7, female 3740 was excluded from study due to clinical observations and was replaced with female 9891.

<sup>c</sup> Prior to dose administration on GD 7, female 3754 was excluded from study due to body weight loss and was replaced with female 5960.

Deviation from study protocol: None

**Observations and times:**

**Parameters and endpoints evaluated:**

<u>Mortality</u>	at least twice daily (AM and PM)
<u>Clinical observations</u>	daily during the predose period, before each dose, once daily during the postdose period, and on the day of scheduled euthanasia. for general appearance. Postdose observations were recorded between 1 and 2 hours following dose administration and at the end of the normal working day.
<u>Body weights</u>	on GD 0 (Supplier), on the day of arrival at the Testing Facility and on GD 4 during the predose period. Body weights were also recorded daily during the dose and postdose periods and on the day of scheduled euthanasia.
<u>Food consumption</u>	daily after arrival at the Testing Facility (values not tabulated) and daily during the dose and postdose periods.
<u>Mating Performance</u>	daily during the cohabitation period
<u>Terminal Procedures:</u>	On GD 29 (main), see the details at the table below (copied from the Applicant's application)

Group No.	No. of Female Rabbits	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology
			Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights		
1	3 <sup>a</sup>	GD 20	Pregnancy Status	-	-	-	-	-
2	5 <sup>a</sup>						-	-
3	5 <sup>a</sup>						-	-
4	5 <sup>a</sup>						-	-
Unscheduled Deaths <sup>a</sup>			-	-	-	-	-	
1	20	GD 29	X	X	X <sup>b</sup>	X <sup>c</sup>	-	-
2	20						-	-
3	20						-	-
4	20						-	-
Unscheduled Deaths			-	-	-	-	-	

GD = Gestation Day; X = Procedure conducted; - = Not applicable.  
<sup>a</sup> Rabbits assigned to the toxicokinetic study.  
<sup>b</sup> See Section 9.13.4 (Tissue Collection and Preservation) for tissues retained.  
<sup>c</sup> The gravid uterus was weighed for all rabbits.

<b>Gross pathology:</b>	All animals on GD 21 for examination of the thoracic, abdominal and pelvic viscera
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<b>Histopathology:</b>	<table border="1"> <thead> <tr> <th>Tissue</th> <th>Collected</th> <th>Weighed</th> <th>Comment</th> </tr> </thead> <tbody> <tr> <td>Cervix</td> <td>X</td> <td>-</td> <td>Retained with uterus for all main study nonpregnant rabbits.</td> </tr> <tr> <td>Gravid Uterus</td> <td>-</td> <td>X</td> <td>Weighed for all pregnant rabbits at scheduled euthanasia.</td> </tr> <tr> <td>Ovaries</td> <td>X</td> <td>-</td> <td>All nonpregnant rabbits assigned to the main study.</td> </tr> <tr> <td>Uterus</td> <td>X</td> <td>-</td> <td>Retained with cervix for all main study nonpregnant rabbits.</td> </tr> </tbody> </table> <p>X = Procedure conducted; - = Not applicable.</p>	Tissue	Collected	Weighed	Comment	Cervix	X	-	Retained with uterus for all main study nonpregnant rabbits.	Gravid Uterus	-	X	Weighed for all pregnant rabbits at scheduled euthanasia.	Ovaries	X	-	All nonpregnant rabbits assigned to the main study.	Uterus	X	-	Retained with cervix for all main study nonpregnant rabbits.
Tissue	Collected	Weighed	Comment																		
Cervix	X	-	Retained with uterus for all main study nonpregnant rabbits.																		
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Ovaries	X	-	All nonpregnant rabbits assigned to the main study.																		
Uterus	X	-	Retained with cervix for all main study nonpregnant rabbits.																		

<b>Toxicokinetics:</b>	<table border="1"> <thead> <tr> <th rowspan="2">Group No.</th> <th colspan="6">Sample Collection Time Points (Approximate Time Postdose) on GD 19</th> </tr> <tr> <th>1 hour</th> <th>2 hour</th> <th>4 hour</th> <th>7 hour</th> <th>12 hour</th> <th>24 hour</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>X</td> <td>X</td> <td>X</td> <td>-</td> <td>-</td> <td>-</td> </tr> <tr> <td>2</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> </tr> <tr> <td>3</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> </tr> <tr> <td>4</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> <td>X</td> </tr> </tbody> </table> <p>X = Sample collected.</p>	Group No.	Sample Collection Time Points (Approximate Time Postdose) on GD 19						1 hour	2 hour	4 hour	7 hour	12 hour	24 hour	1	X	X	X	-	-	-	2	X	X	X	X	X	X	3	X	X	X	X	X	X	4	X	X	X	X	X	X
Group No.	Sample Collection Time Points (Approximate Time Postdose) on GD 19																																									
	1 hour	2 hour	4 hour	7 hour	12 hour	24 hour																																				
1	X	X	X	-	-	-																																				
2	X	X	X	X	X	X																																				
3	X	X	X	X	X	X																																				
4	X	X	X	X	X	X																																				

Procedure	Frequency of Testing	
	Main Phase Animals	Toxicokinetic Animals
Cageside Observations	≥ 2 Daily	≥ 2 Daily
Clinical Observations	GD 8, 11, 14, 17, 21, 24, 27, and 30	GD 8, 11, 14, 17, and 21 <sup>a</sup>
Body Weight	Daily	GD 8, 11, 14, 17, and 21 <sup>b</sup>
Food Consumption	Daily	Not required

Reproductive parameters: The ovaries and uterus/cervix were examined for number and distribution of corpora lutea and implantation sites, placentae (size, color, or shape), live and dead fetuses, and early and late resorptions.

Fetal examination: Each fetus (live or dead) was sexed, weighed, and examined for external malformations/variations, visceral/skeletal malformations/variations).

Statistical evaluations:

Continuous data (e.g., body weights, body weight changes, food consumption and litter averages for percent nonviable conceptuses) were analyzed using Bartlett's Test of Homogeneity of Variances<sup>7</sup> and the Analysis of Variance, when appropriate [i.e., Bartlett's Test was not significant ( $p > 0.001$ )]. If the Analysis of Variance was significant ( $p \leq 0.05$ ), Dunnett's Test was used to identify the statistical significance of the individual groups using two-tailed probability criteria. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant ( $p \leq 0.001$ )], the Kruskal-Wallis Test was used ( $\leq 75\%$  ties). In cases where the Kruskal-Wallis Test was statistically significant ( $p \leq 0.05$ ), Dunn's Method of Multiple Comparisons<sup>11</sup> was used to identify the statistical significance of the individual groups using two-tailed probability criteria. If there were greater than 75% ties, Fisher's Exact Test was used to analyze the data using two-tailed probability criteria.

Count data (e.g., number of corpora lutea, implantations, live fetuses, etc.) were evaluated using the procedures described above for the Kruskal-Wallis Test and Dunn's Method of Multiple Comparisons.

## Results

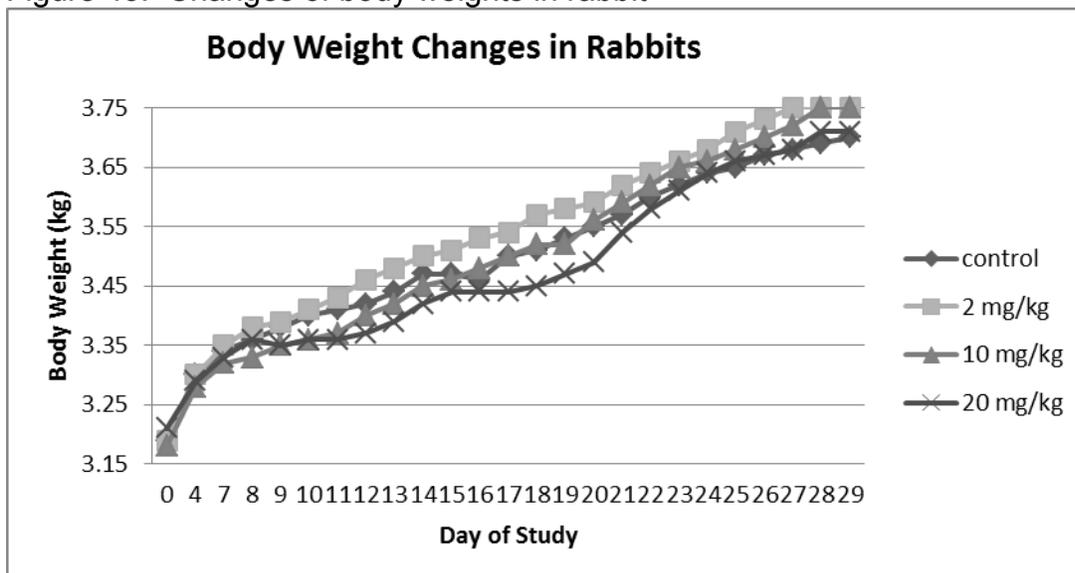
### In life observations:

Mortality: none

Clinical signs: unremarkable

Body weight:

Figure 43. Changes of body weights in rabbit



**Summary:** At 20 mg/kg, there was a statistically significant reduction ( $p \leq 0.05$ ) in the average maternal body weight gain for the dosing interval of GD 7 to 10 (50% of controls), followed by a reduction in the average maternal body weight gain for the dosing interval of GD 10 to 13 (75% of controls). Overall, the average maternal body weight gain was reduced for the dosing interval of GD 7 to 20 compared to controls (76% of controls). The observed reduction of body weight recovered after the cessation of dosing (GD 20 to 24).

Food consumption:

$\leq 20$  mg/kg: unremarkable

20 mg/kg: Absolute (g/day) and relative (g/kg/day) maternal food consumption were reduced for the dosing interval of GD 7 to 20 compared to controls (88% and 89% of controls, respectively). Within the dose period, food consumption (g/day and g/kg/day) was reduced at each tabulated interval between GD 7 through 20 (86% to 92% of controls), but the reductions were only statistically significant (absolute,  $p \leq 0.05$ ) for the dosing interval of GD 10 to 13.

Maternal food consumption recovered during the postdose period (GD 20 to 29).

Note: The reductions in maternal food consumption in the 20 mg/kg dose group correlated with reduced maternal body weight gain that also occurred during the dosing period.

**Doe:**

*Necropsy evaluation:* unremarkable

*Uterine and Ovarian parameters*

Changes of uterine weight: unremarkable

Uterine or ovarian parameters: unremarkable

**Embryo-fetal data:***Mean fetal body weight: unremarkable**Fetal morphological data:*

Table 64. Fetal morphological changes in rabbits

Index		Fetus				Litter			
Group (mg/kg)		0	2	10	20	0	2	10	20
Number examined		173	190	179	160	19	20	20	19
Live		173	190	179	160	19	20	20	19
dead		0	0	0	0	0	0	0	0
<b>External malformation</b>									
Body									
Skin discolored					1				1
					0.6				5.3
Abdominal distention					1 <sup>a</sup>				1
					0.6				5.3
Fore and/or hindlimb(s):									
Digit(s) short					1 <sup>b</sup>				1
					0.6				5.3
<b>Soft tissue malformation</b>									
Heart									
Ventricle small					1 <sup>c</sup>				1
					0.6				5.3
Interventricular septal defect					1 <sup>c</sup>				1
					0.6				5.3
Vessels									
persistent truncus arteriosus					1 <sup>c</sup>				1
					0.6				5.3
Lungs									
Small					1 <sup>c</sup>				1
					0.6				5.3
Contains fluid					1				1
					0.6				5.3
Other									
Abdominal cavity contains fluid					1 <sup>c</sup>				1
					0.6				5.3
Thoracic cavity contains fluid					1 <sup>c</sup>				1
					0.6				0.6
<b>Skeletal variations</b>									
Skull									
Nasals midline suture									

displaced	-N	1		2		1		2
	-%	0.5		1.2		5.0		10.5
Nasal frontal suture Irregular	-N	1		1		1		1
	-%	0.5		0.6		5.0		0.6
Frontals, contain an Interfrontal	-N		1				1	
	-%		0.6				5.0	
Parietal, contains an Intraparietal	-N		1				1	
	-%		0.6				5.0	
Hyoid Ala, short	-N		1					1
	-%		0.6					5.3
Cervical vertebrae Cervical rib present at 7th cervical vertebrae	-N		3				1	
	-%		1.7				5.0	
Caudal vertebrae Misaligned	-N	1	3			1	3	
	-%	0.5	1.7			5.0	15	
Incompletely ossified	-N		1 <sup>d</sup>				1	
	-%		0.6				5	
Forelimb Digit, absent	-N		1 <sup>e</sup>	1 <sup>f</sup>			1	1
	-%		0.6	0.6			5.0	5.3
Metacarpal, absent	-N		1 <sup>e</sup>	1 <sup>f</sup>			1	1
	-%		0.6	0.6			5.0	5.3
Phalanx, absent	-N		1 <sup>e</sup>	1 <sup>f</sup>			1	1
	-%		0.6	0.6			5.0	5.3
Phalanx, small	-N			3 <sup>b</sup>				2
	-%			1.9**				10.5
Ossification sites (Ossification sites per fetus per litter)								
Group (mg/kg)		0		2		10		20
Vertebrae								
Thoracic -(% of control)						1.9*		2.2**
Lumbar -(% of control)								-3.9*
Caudal -(% of control)						2.0*		2.1*
Ribs (pairs)								
-(% of control)								2.4**
Sternum								
Sternal centers								
-(% of control)								3.4**

<sup>a</sup>: Fetus 3770-4 had other soft tissue alterations;

<sup>b</sup>: Fetus 3776-1 had other skeletal alterations;

c. Fetus 3770-4 had other soft tissue alterations

d. Fetus 3759-9 had other skeletal alterations

e. Fetus 3742-6 had other skeletal alterations.

f. Fetus 3769-2 had other skeletal alterations.

\*  $p < 0.05$ ; \*\* $p \leq 0.01$

**Summary:** Administration of 10 mg/kg PD-0332991 resulted in short pollex of forepaws with small phalanges at skeletal examination.

**Note:**

- 1) All soft tissue abnormalities that were observed were considered to be unrelated to PD-0332991 because the finding was not dose-dependent and/or the finding was limited to one fetus in any dose group.
- 2) The Applicant claimed that the changes in skeletal ossification were not considered to be an adverse effect of PD-0332991 because: 1) this is a common skeletal variation (control group had >50% incidence of 13 ribs); 2) there were no fetuses that had less than 12 or more than 13 rib pairs in any dose group; 3) there were no other significant changes in the axial skeleton; and 4) there was no change in the average number of pre-sacral vertebrae (total number of cervical, thoracic and lumbar) among the groups. The reviewer agrees the Applicant's conclusion.

**Toxicokinetics:**

Table 65. A summary of the estimated TK parameters on GD 19 following daily administration of PD-0332991 GD 7 to GD 19 at dose levels of 2, 10 and 20 mg/kg/day.

Dose (mg/kg)	$C_{max}$ (ng/mL)	Dose normalized $C_{max}$	AUC <sub>0-24</sub> (ng.hr/mL)	Dose Normalized AUC <sub>0-24</sub>	$T_{max}$
2	178	89	924	462	1
10	1280	128	7460	746	1.8
20	2470	124	17200	860	2.0

**Summary:**

- Exposure ( $C_{max}$  and AUC) was dose proportional between 10 mg/kg and 20 mg/kg;
- Exposure ( $C_{max}$  and AUC) increased in a greater than dose proportion manner between 2 and 10 mg/kg;
- Peak PD-0332991 concentrations ( $T_{max}$ ) occurred within 2h postdose.

**Conclusion:** Under the conditions of this study, the maternal NOAEL for PD-0332991 is 10 mg/kg ( $C_{max}$  of 1280 ng/mL; AUC<sub>0-t</sub> of 7460 ng•hr/mL) due to the observed reduced maternal body weight gain and food intake at 20 mg/kg. The developmental NOAEL for PD-0332991 was also 10 mg/kg based on small phalanges observed at 20 mg/kg.

**Potential reproductive toxicity of palbociclib based on the mechanism of action**

Maternal toxicities and adverse effect on early fetal development was observed in the conducted rat and rabbit studies. In addition, published literature describing the function of CDK4/CDK6 in development also suggests that IBRANCE can cause fetal harm based on the mechanism of action.

Role of CDK4 in development**Loss of Cdk4 expression causes infertility and insulin deficient diabetes and Cdk4 activation results in  $\beta$ -islet cell hyperplasia**

Sushil G. Rane et al., Nat Gen. (1999); 22:44-52.

## Key findings:

- Cdk4<sup>neo/neo</sup> mice are viable, but small in size and infertile in both males and females;
- Loss of Cdk4 resulted in a reduction in the number of  $\beta$ -islet cells;
- Cdk4<sup>neo/neo</sup> mice develop insulin-deficient diabetes;
- Other findings included neurological defects such as impaired locomotion, staggering, and hyperactivity; abnormalities in thymocytes; changes in maturation and allergen response; and impaired adipocyte differentiation

**Genetic rescue of Cdk4 null mice restores pancreatic b-cell proliferation but not homeostatic cell number**

Javier Martin et al. Oncogene (2003) 22, 5261–5269

- Cdk4 is essential for the postnatal proliferation of pancreatic b cells but not for embryonic neogenesis from ductal epithelial cells.

Review comment: Altered glucose metabolism associated with pancreatic islet cell vacuolation (also identified in the 15-week rat study), and secondary effects in the eye, teeth, kidney, and adipose tissue were identified in the 27-week rat study with palbociclib. Young rats (8 weeks old upon study start in the 27-week study) were used in the rat toxicity studies with palbociclib. Beta cell proliferation in rats displays an age-related decline (from 20% per day in pups to 10% per day in adolescence to 2% per day in early adulthood and 0.07% per day in 1-year old rats) to a level approaching that in humans where beta cell proliferation is minimal at 0.04% per day. With higher rates of beta cell proliferation compared to adult humans, the effects on glucose homeostasis could be related to an increased susceptibility of rat pancreatic beta cells to the actions of a CDK4/6 cell cycle inhibitor. The rapid proliferation of human beta cells diminishes soon after infancy, and cells enter and remain in a quiescent state through adulthood. Therefore, palbociclib may not have adverse effect on glucose metabolism in adults, but may cause insulin deficient diabetes in offspring if palbociclib is used during pregnancy, or if the patient becomes pregnant while taking this drug.

**Mammalian Cells Cycle without the D-Type Cyclin-Dependent Kinases Cdk4 and Cdk6**

Marcos Malumbres et al., Cell (August 20, 2004), Vol. 118, 493–504.

**Key findings:**

- Late embryonic lethality of Cdk4;Cdk6 double mutant mice
  - Embryos defective for Cdk4 and Cdk6 died during the late stages of embryonic development (gestation day 14.5 until birth) due to severe anemia.

**Characterization of the abnormal pancreatic development, reduced growth and infertility in Cdk4 mutant mice**

Richard V Mettus et al., *Oncogene* (2003) 22, 8413–8421

**Key findings:**

Homozygous Cdk4-deficient mice displayed defects in weight gain, fertility and hypoproliferation of specific endocrine cells of the pituitary and pancreas, the latter of which results in a diabetes-like phenotype.

**Cdk4 promotes adipogenesis through PPAR $\gamma$  activation**

Anna Abella et al., *Endocrinology* (2002 Aug), 143(8):3001-8.

**Key findings:**

Primary embryonic fibroblasts from cdk4 $^{-/-}$  mice have lost their capacity to differentiate into adipocytes, and the positive role of cdk4 in adipogenesis is likely the result of its interaction with PPAR $\gamma$ .

**Role of Cdk4 in lymphocyte function and allergen response**

Yu-Hua Chow et al., *Cell Cycle* (December 15, 2010), 9:24, 4922-4930.

**Key findings:**

Cdk4 $^{-/-}$  mice had hypoplastic thymuses with decreased total thymocyte cell numbers and increased CD4/CD8 double negative cells. Cdk4 $^{-/-}$  bone marrow (BM) chimeric mice showed similar findings.

## 11 Other toxicology studies

**Local Tolerance:** no study was reviewed, the following summary was provided by the Applicant.

Palbociclib was evaluated for the potential to cause local irritation when administered intravenously or perivascularly as a parenteral formulation to the ear of New Zealand White rabbits. Palbociclib and its accompanying formulation were well-tolerated when administered as a single IV or perivascular (PV) dose at 50 and 2.5  $\mu$ g, respectively. Tissue irritancy scores were limited to redness and discoloration throughout the study that was mostly comparable among the dose groups, and there were no significant differences in histologic findings between saline, vehicle, and the palbociclib-treated group. Based on the assessment of microscopic pathology, IV and PV administration of palbociclib to the ears of New Zealand White rabbits was not associated with vascular or perivascular irritation at the doses tested.

**Phototoxicity:** no study was reviewed, the following summary was provided by the Applicant.

The phototoxicity potential of palbociclib was evaluated based on its absorbance in the ultraviolet A (UVA) range at 355 nm with a molar extinction coefficient (MEC) of 16751 L

$\text{mol}^{-1}\text{cm}^{-1}$  when calculated at pH 7.4, and affinity for pigmented tissues (eg, eye and skin) following systemic administration. Palbociclib was evaluated in a non-GLP 3T3 fibroblast Neutral Red Uptake (NRU) assay and based on photo irritation factor (PIF) and mean photo effect (MPE) values of 1.44 and 0.003, respectively, was determined to be non-phototoxic.

### Studies on Metabolites

Table 66. Summary of toxicokinetic parameters in rats (PF-05089326, a metabolite): part of 15-week rat study

Study day	Dose level (mg/kg/dose)	Gender	$C_{\text{max}}$ (ng/mL)	Dose normalized $C_{\text{max}}$	$\text{AUC}_{0-24}$ (ng.h/mL)	Dose Normalized $\text{AUC}_{0-24}$	$t_{\text{max}}$ (h)
1	10	M	2.1	0.2	19.8	2	4
	50	F	3.6	0.07	25.9	0.5	3
	30	M	14.4	0.48	196	6.5	5
	100	F	7.3	0.07	59	0.6	7
	100	M	24.0	0.24	386	3.9	8
	200	F	7.1	0.04	69.6	0.35	6
49	10	M	6.7	0.7	47.6	4.8	4
	50	F	3.9	0.08	26.4	0.5	2
	30	M	23.2	0.77	301	10	5
	100	F	6.1	0.06	52.8	0.53	4
	100	M	49.7	0.5	927	9.27	6
	200	F	8.0	0.04	116	0.58	4
105	10	M	6.9	0.7	52.1	5.21	4
	50	F	3.2	0.06	28.6	0.57	2
	30	M	27.7	0.9	463	15.4	5
	100	F	7.3	0.07	83.8	0.84	7
	100	M	55.1	0.55	895	8.95	5
	200	F	10.0	0.05	192	0.96	7

#### Conclusion:

- On Days 1, 49, 105 plasma exposures ( $C_{\text{max}}$  and mean  $\text{AUC}_{0-24}$ ) were increased more than proportionally to the dose increases from 10 mg/kg to 30 mg/kg, but increased less than proportionally to the dose increases from 30 mg/kg to 100 mg/kg in male rats; plasma exposures ( $C_{\text{max}}$  and mean  $\text{AUC}_{0-24}$ ) were generally increased proportionally to the dose increases at the tested ranges (50 mg/kg to 200 mg/kg) in female rats.
- Higher exposure for the males was observed on all occasions compared to the females;
- No apparent accumulation was observed following repeated dosing;
- $T_{\text{max}}$  ranged from 4 to 8 hours in male rats, and 4 to 7 hours in female rats.

**Studies on Impurities****Study title:** Genetic toxicology–bacterial mutagenicity assay of**Note:** (b) (4) is a drug substance impurity**Key findings:** Under the study conditions, (b) (4) was not mutagenic to test strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2uvrA pKM101(*E. coli*) with or without metabolic activation.**Study no.:** 2013-1QA-GN**Volume #, and page #:** electronic submission, page 1-44**Conducting laboratory and location:** Pfizer Worldwide Research & Development  
Drug Safety Research & Development  
Eastern Point Road  
Groton, CT USA**Date of study initiation:** January 28, 2013**GLP compliance:** no**QA reports:** yes ( ) no ( x)**Drug, lot #, and % purity:** (b) (4)  
Lot # 705873-057-1  
Purity 99.9%**Methods:** plate incorporation**Strains:** TA98, TA 100, TA1535, TA1537, and WP2uvrA pKM101**Concentrations used in definitive study:**

0.05, 0.15, 0.50, 1.5, and 5.0 mg/plate.

**Basis of concentration selection:** bacteriotoxicity or ICH S2 criteria for the highest concentration of 5000 µg/plate**Metabolic activation system:** Liver S9 mix from Aroclor 1254-induced rats**Negative controls:** dimethylsulfoxide (DMSO)**Positive controls:**

Indicator Strain	Positive Control Without S9 (dose)	Positive Control With S9 (dose)
TA 1535	Sodium nitrite (2.0 mg/plate)	2-Anthramine (0.005 mg/plate)
TA 1537	9-Aminoacridine (0.05 mg/plate)	2-Anthramine (0.01 mg/plate)
TA 98	2-Nitrofluorene (0.005 mg/plate)	2-Anthramine (0.01 mg/plate)
TA 100	Nitrofurantoin (0.002 mg/plate)	2-Anthramine (0.005 mg/plate)
WP2uvrA pKM101	N-Ethyl-N'-nitro-nitrosoguanidine (0.005 mg/plate)	2-Anthramine (0.1 mg/plate)

**Incubation and sampling times:** at 37°Capproximately 48 hours for *E. coli* and ~ 72 hours for *Salmonella***Results**Study validity:

No. of replicates: 3

Counting method: using an Accucount 1000 (count correction by area) in conjunction with AAA Version 3.9 or manually (no count correction), if necessary.

Criteria for positive results: a positive direct mutagenic response is defined as a dose-related, reproducible, three-fold increase in the average number of revertant colonies per plate compared to the average number per non-activated negative control plate.

Control selection for bacteria tester strains was adequate based upon Guideline For Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses. Drug concentration selection was adequate based upon observed bacteriotoxicity or ICH S2 criteria.

Study outcome:

There was no evidence of significant dose-related increases in the number of revertant colonies compared to the negative controls with any of the strains tested in either the absence or presence of S9.

**Comments and conclusions:** The study was valid under the conditions of this study. (b) (4) is not mutagenic to test strains TA98, TA100, TA1535, TA1537 and WP2uvrA pKM101 in the Ames reverse-mutation assay.

**Study title:** *In vitro* micronucleus assay of (b) (4)

**Key findings:** (b) (4) was positive for the induction of micronuclei in the cultured TK6 cells *in vitro* micronucleus assay.

**Study no.:** eTK62013-5

**Methods:** TK6 cells (a Human B lymphoblastoid cell line) were exposed to (b) (4) for 4 hours with or without S9 metabolic activation; for 27 hours without S9 metabolic activation. Slides were prepared, and evaluated under oil using an epi-fluorescence microscope, equipped with a Plan-Neofluor 100X objective. For both the vehicle control and the treated cultures, one thousand binucleated cells were evaluated for the presence or absence of a kinetochore signal in micronuclei. For the positive controls, five hundred binucleated cells were evaluated. The solvent vehicle control used in this study was DMSO (Dimethyl Sulfoxide). Mitomycin C (MMC) was the positive control in the 4 hour exposure without the S9 metabolic activation system (0.3 µM). Noscipine (Nosc) was the positive control in the 27 hour exposure without the S9 metabolic activation system (11.1 µM). Cyclophosphamide (Cyclo) was used as the positive control in the 4 hour exposure in the presence of the S9 metabolic activation system (8.96 µM).

**Results:** The following tables are copied from the Applicant's submission.

Table 67. Study Results: 4 Hour Treatment + 40 Hour Recovery  
(With Metabolic Activation)

Treatment (µM)	Cytotoxicity <sup>a</sup>	%MN <sup>b</sup>	Fold <sup>c</sup>	P value <sup>d</sup>
<u>Negative Control: DMSO</u>				
1%	0	0.6	1	NA
(b) (4)	Lot No.: 705873-057-1			
(b) (4)				
<u>Positive Control: Cyclophosphamide</u>				
8.95	13.4	2.8	4.67	<0.01**
Study Number: eTK62013-5		Test: 9	Day 0: 2/26/13	

Table 68. Study Results: 4 Hour Treatment + 40 Hour Recovery  
(Without Metabolic Activation)

Treatment (µM)	Cytotoxicity <sup>a</sup>	Mean %MN <sup>b</sup>	Fold <sup>c</sup>	P value <sup>d</sup>
<u>Negative Control: DMSO</u>				
1%	0	0.7	1	NA
(b) (4)	<u>(Batch 705873-057-1)</u>			
(b) (4)				
<u>Positive Control: Mitomycin C</u>				
0.15	7.66	2.9	4.14	<0.01**
0.3	27.6	5.5	7.86	<0.01**
Study Number: eTK62013-5		Test: 4	Day 0: 1/29/13	

Table 69. Study Results: 27 Hour Treatment (Without Metabolic Activation)

Treatment (µM)	Cytotoxicity <sup>a</sup>	%MN <sup>b</sup>	Fold <sup>c</sup>	P value <sup>d</sup>
<u>Negative Control: DMSO</u>				
1%	0	0.7	1	NA
(b) (4)	Lot No.: 705873-057-1			
(b) (4)				
<u>Positive Control: Noscapine</u>				
11.1	48.2	10.4	14.9	<0.01**
Study Number: eTK62013-5		Test: 10	Day 0: 2/26/13	

Footnotes / Abbreviations:

<sup>a</sup> Cytotoxicity = (100 - RPD) where RPD = (the number of population doublings in the treated cultures / the number of population doublings in the negative control cultures) x100

<sup>b</sup> %MN = % micronucleated mononucleates of an individual culture

<sup>c</sup> Fold = %MN of treated / % MN of concurrent negative control

<sup>d</sup> P value: Each dose is compared to the vehicle control using a Fisher's Exact Test.

<sup>e</sup> Trend: A Cochran-Armitage Trend Test is used to determine if a trend exists over the full dose range of analyzed cultures  
Statistical Significance: \* P value < 0.05, \*\* P value < 0.01

DMSO= Dimethyl Sulfoxide

**Summary:** Statistically significant and biologically relevant increases in micronucleus induction were observed above background when tested up to concentration levels that induced at least (b) (4) % relative cytotoxicity under 27 hour treatment conditions in the absence of metabolic activation.

**Study title:** Genetic toxicology–bacterial mutagenicity assay of an impurity (b) (4) of parent compound PD-0332991

**Note:** (b) (4) impurity of the parent compound.

**Key findings:** Under the study conditions, (b) (4) was not mutagenic to test strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2uvrA pKM101(*E. coli*) with or without metabolic activation.

**Study no.:** eAMES2013-15

**Volume #, and page #:** electronic submission, page 1-26

**Conducting laboratory and location:** Pfizer Worldwide Research & Development  
Drug Safety Research & Development  
Eastern Point Road  
Groton, CT USA

**Date of study initiation:** January 28, 2013

**GLP compliance:** no

**QA reports:** yes ( ) no ( x)

**Drug, lot #, and % purity:** (b) (4)  
Lot # 705861-171-2  
Purity: 68.9%

**Methods:** plate incorporation

**Strains:** TA98, TA 100, TA1535, TA1537, and WP2*uvrA* pKM101

**Concentrations used in definitive study:**

0.005, 0.015, 0.05, 0.15, 0.50, 1.5, and 5.0 mg/plate.

**Basis of concentration selection:** bacteriotoxicity or ICH S2 criteria for the highest concentration of 5000  $\mu$ g/plate

**Metabolic activation system:** Liver S9 mix from Aroclor 1254-induced rats

**Negative controls:** distilled water (dH<sub>2</sub>O)

**Positive controls:**

Indicator Strain	Positive Control Without S9 (dose)	Positive Control With S9 (dose)
TA 1535	Sodium nitrite (2.0 mg/plate)	2-Anthramine (0.005 mg/plate)
TA 1537	9-Aminoacridine (0.05 mg/plate)	2-Anthramine (0.01 mg/plate)
TA 98	2-Nitrofluorene (0.005 mg/plate)	2-Anthramine (0.01 mg/plate)
TA 100	Nitrofurantoin (0.002 mg/plate)	2-Anthramine (0.005 mg/plate)
WP2 <i>uvrA</i> pKM101	N-Ethyl-N'-nitro-nitrosoguanidine (0.005 mg/plate)	2-Anthramine (0.1 mg/plate)

**Incubation and sampling times:** at 37°C

approximately 48 hours for *E. coli* and ~ 72 hours for *Salmonella*

## Results

Study validity:

No. of replicates: 3

Counting method: using an Accucount 1000 (count correction by area) in conjunction with AAA Version 3.9 or manually (no count correction), if necessary.

Criteria for positive results: a positive direct mutagenic response is defined as a dose-related, reproducible, three-fold increase in the average number of revertant colonies per plate compared to the average number per non-activated negative control plate.

Control selection for bacteria tester strains was adequate based upon Guideline For Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A). Positive controls produced expected responses. Drug concentration

selection was adequate based upon observed bacteriotoxicity or ICH S2 criteria.

Study outcome:

The mean number of revertants per plate for all treated cultures within all tester strains were comparable to background (concurrent negative controls) with no evidence of a dose response.

**Comments and conclusions:** The study was valid under the conditions of this study. (b) (4)  
[REDACTED] is not mutagenic to test strains TA98, TA100, TA1535, TA1537 and WP2uvrA pKM101 in the Ames reverse-mutation assay.

## 12 Integrated Summary and Safety Evaluation

### TOXICOLOGY TABULATED SUMMARY

<i>Repeat Dose Toxicity Studies</i>			
Title	27-week GLP		15-week GLP
Species	Rat		Dog
Test System	Oral gavage		Oral gavage
Schedule	Daily x 21, 28 days/cycle		Daily x 21, 28 days/cycle
Dose (mg/kg/day)	Male: 10, 30, 100 Female: 50, 100, 300		0.2, 0.6, 2
Mortality	100 mg/kg: 7/20 males, 1/20 female The cause of death (5/8 males): Degeneration and/or inflammation in one or more of the feet		-
Clinical sign	rigid stance; swollen feet, legs, abdomen, penis, or perioral area; white incisor teeth; thin appearance; hypoactivity; lateral recumbence; nonformed feces; clear or red oral discharge; pale eyes, feet, ears, tail, or oral mucosa; audible or irregular respiration; cold to touch (entire body or hind feet); discolored (yellow) skin on the ears, entire body, feet, nose, or tail; discolored (red) skin on the feet, nose, penis, or tail; discolored (red) haircoat on the entire head, nose, perioral; and rough haircoat. reversible		-
Body weight	Male: ↓ up to 38% dose dependent	Female: ↓ up to 14% dose dependent	2 mg/kg: males ( ↓ up to 4%) and females ( ↓ up to 5%).
Food consumption	Male: ↓ at 100 mg/kg (↓ up to 28% at week1) Dose dependent	Female: ↓ at 300 mg/kg ( ↓ up to 17% at week5) Dose dependent	-
Ophthalmoscopy	Male: Lens cataract	Females: -	-

	at 100 mg/kg			
Hematology	Male: ↓ WBC (↓up to 59%) ↓RBC (↓up to 37%) ↑RETIC(↑up to 94%) Dose dependent reversible	Female: ↓ WBC (↓up to 17%) ↓RBC (↓up to 18%) ↑RETIC(↑up to 34%) Dose dependent reversible	Male: ↓ WBC (↓up to -460%) ↓RBC (↓up to 26%) ↑RETIC(↑up to 34%) Dose dependent reversible	Female: ↓ WBC (↓up to 17%) ↓RBC (↓up to 10%) ↓RETIC(↑up to 47%) Dose dependent reversible
Clinical chemistry	Male: 100 mg/kg ↑GLU, UN, ↑AST, ALT, ALP Not recovery after 12 weeks	Female: -	-	
Urilysis	-		-	
Organ weight	Male: ↓ spleen, thymus, testes, epididymis ↑ Adrenal reversible	Female: -	Male: ↓ thymus, testes  ↓ testes not recovered	Female: ↓ thymus  reversible
Gross Pathology	100 mg/kg: foot, tooth, adrenal, lung, GI, spleen, kidney, male reproductive system. reversible	Female: -	male reproductive system  Not recovery	-
Histopathology	Male: Hypocellularity in bone marrow, spleen, lymph nodes, thymus; Degeneration in kidney and chronic progressive nephropathy; Degeneration in tooth; Islets cells vacuolar change in pancreas; Vacuolation in liver; Lens degeneration in eyes Degeneration in testes, Epididymides; Adipose atrophy in skin.  Changes in eye and kidney	Female: Hypocellularity in bone marrow, lymph nodes; Islets cells vacuolar change in pancreas; lens degeneration in eyes.  Reversible	Male: Hypocellularity in bone marrow, thymus; Degeneration in testes, Hypospermia in epididymides.  Not recovered for testes and	Female: Hypocellularity in bone marrow  Reversible

	did not recover, others reversible		epididymides, others reversible	
Review comment	<ul style="list-style-type: none"> <li>Compared to the 15-week study: Pancreatic islet cell vacuolation was observed in both 15-week and 27-week studies, however eye lens degeneration, degeneration of tooth ameloblasts, and renal tubuloepithelial cell vacuolation correlated with increased serum glucose levels and glucosuria were only observed in the 27-week studies. The cause of death in 27-week study was associated with treatment induced hyperglycemia.</li> <li>Exposure (AUC) at HD in males was about 16 times the human exposure at the therapeutic dose, and exposure (AUC) at HD in females was about 7 times the human exposure at the therapeutic dose</li> </ul>		<ul style="list-style-type: none"> <li>Similar drug related toxicities were observed in 39-week study at doses up to 3 mg/kg;</li> <li>Exposure (AUC) at HD was about 0.9 of the human exposure at the therapeutic dose.</li> </ul>	
<b>Genetic Toxicology Studies</b>				
Ames: negative; Chromosomal Aberrations In Cultured Human Peripheral Blood Lymphocytes: negative In Vivo Micronucleus Assay (part of three-week rat study): positive at ≥ 100 mg/kg				
<b>Carcinogenicity studies</b>				
Not conducted to support this indication				
<b>Reproductive and Developmental Toxicology Studies</b>				
Fertility and early embryonic development: negative in female at doses up to 300 mg/kg Embryonic fetal development: rats- ↓ maternal body weight gain, maternal food intake and fetal body weights at 300 mg/kg/day An increased fetal incidence of cervical ribs at the 7 <sup>th</sup> vertebra (skeletal variation) at ≥ 100 mg/kg (palbociclib-related but non-adverse) AUC at 300 mg/kg/day was approximately 3 times the human exposure at the therapeutic dose. rabbits- ↓ maternal body weight gain, maternal food intake at 20 mg/kg/day A low incidence of small phalanges on the forepaws at 20 mg/kg/day, 9 times the human exposure (AUC) at the recommended dose				

### Primary Pharmacology

Palbociclib is a CDK4/cyclinD1 and CDK6/cyclinD2 inhibitor. Palbociclib inhibited the phosphorylation of the retinoblastoma (Rb) protein, and induced cell cycle arrest at the G1 phase, thereby preventing new DNA synthesis and inhibiting cell proliferation. Single agent palbociclib exhibited dose-dependent anti-tumor activity in animal tumor models with various cancer types, but not in the tested Rb-negative tumors, suggesting that palbociclib as an inhibitor of CDK4 and CDK6 attenuated cell cycle progression and tumor growth in an Rb-dependent manner. The combination of palbociclib with anti-estrogen agents demonstrated additive inhibition of cell proliferation in estrogen receptor positive (ER+) breast cancer cells by the dual inhibition of CDK4/6 and ER, and reduction of key pathway components responsible for regulating Rb and ER signaling. The anti-tumor activity of palbociclib was enhanced by concurrent use of anti-estrogen therapeutics.

Safety pharmacology assessments included evaluating the effects of palbociclib on the central nervous system in rats and respiratory and cardiovascular systems in dogs. No significant effects on the function of the central nervous system in rats were observed after a single intravenous administration of palbociclib at doses up to 300 mg/kg. Significant respiratory effects including apnea were observed in dogs at 5 mg/kg. Changes in respiratory parameters at 5 mg/kg also included transient increases in minute volume and respiratory rate, and decreases in compliance, peak expiratory flow, and tidal volume. The effect on respiratory function was observed at an unbound  $C_{max} \geq 842$  ng/mL, approximately 50 times the human clinical exposure at 125 mg QD based on mean unbound  $C_{max}$  (17 ng/mL). The treatment-related cardiovascular effects of palbociclib identified in dogs included increases in QT (up to 14 msec) and QTc (up to 9 msec) interval at  $\geq 3$  mg/kg with the systemic exposure at approximately 4 times the human clinical exposure at 125 mg QD based on mean unbound  $C_{max}$ . Decreases in heart rate (HR; up to 8 beats per minute [bpm]) that corresponded with increases in RR interval (up to 73 milliseconds [msec]) and increases in systolic blood pressure (up to 6 mmHg) were observed at 10 and 30 mg/kg between 4.5 and 20 hours postdose with the systemic exposure greater than 8 times the human clinical exposure at 125 mg QD based on mean unbound  $C_{max}$ .

## PK/ADME

In nonclinical species (rats, dogs, and monkeys), oral pharmacokinetics studies have shown that palbociclib was absorbed with  $T_{max}$  values of 3.5 hours in rats, 9 hours in dogs, and about 3 hours in monkeys; The half-life for palbociclib was 2.3, 21, and 5.3 hours in rats, dogs, and monkeys, respectively. Oral bioavailability was 53%, 37%, and 23% in rats, dogs, and monkeys, respectively. Palbociclib exhibited low to moderate plasma clearance (7.2 to 38 mL/min/kg) and a large volume of distribution (5.1 to 7.1 L/kg), suggesting extensive distribution to tissues. In toxicokinetic studies conducted in rats and dogs, increase in drug exposure was generally dose-dependent. Higher drug exposure was observed in male rats when compared to female rats; however, this sex-related difference was not observed in dogs.

Radioequivalents were widely distributed to most tissues and fluids after an oral dose of [ $^{14}$ C]palbociclib to pigmented Long Evans (L-E) rats, with tissue radioactivity levels consistently greater than those observed in blood. Radioequivalents in central nervous system (CNS) tissues were below those observed in blood except for meninges, pituitary, choroid plexus, and cerebrospinal fluid (CSF). [ $^{14}$ C]palbociclib derived radioequivalents were the highest in the uvea. Plasma protein binding of palbociclib was moderate in mouse, rat, rabbit, and human plasma (mean fraction unbound [fu] ranged from 0.0733 to 0.16), and was low in dog plasma (mean fu of 0.4). Palbociclib showed a modest preferential distribution to blood cells over plasma in humans ( $K_p$  of 2.44), but similar distribution of palbociclib between blood cell and plasma compartments was observed in the evaluated nonclinical species ( $K_p$  between 0.94 to 1.36).

Palbociclib was metabolized to multiple metabolites in a qualitatively similar manner in rat and human hepatocytes. In vitro, palbociclib was primarily metabolized by sulfotransferase (SULT)2A1 and cytochrome P450 (CYP)3A enzymes. In rats, dogs, and humans, [ $^{14}$ C]palbociclib was mainly eliminated via the feces with urinary excretion as a minor route of elimination. In rats, the high fecal elimination occurred via biliary excretion as evidenced by recovery of 50% (males) to 81% (females) of dose in bile within 48 hours after dosing to rats.

Palbociclib demonstrated little or no reversible and/or time-dependent inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6 enzyme activities. Thus, palbociclib is unlikely to have a pharmacokinetic drug-drug interaction (DDI) with drugs that are metabolized by these CYP enzymes. PD-0332991 demonstrated time dependent inhibition of CYP3A.

## Toxicology

*General toxicology:* Palbociclib was assessed in single dose toxicity studies and repeated dose studies in rats with up to 27 weeks in duration and in dogs with up to 39 weeks in duration. Repeat-dose toxicology studies were conducted with daily administration for 21 days followed by 7-day non dosing period.

Single doses up to 500 mg/kg were tolerated in the rat. Body weight loss, fecal changes, hypoactivity, dyspnea, and mortality were observed at  $\geq 1000$  mg/kg in rats. Single doses up to 30 mg/kg were tolerated in the dogs. Emesis, decreased body weight and food consumption, fecal changes, and hematology changes (decreases in red blood cell [RBC] parameters, leukocytes, and platelets) that correlated with

decreased bone marrow cellularity were observed in dogs that received single doses of  $\geq 30$  mg/kg.

In repeat-dose rat and dog toxicity studies, the primary palbociclib-related toxicities were observed in the bone marrow, lymphoid tissues, and male reproductive organs. Minimal to marked decreases in cellularity in the bone marrow and lymphoid tissues (thymus, spleen, mesenteric lymph node, gut-associated lymphoid tissue) were associated with the hematological changes that included decreases in leukocytes (neutrophils, monocytes, eosinophils, and lymphocytes), red blood cell parameters, and platelets, and were observed with dose-related severity in studies of  $\geq 2$  weeks duration in both the rat and dog. Male reproductive organ effects included a dose-related increase of degeneration of seminiferous tubules, hypospermia and increased intratubular cellular debris of the epididymis, and decreased prostate weights that correlated with atrophy or decreased content. The effects on male reproductive organs were identified in the rat and/or dog in studies of  $\geq 3$  weeks duration. Partial to complete reversibility of toxicities to the hematolymphopoietic and male reproductive systems was demonstrated following a recovery period (4-12 weeks), with the exception of the male reproductive organ findings in dogs. Above toxicities were observed at clinically relevant exposures. Additional toxicities included gastrointestinal, liver, kidney, endocrine/metabolic, respiratory, and adrenal effects in rats and/or dogs. Minimal or mild degeneration and regeneration of kidney tubule epithelial cells was observed with increased incidence and/or severity following 15 or longer of dosing in rats and minimal degeneration was observed in a 2-week dog study at a severely toxic dose. In addition, altered glucose metabolism associated with pancreatic islet cell vacuolation and secondary effects in the eye, teeth, kidney, and adipose tissue were identified in the 27-week rat study with palbociclib at doses of  $\geq 30$  mg/kg/day in males and at 300 mg/kg/day in females. The exposures in male and female rats at these doses were approximately 11 and 7 times the human exposure (AUC) at the recommended dose, respectively. Some of these findings were present in the 15-week repeat-dose toxicology study in rats, but with decreased incidence and severity. The rats used in these studies were approximately 7 weeks old at the beginning of the studies. In the 27-week rat study, rats were died due to degeneration and/or inflammation of the feet and other adverse effects which are considered to be associated with treatment induced hyperglycemia. The findings of pancreatic islet cell vacuolation, eye lens degeneration, degeneration of tooth ameloblasts, and renal tubuloepithelial cell vacuolation correlated with increased serum glucose levels and glucosuria, suggesting a role of increased glucose levels, or increased glucose together with the potential primary effects of palbociclib on these organs in the pathogenesis of these findings. The lens degeneration and chronic progressive nephropathy remained at the recovery euthanasia, suggesting permanent and/or progressive nature of these effects.

No additional target organ findings were identified in the 39-week dog study with palbociclib (at up to 3 times human clinical exposure) beyond the hematolymphopoietic and male reproductive organ effects previously characterized in shorter duration studies.

#### *Genetic toxicology:*

Palbociclib was assessed in genetic toxicology assays consisting of the microbial reverse mutation, in vitro cytogenetic (human lymphocytes), in vitro micronucleus (CHO-

WBL cells) and in vivo rat micronucleus assays. All in vitro tests were conducted with and without exogenous metabolic activation using concentrations up to those limited by cytotoxicity or insolubility.

Palbociclib was not mutagenic in the bacterial reverse mutation assay when tested up to 5 mg/plate. Palbociclib was also negative for clastogenicity and polyploidy in a human lymphocyte aberration. Palbociclib caused micronuclei formation in CHO-WBL cells via an aneugenic mechanism. Significant increases in micronucleated polychromatic erythrocytes were observed in male rats at doses  $\geq$  100 mg/kg/day in an in vivo rat micronucleus assay.

#### Carcinogenicity:

Carcinogenicity studies were not conducted or required to support the proposed indication.

#### Reproductive toxicology:

Palbociclib had no effects on estrous cycling or mating and fertility in female rats at up to 300 mg/kg/day. In addition, there were no effects on any ovarian or uterine parameter or embryonic survival in any dose group tested. The NOAEL for reproductive toxicity was identified as 300 mg/kg/day, with associated Day 14 mean  $C_{max}$  and AUC<sub>24</sub> of 627 ng/mL and 8010 ng•h/mL, respectively.

Maternal and embryo-fetal effects of palbociclib were evaluated in presumed pregnant female Sprague-Dawley rats and New Zealand White rabbits. Maternal body weight gain and food intake were reduced up to 14% relative to control at 300 mg/kg/day during the treatment interval in rats. Reduced fetal body weights (5% relative to control) were also observed at 300 mg/kg/day. An increased fetal incidence of cervical ribs at the 7<sup>th</sup> vertebra (skeletal variation) at  $\geq$  100 mg/kg was considered palbociclib-related but non-adverse since the ossification sites were small and are not considered to have postnatal significance. The maternal NOAEL in rats was 100 mg/kg/day due to decreased maternal body weight gain and maternal food intake at 300 mg/kg/day. The GD17 mean  $C_{max}$  and AUC<sub>24</sub> at 300 mg/kg/day was 456 ng/mL and 7310 ng•h/mL (4 times human exposure at the therapeutic dose), respectively. In rabbit, maternal body weight gain and food intake were reduced up to 24% relative to control at 20 mg/kg/day during the treatment interval. An increased incidence of small phalanges (3 fetuses from 2 litters) and other skeletal variations in the forelimbs relative to control were observed during skeletal examination at 20 mg/kg/day. An increased incidence of rabbit fetuses with 13 ribs was considered palbociclib-related but non-adverse at 10 and 20 mg/kg/day, as this finding is among the most common skeletal variations in the rabbit and was not coincident with other changes in the axial skeleton. The maternal and developmental NOAEL was 10 mg/kg/day based on reduced maternal body weight gain and food intake, and the low incidence of fetuses with small forepaw phalanges at 20 mg/kg/day. The GD19 mean  $C_{max}$  and AUC<sub>24</sub> at 20 mg/kg/day was 2470 ng/mL and 17200 ng•h/mL (4 times human exposure at the therapeutic dose), respectively.

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/s/  
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WEI CHEN  
01/16/2015

TODD R PALMBY  
01/17/2015

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

**NDA Number:** 207,103

**Applicant:** Pfizer

**Stamp Date:** August 13, 2014

**Drug Name:** Ibrance® (palbociclib)

**NDA Type:** 505(b)(1)

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

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

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement  
010908

## PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	*x		* Issues generally identified during review
11	Has the applicant addressed any abuse potential issues in the submission?			n/a
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			n/a

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? \_\_\_yes\_\_\_**

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

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

Wei Chen, Ph.D	09/09/2014
Reviewing Pharmacologist	Date
Todd Palmby, Ph.D	09/10/2014
Team Leader/Supervisor	Date

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement 010908

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/s/  
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WEI CHEN  
09/10/2014

TODD R PALMBY  
09/11/2014