CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205552Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Imbruvica (ibrutinib)

Date: August 21, 2013
To: File for NDA 205552
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

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

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/s/

JOHN K LEIGHTON
08/21/2013

MEMORANDUM

Date: August 20, 2013
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 205552
Drug: IMBRUVICA (ibrutinib) capsules
Indications: treatment of patients with MCL who have received at least one prior therapy (b) (4)
Applicant: Pharmacyclics, Inc.

Ibrutinib is a small molecule tyrosine kinase inhibitor developed for the treatment of mantle cell lymphoma (MCL) (b) (4) Ibrutinib inhibits Bruton tyrosine kinase (Btk), an enzyme in the B cell receptor (BCR) signaling pathway. Btk is involved in B-lymphocyte activation and in the maintenance of some B-cell malignancies. Based on an *in vitro* kinase assay conduced, ibrutinib can also inhibit Bmx/Etk, another member of this kinase family, the function of which is not fully understood. It can also inhibit EGFR, and some members of the SRC family of kinases (e.g. Hck and Yes); however, with up to 10 fold less activity. In xenograft and/ or cell culture studies, ibrutinib showed anti-cancer activity against cells derived from B-cell malignancies, including MCL and CLL lines. Ibrutinib inhibited the adhesion of MCL and CLL cells to fibronectin and vascular cell adhesion molecule-1 (VCAM-1), suggesting the potential for ibrutinib to affect the trafficking of B-cells.

Pharmacology, safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism and excretion), and toxicology studies were conducted in *in vitro* systems and/or in animal species. Animal toxicology studies were conducted in appropriate species, using the administration route and dosing regimens that adequately addressed safety concerns in humans. Ibrutinib-related toxicities in rats and dogs included: GI toxicities (e.g. ulceration and inflammation), adverse findings in the lymphoid tissues (e.g. depletion, necrosis, and inflammation), and epidermal necrosis and exudate. Other findings with unknown association to treatment included muscle degeneration in the stomach, effects on bone (e.g. thinning of cortical bone), and pancreatic acinar atrophy/ reduced zymogen granules.

Transient lymphocytosis reported in patients treated with IMBRUVICA may be due to reduced homing of leukocytes as expected based on the pharmacology studies. GI, skin and musculoskeletal disorders have been reported in patients and are listed in the label. The ongoing studies in patients will provide additional information on the toxicities associated with ibrutinib.

Ibrutinib was not mutagenic or clastogenic when tested in the battery of genotoxicity studies. Several impurities were tested in the bacterial mutagenicity (Ames) assay and/or assessed for mutagenicity through SAR (structure- activity relationship) computational methods. The impurities were considered negative for mutagenicity. Ibrutinib caused fetal malformations in rats when given to pregnant animals during the period of organogenesis, at a maternally toxic dose. Pregnancy category D is recommended.

Fertility studies using ibrutinib have not been conducted. The general toxicology studies in rats and dogs did not demonstrate adverse findings in male or female reproductive organs.

The nonclinical studies needed to support product labeling were reviewed by Drs. Shwu-Luan Lee, Brian Chiu, Margaret Brower, and George Chang. The nonclinical findings are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

Recommendation: I concur with the pharmacology/toxicology reviewers that from a nonclinical perspective, IMBRUVICA may be approved and that no additional nonclinical studies are needed to support approval of IMBRUVICA for the proposed indications.

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/s/

HALEH SABER
08/20/2013

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:	NDA 205552
Supporting document/s:	N-000
Applicant's letter date:	May 31, 2013
CDER stamp date:	April 30, 2013
Product:	Ibrutinib (Imbruvica)
Indication:	Mantle cell lymphoma (MCL) [redacted] (b) (4) at least one prior therapy
Applicant:	Pharmacyclics, Inc.
Review Division:	Division of Hematology and Oncology Toxicology (DHOT), for Division of Hematology Products (DHP)
Reviewer:	Shwu-Luan Lee, Ph.D., Haw-Jyh (Brian) Chiu, Ph.D., George Ching-Jey Chang, Ph.D., Margaret E. Brower, Ph.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John Leighton, Ph.D., DABT (DHOT) Ann Farrell, MD (DHP)
Project Manager:	CAPT Diane Hanner

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not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 205552.

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1 Executive Summary

1.1 Recommendations

There are no pharmacology/toxicology issues which preclude approval of Imbruvica for the proposed indication.

1.1.1 Approvability

Recommending approval

1.1.2 Additional Non Clinical Recommendations

No additional non-clinical studies are required for the proposed indications.

1.1.3 Labeling

Recommendations on labeling have been provided within team meetings and communicated to the Applicant. See the approved label.

1.2 Brief Discussion of Nonclinical Findings

Ibrutinib (PCI-32765) is an irreversible inhibitor of Bruton's tyrosine kinase (Btk); it binds covalently to a cysteine in the active site of Btk. The IC_{50} of ibrutinib was 0.46 nM in the in vitro kinase assay. Ibrutinib may also inhibit Bmx/Etk, another member of this tyrosine kinase family as indicated by the IC_{50} of 0.76 nM and B lymphocyte kinase, BLK (IC_{50}= 0.52 nM). The following kinases were inhibited by ibrutinib with IC_{50}s up to 4 nM: CSK, FGR, Brk, and HCK. Ibrutinib showed anticancer activity against B cell tumor cells in cell culture or xenograft studies. The tumor cells tested included mantle cell lymphoma (e.g. MINO), chronic lymphocytic leukemia (TCL1-192, a line of murine leukemia cells), non-Hodgkin lymphoma/follicular lymphoma (e.g. DOHH2), and diffuse large B-cell lymphoma (e.g. OCI-LY10) cell lines.

Ibrutinib inhibited the adhesion of mantle cell lymphoma (MCL) (b) (4) cell lines as well as the normal B cells to fibronectin and vascular cell adhesion molecule-1 (VCAM-1). Therefore, ibrutinib may affect the trafficking and localization of leukocytes; this effect may explain lymphocytosis reported in patients treated with ibrutinib.

Ibrutinib inhibited hERG channel currents with an IC_{50} value of approximately 1 μM and may be considered a low-potency blocker. In a single-dose safety pharmacology study in Beagle dogs, an oral ibrutinib dose up to 150 mg/kg did not induce QT interval prolongation; increases in the RR interval were observed. Dose-dependent RR interval prolongation and decreased heart rate was reported in dogs in the 13-week toxicology

study during Weeks 1 and 12. The effect occurred at 1 hour post-dose. One of the major metabolites of PCI-32765, PCI-45227, inhibited hERG channel currents with an IC_{50} value of 9.6 μM, i.e. ten fold less potency for blocking the Ikr current compared to the parent drug. QTc prolongation was not reported in patients treated with ibrutinib.

The ADME studies were not reviewed; however, based on the summary information provided by the Applicant, orally administered ibrutinib was absorbed fairly rapidly (t_{max} of ~ 5 min-2.5 hr in rats and 0.5-4 hr in dogs), with variable oral bioavailability (~7% to 23%) in animal species. Ibrutinib was highly bound to plasma proteins in all species tested (96-99%): mouse, rat, rabbit, dog, and human plasma. Following an oral dose of ^{14}C-ibrutinib in Long Evans male rats, radioactivity was high in the following tissues and organs: small intestine, esophagus, liver, urinary bladder, and kidney. Ibrutinib-derived radioactivity was not detectable in tissues of the central nervous system with the exception of the olfactory lobe, indicating a limited ability of ibrutinib and/or its metabolites to cross the blood-brain barrier in rats. The distribution in the milk of lactating rats was not investigated. There was no clear difference in the distribution of radioactivity in the pigmented tissues when compared to the non-pigmented tissues, in treated rats. A total of 41 metabolites were identified in the *in vitro* or *in vivo* studies. The main circulating metabolites in rat plasma were M15, M5 and M37 (PCI-45227). Following oral doses of ^{14}C ibrutinib in rats, up to 47% of the radioactivity was excreted in bile, with M21 (a sulfate conjugate of M35) as the main metabolite. While very low radioactivity was found in urine (<2% of the dose), the main metabolites in feces were M34, M35 and M17 (approximately 8-16% of the dose). In human liver microsomes, ibrutinib was predominantly metabolized by CYP3A4/5. The primary route of elimination of ibrutinib was via feces (hepatobiliary) in animals. Accumulations of ibrutinib and one of its major metabolites, PCI-45227 (M37), occurred following repeated oral doses of ibrutinib in both rats and dogs.

The general toxicology studies in rats and dogs identified GI tract, lymphoid tissues, bone and skin as the main target of toxicities. The major findings are as follows:

- Gastro-intestinal tract:
  The most prominent effect of ibrutinib in rats and dogs was dose-dependent GI toxicities. The toxicities were observed following multiple oral administrations of the drug, and were considered the cause of mortalities in the rat (acute inflammation and ulceration of the small intestines) and the dog (enterocolitis). GI histopathology findings included atrophy of squamous epithelium with progression to ulceration in the nonglandular stomach, acute inflammation and ulceration of the intestinal tract, as well as muscle degeneration in the stomach.
- Hematopoietic/lymphoid system:
  Findings included increased white blood cells and differentials (which may be due to ulceration/necrosis and inflammation reported in various tissues or may be due to reduced homing of B cells); lesions in lymphoid tissues; lymphoid depletion; inflammation, necrosis, and atrophy in lymph nodes, spleen, as well as thymus. Immunophenotyping incorporated into the 13-week study in rats indicated lower absolute B cells and increased T- and natural killer cells in peripheral blood.

- Skin
  In rats, epidermal necrosis, surface exudate, dermal abscess, and acute and subacute inflammation were observed at the end of a 4-week treatment; squamous atrophy of epithelium was noted at the end of the 13-week study. There were no remarkable findings in the skin of dogs.
- Liver
  Hepatotoxicity was evident in rats in the 4-week study, as shown by increased ALTAST and hepatic necrosis. Liver toxicities were not observed in the 13-week study in rats or in studies in dogs.
- Bone
  Minimal to mild decreases in cortical and trabecular bones were found in rats treated for 13 weeks.
- Other target organs:
  Acinar atrophy and decrease in zymogen granules in pancreas was observed in rats. Bilateral corneal dystrophy/degeneration (opacity of the cornea) was found in dogs treated with ibrutinib for 4 weeks and in one dog in the 13-week study. The association of these findings to the ibrutinib treatment is unclear.

Ibrutinib was not mutagenic in bacterial Ames test or clastogenic in a chromosome aberration test in Chinese Hamster Ovary cells (CHO). Ibrutinib did not increase micronucleus formation in mice after oral doses up to 2000 mg/kg. The mutagenicity of impurities was assessed through Ames test or by 2 computational SAR analyses (DEREK Nexus and MultiCase). The impurities tested were not mutagenic.

Reproductive and developmental toxicities of ibrutinib were investigated in rats and rabbits. Ibrutinib was administered orally to pregnant rats during the period of organogenesis at doses of 10, 40 and 80 mg/kg/day. Increased post-implantation loss and increased resorption occurred at the high dose of 80 mg/kg. Fetal toxicities (visceral malformations and variations, and skeletal variations) were observed at the high dose of 80 mg/kg. Reduced fetal weight was seen at ibrutinib doses at 40 mg/kg and 80 mg/kg. The dose of 80 mg/kg resulted in maternal toxicities. The dose of 80 mg/kg/day in animals resulted in exposures (total AUC) approximately 14 times the AUC in patients with MCL (ibrutinib dose of 560 mg/day) and 20 times the AUC in patients with CLL (ibrutinib dose of 420 mg/day). The exposure at 40 mg/kg/day was approximately 6 times the AUC in patients with MCL and 8 times the AUC in patients with CLL.

In a non-GLP study conducted in rabbits, ibrutinib was administered orally to pregnant animals during the period of organogenesis at doses of 10, 30, and 100 mg/kg/day. At the ibrutinib dose of 100 mg/kg, which is greater than the maternally-toxic dose (≥30 mg/kg/day), there were embryo-fetal toxicities. Findings included increases in resorption and implantation loss, decreases in viable fetuses and fetal body weights, as well as spontaneous abortions.

Ibrutinib did not cause adverse findings in male or female reproductive organs in general toxicology studies. Testicular seminiferous tubule degeneration was found in one dog that was treated with 220 mg/kg of ibrutinib and sacrificed early due to poor conditions. This finding was possibly an indirect effect of ibrutinib due to the poor condition of the animal.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

936563-96-12.1.2 Generic Name

Ibrutinib

2.1.3 Code Name

PCI-32765-00

2.1.4 Chemical Name

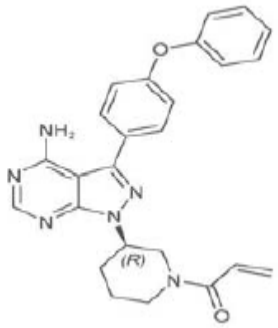
R enantiomer of 1-[3-[4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-1-piperidinyl]-2-propen-1-one (free base)

2.1.5 Molecular Formula/Molecular Weight

$C_{25}H_{24}N_6O_2$/440.5 g/mol

2.1.6 Structure

Figure 1- Structure of ibrutinib (PCI-32765)



PCI-32765 is an R-enantiomeric chiral compound.

2.1.7 Pharmacologic class

Kinase inhibitor

Mechanism of action: Bruton's tyrosine kinase inhibitor

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 102688; DMF (b) (4)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Table 1- Clinical formulation

(Table from the Applicant, eCTD Module 3, Sction 3.2.P.1)

Component	Quality Reference	Function	Quantity/Unit Dose (mg/capsule)
Ibrutinib	In house specification	Active	140 [a]
Microcrystalline cellulose	NF, Ph. Eur., JP	(b) (4)	
Croscarmellose sodium	NF, Ph. Eur., JP		
Sodium lauryl sulfate	NF, Ph. Eur., JP		
Magnesium stearate[b]	NF, Ph. Eur., JP		
Size 0, white, opaque, hard gelatin capsule with black "ibr 140 mg" print [c]	In house specification	Capsule shell	1 capsule
		Total	330.0

[a] The ibrutinib quantity per capsule may be adjusted based on its purity (b) (4) (b) (4) with a corresponding adjustment to the microcrystalline cellulose quantity.

[b] (b) (4)

[c] Capsules are composed of gelatin, NF, Ph. Eur. and titanium dioxide, USP/Ph.Eur/JP; the capsule shells are printed with (b) (4) printing inks (see Table 2 and Table 3 for ink compositions).

2.3.2 Comments on Novel Excipients

None. All excipients are USP or NF grade.

Reviewer's note: the amount of microcrystalline cellulose, i.e., (b) (4) mg/capsule is within the range of FDA approved drug for this excipient in hard gelatin capsule (up to (b) (4) /capsule).

2.3.3 Comments on Impurities/Degradants of Concern

There are five impurities in the drug substance at levels higher than the qualification threshold described in ICH Q3A. Among them, (b) (4) and (b) (4) were also degradants found in the drug product (DP). However, the contents in the DP were below qualification threshold. All impurities above the qualification threshold of ICH Q3A were assessed for genotoxicity. Genotoxicity assessment was also done for impurities (b) (4) and (b) (4) which were below qualification threshold. The table below is the summary of safety assessment conducted for the impurities of concern:

Table 2- Summary of impurities/degradants in drug substance and drug product

Impurity (ID #)	Description	Specification (NMT % w/w)	Comment
(b) (4)	Actual DS Impurity - Synthesis impurity (b) (4)	(b) (4)	• Derek and MultiCase were conducted. The impurity is considered negative for mutagenicity
	Actual DS Impurity Synthesis Impurity Degradation product		• Qualified in a general toxicity study • Ames negative
	Actual DS impurity - Synthesis impurity (b) (4)		• Qualified in a general toxicity study • Ames negative;
	Actual DS Impurity Synthesis impurity (b) (4) Degradation product		• A general toxicity: study with this impurity is ongoing • Derek and MultiCase: were conducted. The impurity is considered negative for mutagenicity
	Actual DS Impurity Degradation product (ibrutinib dimer)		• A general toxicity: study with this impurity is ongoing • Derek and MultiCase: were conducted. The impurity is considered negative for mutagenicity
	Potential DS Impurity* Carryover impurity from starting material (b) (4)		• Below qualification threshold • Ames negative
	Potential DS Impurity* Synthesis impurity (b) (4) Degradation product		• Below qualification threshold • Ames negative

DS: drug substance; DP: drug product

Reviewer's note:
The proposed specification limits (% w/w) of impurities (b) (4) and (b) (4) are acceptable, according to ICH guidance S9.

The highest daily intake of these impurities at the proposed specification limit in patient at recommended dose of 560 mg per day would be:

Impurity	Drug product
[redacted]	(b) (4)

While the specification limits are acceptable for the proposed cancer indications, the Applicant is currently conducting a general toxicology study to further assess the safety profile of these impurities. These data are not needed for the current indications; however, they may be needed later; e.g. if the Applicant seeks approval in indications not covered by ICH S9.

2.4 Proposed Clinical Population and Dosing Regimen

- Indication: for the treatment of mantle cell lymphoma (MCL) [redacted] (b) (4) in patients who have received at least one prior therapy
- Dosing regimen:
  - MCL: 560 mg once daily

[redacted] (b) (4)

2.5 Regulatory Background

The Applicant submitted a rolling NDA, the pharmacology/toxicology data, Module 4, was submitted on April 30, 2013.

3 Studies Submitted

3.1 Studies Reviewed

Study Number	Study Title
Primary Pharmacology	
08-088-Z-X-BIO	*In vitro* pharmacodynamic studies with PCI-32765
	Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase, Pan *et al.*, Chem Med Chem 2:58-61, 2007
	Chronic active B-cell receptor signaling in diffuse large B-cell lymphoma, Davis E, Ngo V, Lenz G, *et al.,* Nature, 463:88-94, 2010
	The clinically active BTK inhibitor PCI-32765 targets B-cell receptor- and chemokine-controlled adhesion and migration in chronic lymphocytic leukemia, de Rooij *et al.*, Blood 119: 2590-2594, 2012
07-114-M-IV-IP-PO-PDI	*Ex vivo* labeling study of splenocytes using a btk fluorescent probe (PCI-33880) and inhibition of labeling following administration of PCI-32765 in

	mice by the intravenous, intraperitoneal or oral route
07-119-M-PO/IV/SC-PDI	*Ex vivo* time course inhibition study of PCI-32765 administered orally, subcutaneously or intravenously to Balb/C mice followed by splenocyte harvest and fluorescent probe labeling
07-130-M-SC/PO-PDI	*Ex vivo* dose response inhibition study of PCI-32765 administered orally, or subcutaneously to Balb/C mice followed by splenocyte harvest and fluorescent probe labeling
07-132-M-PO-PDI	*Ex vivo* labeling study of DOHH2 tumor tissue using a Btk fluorescent probe (PCI-33880) and inhibition of labeling following administration of PCI-32765 in mice by the oral route
10-089-M-PO-EFI	Evaluation of tumor inhibition in OCL-LY 10 lymphoma tumor bearing female SCID mice administered ibrutinib by oral gavage
07-098-M-IV/IP/SC-PDI	Intravenous or intraperitoneal route efficacy study of PCI-32765 in DOHH2-tumor bearing CB17-SCID mice
07-137-M-SC/PO-PDI	Efficacy and pERK inhibition study of PCI-32765 administered orally to DOHH2 tumor bearing CB17 Fox Chase SCID mice
07-132-M-PO-PDI	Ex vivo labeling study of DOHH2 tumor tissue using a Btk fluorescent probe (PCI-33880) and inhibition of labeling following administration of PCI-32765 in mice by the oral route
12-035-M-PO-EFI	Study title: Efficacy study using the mino mantle cell lymphoma tumor line in CB17 SCID mice administered ibrutinib continuously via medicated feed
	The Bruton tyrosine kinase inhibitor PCI-32765 thwarts chronic lymphocytic leukemia cell survival and tissue homing in vitro and *in vivo*, Ponader et al., Blood 119: 1182-1189, 2012
Secondary Pharmacology	
05-0383-V-X-RB	Hit profiling screen data report Celera Genomics report ((b) (4)
07-074-Hu-X-RB	Lead profiling screen data report (b) (4))
Safety Pharmacology	
06-024-R-PO-SP	An oral (gavage) central nervous system pharmacological profile of PCI-32765 in rats
07-077-R-PO-SP	Respiratory assessment of PCI-32765 administered by oral gavage to plethysmograph-restrained female Sprague Dawley rats
07-079-HEK-X-CT	Effects of PCI-32765 on cloned hERG potassium channels expressed in human embryonic kidney cells
10-015-HEK-X-CT	Effect of PCI-45227 on cloned hERG potassium channels expressed in human embryonic kidney cells
06-026-D-PO-SP	Cardiovascular assessment of orally administered PCI-32765 in conscious radiotelemetry-implanted male Beagle dogs
General toxicology	
Repeat dose toxicology	
06-017-R-PO-TX (b) (4) -622011	A 28 day oral (gavage) toxicity study of PCI-32765 in rats with a 28-day recovery period
10-068-R-PO-TX (b) (4) -622033)	A 13-week oral (gavage) toxicity study of PCI-32765 in rats with a 6-week recovery period
06-018-D-PO-TX (b) (4) -622012)	A 28 day oral (gavage) toxicity study of PCI-32765 in dogs with a 28-day recovery period
10-069-D-PO-TX ((b) (4) -622034)	A 13-week oral (gavage) toxicity study of PCI-32765 in Beagle dogs with a 13-week recovery period
	Oval corneal opacities in beagles. III. Histochemical demonstration of stromal lipids without hyperlipidemia, Roth *et al*., Invest Ophthalmol Vis Sci 1 (part 1): 95-106, 1981
	Inflammation-mediated osteopenia (IMO) during acute inflammation in rats is due to a transient inhibition of bone formation, Pfeilschifter *et al*.,

	Calcif Tissue Int. 41:321-325, 1987.
Genotoxicity	
06-027-Sal-X-MU	Bacteria reverse mutation assay
07-038-CHO-X-MU	In vitro mammalian chromosome aberration test
08-071-CHO-X-MU	In vitro mammalian chromosome aberration test
07-037-M-PO-MU	Mouse bone marrow erythrocyte micronucleus test following oral administration of PCI-32765
Genotoxicity for impurities	
11-113-Sal-X-Mu	Bacterial reverse mutation assay of (b) (4)
11-098-Sal-X-Mu	Bacterial reverse mutation assay of (b) (4)
12-170-Sal-X-MU-IMP	Bacterial reverse mutation assay of (b) (4)
11-099-Sal-X-Mu	Bacterial reverse mutation assay of (b) (4)
Reproductive and developmental toxicology	
11-132-R-PO-TT	An oral (gavage) embryo fetal development study of PCI-32765 in rats
10-064-B-PO-TTE (non-GLP)	An oral (gavage) concentration range-finding study of the effects of PCI-32765 on embryo/fetal development in rabbits

3.2 Studies Not Reviewed

Study Number	Study Title
Primary Pharmacology	
13-042-Hu-X-ENZ	Inhibition of BTK by PCI-32769 in Reaction Biology Kinase Assay
13-047-Hu-X-ENZ	Inhibition of BTK by PCI-45227 in Reaction Biology Kinase Assay
08-082-M-PO-PDI	A time course evaluation of BTK active site binding by PCI-32765 using an ex vivo probe labeling pharmacodynamic marker assay in mouse splenocytes
08-091-M-PO-PDI	An evaluation of BTK occupancy by PCI-32765 following continuous administration in BALB/c Mice
12-098-R-PO-PDI	Btk active-site occupancy in splenocytes from rats dosed orally with PCI-32765 -dose response study
08-087-M-PO-PDI	An evaluation of BTK down regulation by PCI-32765 following repeat-dose oral administration in Balb/c mice
PK/ADME*	
11-022-R-PO-AME (FK10242)	The metabolism, excretion and stability of ^{14}C-JNJ-54179060 (PCI-32765) in the male and female Sprague-Dawley rat after a single oral administration of ^{14}C- JNJ-54179060 at 10 mg/kg.
07-039-R-PO-ADME	Distribution, metabolism, and excretion of [^{14}C]-PCI-31523 following oral or intravenous administration to rats. Document ID No.:EDMS-ERI-59069388
12-081-R-PO-EXC (FK10301)	The biliary excretion of ^{14}C-JNJ-54179060 in the male Sprague-Dawley rat after a single oral administration of ^{14}C-JNJ-54179060 at 10 mg/kg. Document ID No.: EDMS-ERI-60413627
12-188-Hu-PO-MT (FK10267)	The analysis of the radioactivity levels and metabolite profiling and identification in biological samples collected during clinical trial PCI-32765CLL1004, investigating the absorption, metabolism and excretion of 14C-JNJ-54179060 after a single oral dose of 140 mg. Document ID No.: EDMS-ERI-61014961
11-134-R-PO-WBA (FK10245)	^{14}C-JNJ-54179060: Tissue distribution in the male pigmented rat determined by whole-body autoradiography following a single oral dose. Document ID No.: EDMS-ERI-47954038
12-083-Hu-X-PB (FK10375)	The distribution of JNJ-54179060 in blood and the plasma protein binding of JNJ-54179060 in different animal species and human. Document ID

	No.: EDMS-ERI-59384312
07-153-Hu-X-MTI	Covalent Protein Binding and Metabolism Assessment of 14C-PCI-31523 (PCI-32765) in Various Recombinant CYP450 Preparations and Human-Liver Microsomes. Document ID No.: EDMSERI-59147862.
12-080-V-X-MT (FK10269)	The in vitro metabolism of ^{14}C-JNJ-54179060 in liver microsomes and hepatocytes of rat, Rabbit, dog and human. Documen ID No.: EDMS-ERI-60496556
(b) (4)	
12-013-Hu-X-MT	Cytochrome P450 Reaction Phenotyping using Recombinant CYP enzymes (9 Isoforms). Document ID No.: EDMS-ERI-60052035
12-014-Hu-X-MT	Study to Investigate Human Microsomal Metabolic Stability of PCI-32765 in the Presence of CYP Specific P450 Inhibitors. Document ID No.: EDMS-ERI-60080299
11-041-Hu-X-MTI	To Determine Vmax and Km for Generating the Metabolite PCI-45227 from PCI-32765 in Recombinants Expressing CYP450 2D6 or 3A4/A5 in the Presence of Epoxide Hydrolase. Document ID No.:EDMS-ERI-60052030
07-123-V-X-MT	Study to Investigate the Stability of the Test Compound, PCI-32765, in Rat, Dog, Monkey and Human Liver Microsomes. Document ID No.: EDMSERI-60052034
12-080-V-X-MT (FK10269)	The In Vitro Metabolism of ^{14}C-JNJ-54179060 in Liver Microsomes and Hepatocytes of Rat, Rabbit, Dog and Human. Document ID No.: EDMS-ERI-60496556
06-029-Hu-X-CYP	ADME-Tox: CYP Inhibition –Study of Compound PCI-32765. Document ID No.:EDMS-ERI-60119627
12-159-V-PO-MT (FK10390)	Assessment of Senantiomer exposure after oral dosing of the Renantiomer JNJ-54179060 (PCI-32765) in plasma from different animal species and human. Document ID No.:EDMS-ERI-60873698
10-075-Hu-X-INDC	In vitro evaluation of PCI-32765 and PCI-45227 as inducers of cytochrome P450 expression in cultured human hepatocytes. Document ID No.: EDMS-ERI-60119623
General toxicology	
Single dose toxicity	
08-014-M-PO-AT1	Oral single dose toxicity study of PCI-32765 in mice
06-022-R-PO-AT	An acute oral toxicity study in male and female rats administered PCI-32765
07-109-R-IV-AT1	Intravenous single dose toxicity study of PCI-32765 in female rats
Repeat-dose toxicity	
07-146-R-PO-TX1	Two-Week Oral Toxicity Study of PCI-32765 in Rats. Document ID No.: EDMS-ERI-58330576
11-042-R-PO-TX	A 14-day oral (gavage) toxicity study of PCI-32765 in Sprague Dawley rats. Document ID No.: EDMS-ERI-58351953
11-136-B-PO-TXE	An oral (gavage) 7-day tolerability study of PCI-32765 in non-pregnant New Zealand white rabbits
06-015-D-SC/PO-TXE	An escalating single-dose/14-day toxicity and toxicokinetic study of subcutaneously or orally administered PCI-32765 in Beagle dogs
11-043-PO-TX	A 14-day oral (gavage) toxicity study of PCI-32765 in Beagle dogs
Reproductive and development toxicology	
10-063-R-PO-TTE	An oral (gavage) dose range-finding study of PCI-32765 on embryo/fetal development in rats

***The studies in PK/ADME section submitted by the Applicant (Section 4.2.2.2.)`were not reviewed. Only the pivotal studies summarized in Section 5 of current review, are listed in the table.**

3.3 Previous Reviews Referenced

IND 102688

4 Pharmacology

4.1 Primary Pharmacology

Background and proposed mechanism of action
B cell lymphoma and chronic lymphocytic leukemia are thought to arise from mutation acquired during B cell development; as such, signaling through the B cell receptor (BCR) is needed to sustain the viability of B cell malignancies. Bruton's tyrosine kinase (Btk) is an enzyme in the B cell receptor signal transduction pathway involved in the maintenance of B cell malignancies. PCI-32765 is an irreversible inhibitor of Bruton's tyrosine kinase. The irreversibility of the drug is attributed to an electrophilic group which binds covalently to a specific cysteine (Cys-481) in the active site of Btk (Pan *et al.*, Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. Chem Med Chem 2:58-61, 2007).

***In vitro* studies:**

Study title: *In vitro* pharmacodynamic studies with PCI-32765 (Study # 08-088-Z-X-BIO)

- Kinase inhibition profile

Binding affinity and specificity of PCI-32765 and racemate PCI-31523 to a panel of kinases was assessed via screening panels of kinases.

Screening the Ambit kinome-scan
The Ambit kinome-scan is an active-site dependent competition-binding assay in which human kinases of interest are fused to a proprietary tag. The amount of kinase bound to an immobilized, active-site directed ligand was measured in the presence and absence of the PCI-31523 (the racemic mixture containing PCI-32765 [R] and PCI-32769 [S]). PCI-31523 inhibited 17 of 155 kinases with an $IC_{99} < 10\ \mu M$.

Inhibition of kinase activity by PCI-32765
Inhibition in purified kinase enzymes which identified in the Ambit kinase-scan (above) was further confirmed by measuring the inhibition IC_{50} values via incubation of PCI-32765, ^{33}P-ATP, and substrate (purified enzyme). The results of the assays performed with PCI-32765 against Btk and selected members of the closely related Tec and Src/Abl family kinases are shown in Table below (Table from the Applicant).

Table 3- IC_{50} values of PCI-32765 vs. selected kinases

Kinase	PCI-32765 IC_{50} (nM)	Kinase	PCI-32765 IC_{50} (nM)
BTK	0.46	HCK	3.67
ITK	10.70	Lyn	200.45
Bmx/ETK	0.76	YES	6.51
TEC	77.76	ABL	86.12
EGFR	5.55	Brk	3.34
JAK3	16.13	CSK	2.25
BLK	0.52	FER	8070.00
LCK	33.24	FLT3	72.90
C-src	170.75	JAK2	>10000
FGR	2.31	SYK	>10000
Fyn	95.55		

The IC_{50} values supported the notion that PCI-32765 exerted high inhibition on Btk (IC_{50} = 0.46 nM). Other targets with relatively low IC_{50}s are: Bmx/ETK (0.76), BLK (0.52), and CSK (2.25), FGR (2.31), Brk (3.34), and HCK (3.67).

The S-enantiomer of ibrutinib, PCI-32769, is 3.6 times less effective than ibrutinib in the inhibition of Btk activities (Study# 13-042-Hu-X-ENZ: Inhibition of Btk by PCI-32769 in Reaction Biology Kinase Assay). Also, based on IC_{50} values, it is estimated that metabolite PCI-45227, is approximately 15 times less active than the parent compound, ibrutinib, in the inhibition of Btk (IC_{50} of 6.16 nM and 0.36 nM, for PCI-45227 and ibrutinib, respectively). On the other hand, in comparison to ibrutinib, metabolite PCI-45227 is somewhat more selective for Btk inhibition relative to other kinases such as EGFR and Lck (Study # 13-047-Hu-X-ENZ - Inhibition of Btk by metabolite PCI- 45227 in Reaction Biology Kinase Assay).

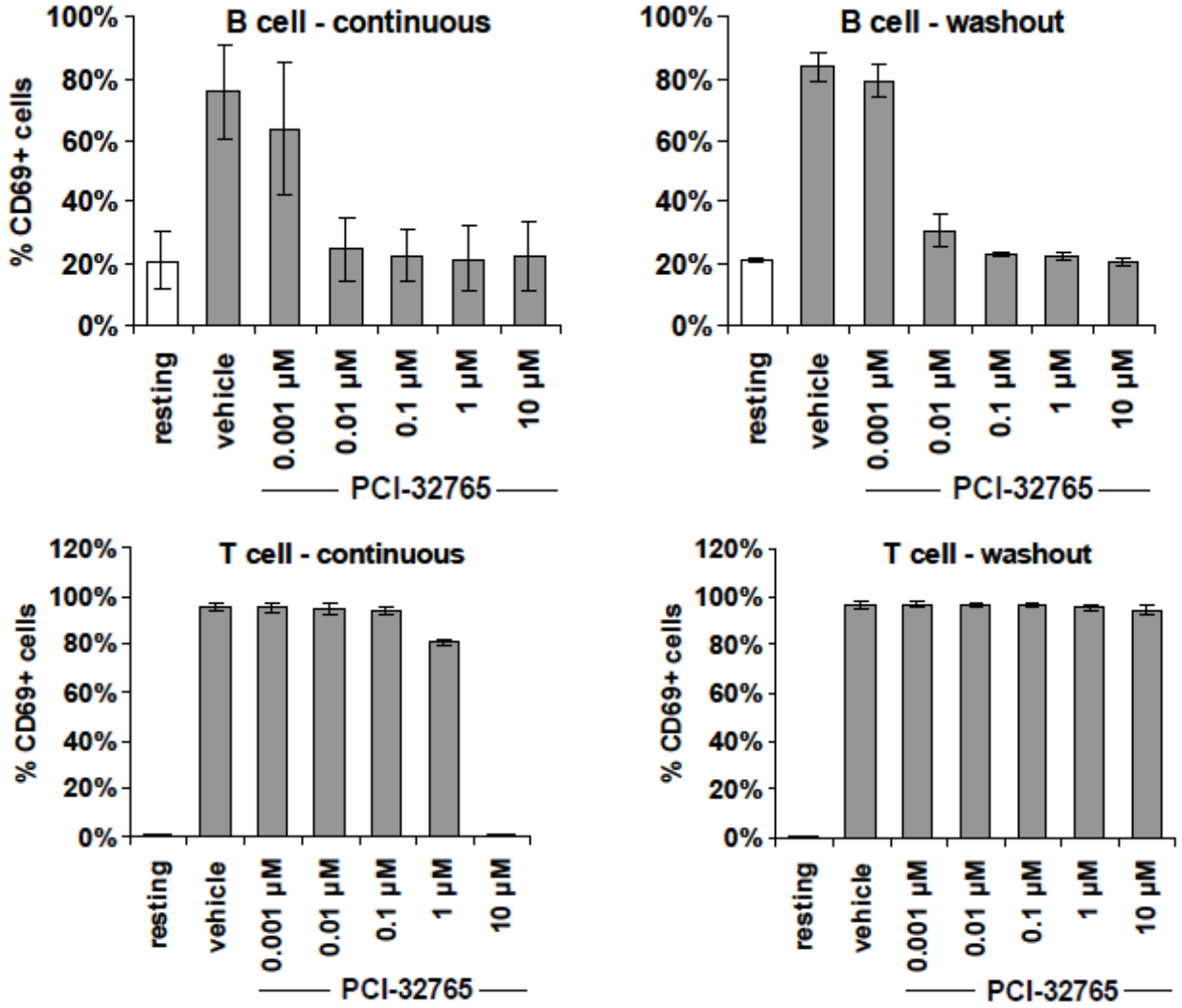
- Inhibition of Btk-mediated cell signaling and B-cell activities

Inhibition of phosphorylation of the Btk substrate PLC-γ and the downstream kinase ERK was examined in the B cell lymphoma cell line DOHH2. PCI-32765-pretreated cells were stimulated at the BCR with anti-IgG antibody. Phosphorylation was detected 2 minutes after stimulation using phospho-specific antibodies. PCI-32765 inhibited, in a concentration-dependent fashion, the phosphorylation of the Btk physiological substrate, PLC-γ, and a downstream kinase, ERK, with IC_{50} values of 20 nM and 15 nM, respectively (data not shown).

The inhibition of B-cell and T-cell activities was further demonstrated in isolated human blood cells. Human CD20+ B cells were stimulated by anti-IgM antibody, and then

subjected to a 1-hour (pulse) exposure to PCI-32765. PCI-32765 at 10 nM completely prevented upregulation of the early lymphocyte activation marker CD69 for at least 18 hours (see Figure 2 below). The result demonstrated a sustained inhibition of B cell activation by PCI-32765 (with an IC_{50} < 10 nM). After the washout period, ibrutinib retained its activity, consistent with an irreversible mechanism of action, potentially through irreversible binding to the target. By contrast, when primary T cells (which do not express Btk) were stimulated at the T cell receptor (TCR) by anti- CD3/anti-CD28 antibody, a 1000-fold higher concentration of PCI-32765 was required to inhibit TCR signaling (also measured by CD69), and this inhibition was lost following washout (see Figure 2 below). No change in cell viability was observed in B cells or T cells following 18 hours of exposure to PCI-62765.

Figure 2- Inhibition of B and T Cell Activation by PCI-32765



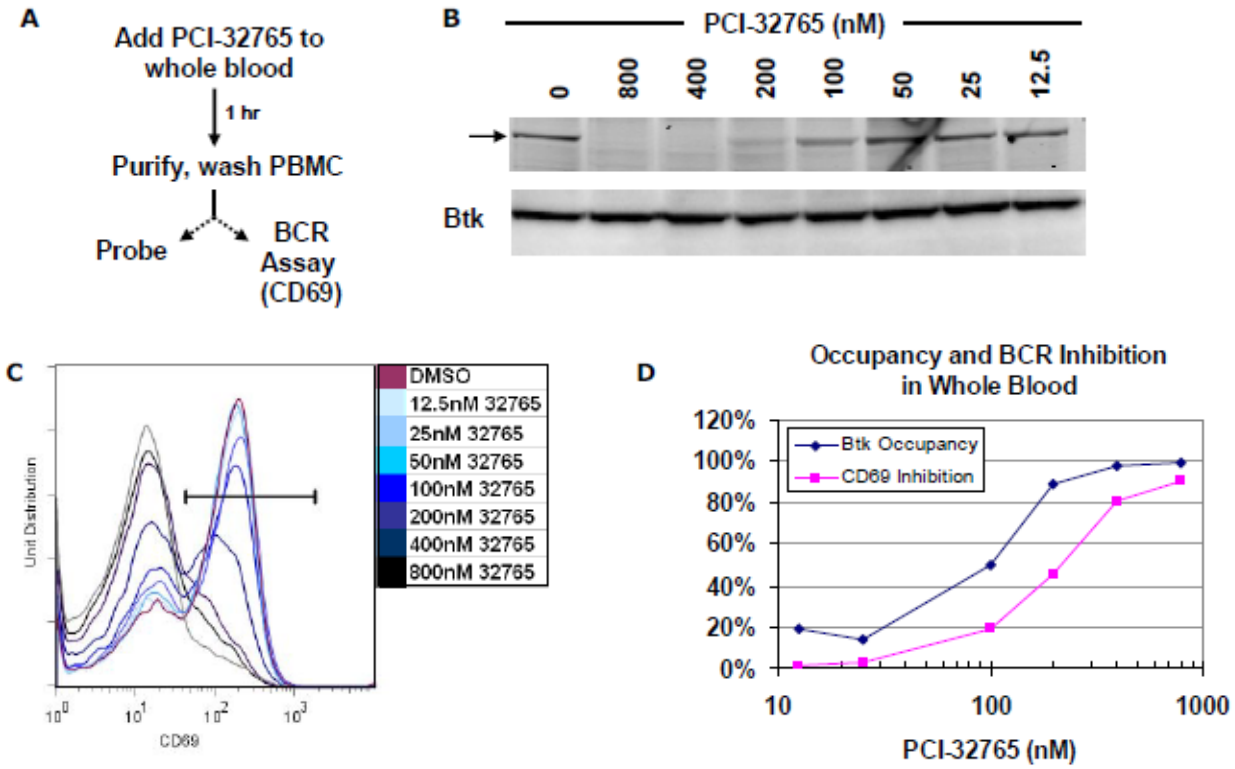
Note: CD69 expression determined by flow cytometry. Error bars represent standard deviation from at least two experiments using at least two separate donors.

- Correlation between binding to Btk active sites and B-cell inhibition by PCI-32765

Covalent binding to the active site of Btk (Cys-481) by PCI-32765* was confirmed *in vitro* using an active site-directed fluorescent probe; fluorescent signals were blocked by the presence of covalently bound PCI-32765 in cell-lysates (data not shown) as well as isolated human PBMCs. Btk is required for the inhibition of anti-IgM induced B cell activation, as measured by CD69 upregulation. Covalently bound PCI-32765 at Btk active sites reduces the level of available (active) Btk. The active site probe assay was thus used to determine the concentration of PCI-32765 in whole blood required to occupy the active site of Btk in PMBCs. It was found that PCI-32765 at 200 nM led to near complete occupancy of Btk in PBMCs, as well as approximately 50% inhibition of CD69 staining. There was a correlation between Btk occupancy and inhibition of B cell activation as assayed by CD69 inhibition (see Figure 3 below; from the submission).

*Reviewer's note:
PCI-45227 is a prominent dihydrodiol metabolite (M37) observed in plasma from laboratory animals and humans with an inhibitory activity towards Btk approximately 15 times lower than that of ibrutinib, based on the IC_{50} values.

Figure 3- Active-site occupancy of Btk by PCI-32765 in whole blood correlates with Inhibition of B cell activation



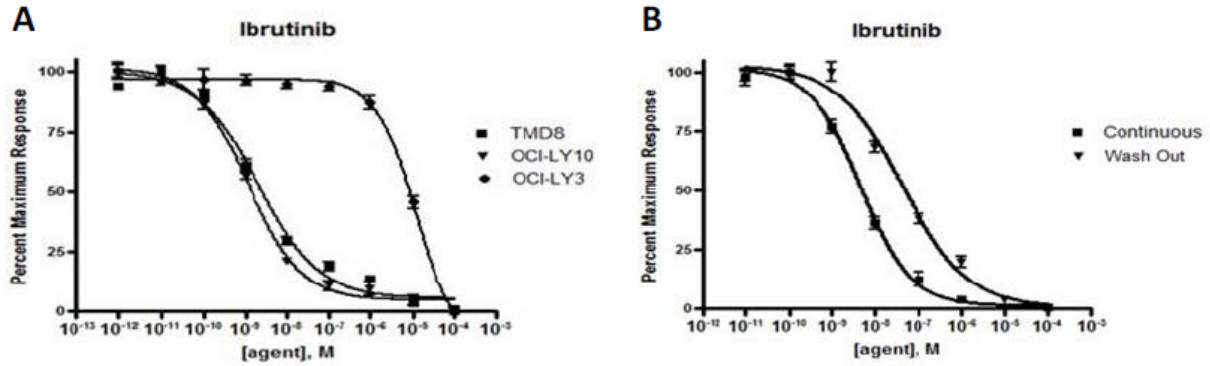
Note: (A) Experimental procedure; (B) Active-site probe assay showing concentration-dependent inhibition of probe binding (arrow) by PCI-32765; (C) CD69 expression measured by flow cytometry; (D) Percent inhibition of CD69 expression plotted with Btk occupancy.

Effect of ibrutinib on the growth of DLBCL cell lines in culture

The investigation of Davis *et.al.*in a subtype of diffuse large B cell lymphoma (DLBCL), an activated B cell-like (ABC) DLBCL, suggested the pathogenic role of BCR signaling in maintaining the function of NF-κB and survival of ABC DLBCL clones (Davis E, Ngo V, Lenz G, *et al.* Chronic active B-cell receptor signaling in diffuse large B-cell lymphoma. Nature, 463:88-94, 2010). Death of ABC DLBCL cell lines following knockdown of BCR signaling components, including Btk, supported the notion that Btk may be a critical downstream effector of the survival pathway in the ABC DLBCL cell line. OCI-Ly3, OCI-Ly10, and TMD8 human DLBCL lines of activated B-cell phenotype (ABC) were used to study the effect of ibrutinib. Ibrutinib inhibited the growth of OCI-LY10 and TMD8 cells containing wild-type CARD11 at low concentrations (EC_{50} = 2 and 1 nM, respectively); however, it did not inhibit growth of OCI-LY3 cells carrying an activating CARD11 mutation downstream of Btk. Inhibition of OCI-LY3 required > 1,000-fold higher concentrations (EC_{50} = 12,000 nM) (panel A, figure below). Mutations in CARD11, an oncogene, drive the activation of NF-κB and enhance the survival of ABC DLBCL cells. A one-hour pulse of ibrutinib, followed by washout of the compound, was sufficient to inhibit the growth of OCI-LY10 (EC_{50} = 50 nM), a result consistent with irreversible inhibition of Btk (panel B, figure below).

Figure 4- Inhibition of growth and occupancy of Btk in ABC DLBCL cell lines

(Figure from the Applicant)



- The effect of ibrutinib on neoplastic B cell adhesion and migration

The homing and trafficking of B cells is tightly controlled and regulated by the interaction of chemokine receptors (e.g. CXCR4) and adhesion molecules (integrins). BCR signaling within lymphoid tissues has been associated with the control of homing and adhesion. The interaction of neoplastic B cells with stromal cells in the microenvironment is important in the progression of various B-cell malignancies.

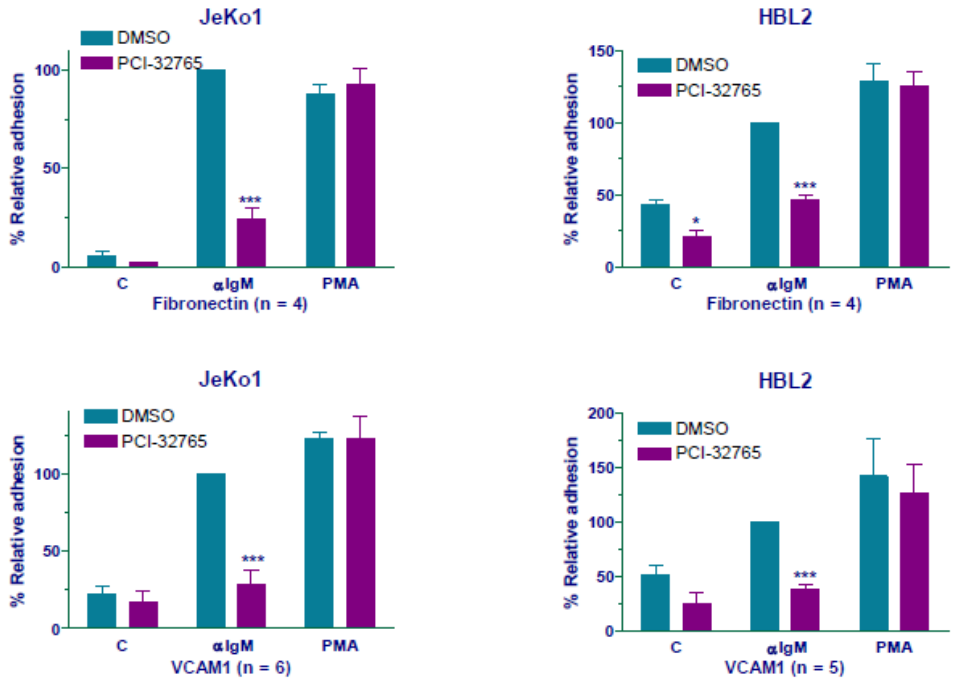
<u>Ibrutinib Inhibits BCR- and chemokine-mediated adhesion and migration in MCL Cell Lines</u>

Study title: *In vitro* pharmacodynamics studies with ibrutinib (PCI-32765) (Study #08-088-Z-X-BIO) (eCTD, Module 4, Section 4.2.1.1.)

In the mantle cell lymphoma (MCL) cell line Mino, ibrutinib inhibited both auto-phosphorylation (pY223) and trans-phosphorylation (pY551) of Btk and the phosphorylation of downstream signaling proteins PLCγ2, Akt, Erk and Jnk following stimulation with anti-IgM or with the cytokines CXCL12 and CXCL13. Similar inhibitory effects were also observed in normal human CD19+B cells and in human primary MCL cells (data not shown).

Anti-IgM (BCR) stimulated adhesion of Jeko1 and HBL2 cells onto fibronectin or VCAM-1 was inhibited (50-70%) by ibrutinib (100 nM) (see figure below from the Applicant). Similar inhibitory effects of ibrutinib were observed when adhesion was activated by cytokines CXCL12 and CXCL13 (data not shown).

Figure 5- Inhibition of BCR-stimulated MCL cell line adhesion by ibrutinib



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- Anti-proliferative effects in B cell lymphoma cell lines

The anti-tumor activity of PCI-32765 was assessed in 15 human B cell lymphoma derived cell lines. Plated cells were incubated with PCI-32765 at concentrations ranging from 0.039-10 μM. The concentration required to inhibit cell growth by 50% (GI_{50}) was determined by linear interpolation and is reported in the table below (from the Applicant). Based on the GI_{50} values, PCI-32765 appeared to exert more inhibitory effects on certain subtypes of B-cell lymphomas under the condition of the study.

Table 4- Growth inhibition of B cell lymphoma derived cell lines by PCI-32765

B-Lymphoma Cell Line	Origin	PCI-32765 GI_{50} (μM)
LY10	DLBCL	0.10
WSU-NHL	FL	0.12
DOHH2	FL	0.12
Mino	Mantle cell	0.15
DHL-6	DLBCL	0.18
WSU-DLCL2	FL	0.5
DHL-4	DLBCL	0.53
DHL10	DLBCL	3.7
Ramos	Burkitt's	5.5
LY19	DLBCL	>10
LY3	DLBCL	>10
DB	FL	>10
Raji	Burkitt's	>10
Granta-519	Mantle cell	>10
Jeko-1	Mantle cell	>10

***In vivo* studies**

- Effects of ibrutinib on active-site binding (ex vivo studies in mouse spenocytes)

The results of three studies listed below will be discussed together. The studies were conducted by Pharmacyclics, Inc. (eCTD Module 4, Section 4.2.1.1.)

Study title: *Ex vivo* labeling study of splenocytes using a btk fluorescent probe (PCI-33880) and inhibition of labeling following administration of PCI-32765 in mice by the intravenous, intraperitoneal or oral route (#07-114-M-IV-IP-PO-PDI)

Study title: *Ex vivo* time course inhibition study of PCI-32765 administered orally, subcutaneously or intravenously to Balb/C mice followed by splenocyte harvest and fluorescent probe labeling (#07-119-M-PO/IV/SC-PDI)

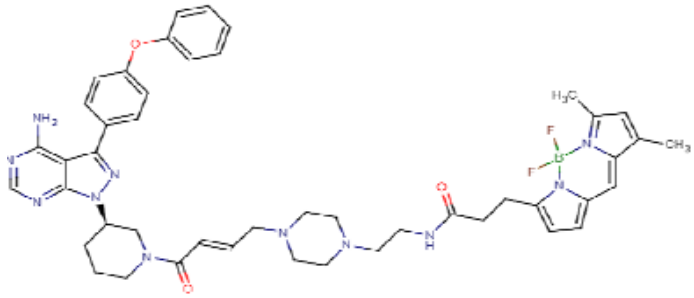
Study title: *Ex vivo* dose response inhibition study of PCI-32765 administered orally, or subcutaneously to Balb/C mice followed by splenocyte harvest and fluorescent probe labeling (#07-130-M-SC/PO-PDI)

PCI-32765 was administered to Balb/C mice via various administration routes (vehicle: 20% HP-β-Cyclodextrin), and the binding affinity and specificity toward Btk active sites

were assessed in isolated splenocytes 2 hours post treatment via monitoring signaling of a Btk fluorescent probe, PCI-33880. Active-site occupancy was measured indirectly by the degree to which the fluorescent probe PCI-33880 was inhibited from binding to Btk.

PCI-32765

PCI-33880



In the *ex vivo* study where fluorescent probe labeling of Btk in the splenocytes was measured. Two hours after dosing with 5 mg/kg of PCI-32765 splenocytes harvested from BALB/c mice were found to exhibit complete Btk occupancy via intravenous or intraperitoneal dosing and complete occupancy after oral dosing at the 50 mg/kg dose level. The effect was mediated through inhibition of labeling of PCI-33880 at the Btk active sites (via occupancy).

The time-course of active-site occupancy was evaluated in mice administered PCI32765 (50 mg/kg) by the oral, subcutaneous, or intravenous routes. Spleens were harvested 1, 3, 5, 7, and 24 hours post-dosing. PCI-32765 occupied Btk active site in splenocytes from 1 hour to at least 7 hours post-dosing. At the 50 mg/kg dose level, there was no apparent difference in effect among the three routes of administration. Partial occupancy of Btk active sites was still apparent at 24 hour post-dosing (data not shown). This suggests that at 50 mg/kg P.O., the dose of ibrutinib resulted in maximal occupancy of Btk in the spleen despite the reduced bioavailability by the oral route.

Study title: *Ex vivo* labeling study of DOHH2 tumor tissue using a Btk fluorescent probe (PCI-33880) and inhibition of labeling following administration of PCI-32765 in mice by the oral route (#07-132-M-PO-PDI)

In a separate study, similar result was obtained. In brief, subcutaneous administration of PCI-32765 inhibited labeling of the fluorescent probe to Btk in the splenocytes harvested 3 hours post-dosing at 1 mg/kg/dose while a 10 fold higher oral dose of PCI-32765 (10 mg/kg/dose) to exert similar inhibitory effects. A partial inhibition of the fluorescent probe was observed at 5 mg/kg/dose for the oral route (data not shown).

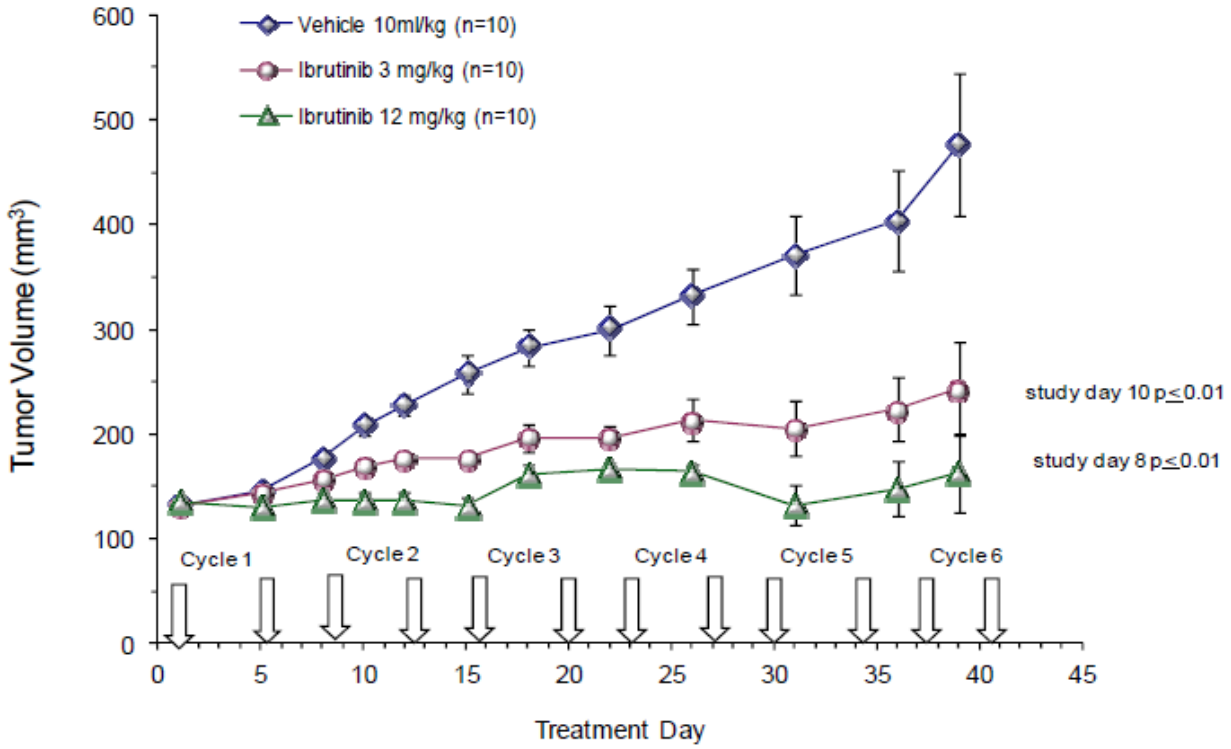
- Studies in xenograft models bearing human lymphoma cells

Study title: Evaluation of tumor inhibition in OCL-LY 10 lymphoma tumor bearing female SCID mice administered ibrutinib by oral gavage (Study # 10-089-M-PO-EFI) (Module 4)

Female SCID mice (n=10/group) were inoculated with the OCL-Ly10 tumor cell line ($1x10^7$ cells), and were treated with oral doses of vehicle (0.5% methyl cellulose in sterile water) or PCI-32765 at 3 or 12 mg/kg (dose volume, 10 mL/kg) once daily for 5 consecutive days, followed by a 2-day no-dosing break. The cycle was repeated for a total of 39 days (i.e., total of 5x 5 days). Tumor volumes at the subcutaneous implantation site (lateral thigh) were measured 2-3 times each week through study Day 39. The drug effect on the activity of Btk in the tumor was assessed (n=3/group) via a Btk probe PCI-33380 (active site labeling, see above) as well as the expression of pERK (Western blot).

Results:
Orally administered PCI-32765 was tolerated and exerted a dose-dependent inhibition of tumor growth (figure from the Applicant's submission).

Figure 7- Tumor growth in SCID female mice bearing OCI-Ly10 administered vehicle or ibrutinib



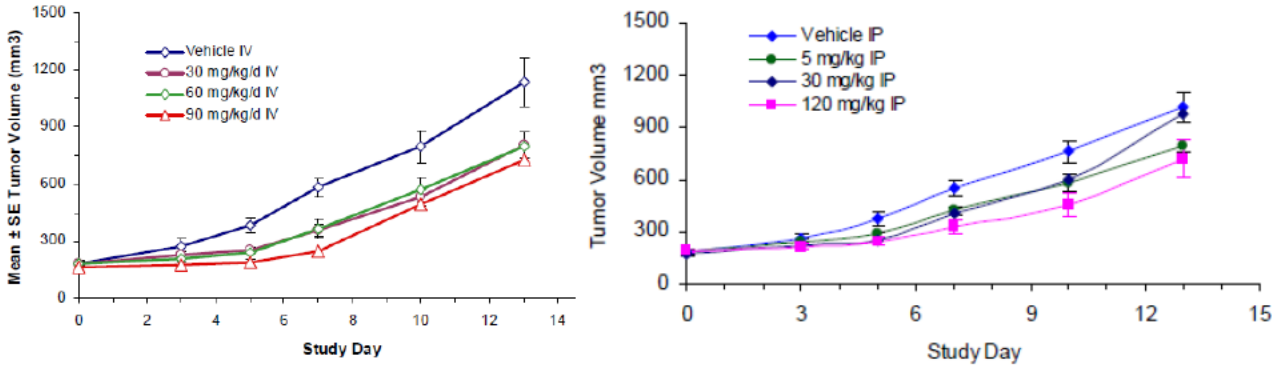
BTK occupancy in the tumor lysates collected 3 hours post-dosing were 43-46% both at the 3 and 12 mg/kg dose levels, indicating no dose response for occupancy between these dose levels.

Studies in the lymphoma xenograft models in SCID mice: # 07-098-M-IV/IP/SC-PDI, 07-137-M-SC/PO-PDI and 07-132-M-PO-PDI.
Similar study designs, as in the study # 10-089-M-PO-EFI, were followed and PCI-32765 was administered via various routes to male SCID mice bearing lymphoma cell line DOHH2 or DLCL2 tumors.

The study results (in the following figures reported by the Applicant) indicate a dose-dependent inhibition of tumor growth by PCI-32765 in the xenograft models.

Study #07-098-M- IV/IP/SC-PDI (#07-098-M-IV/IP-EFI)

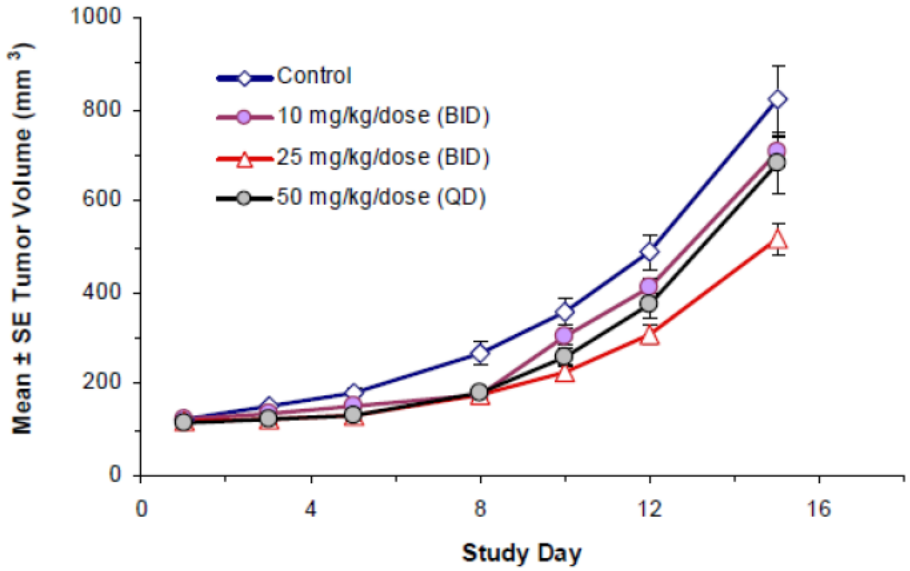
Figure 8- DOHH2 Tumor volume – time profiles in mice dosed with PCI-32765



Administration of Intravenous or intraperitoneal PCI-32765 resulted in a dose-dependent suppression of the DOHH2 tumor growth, with IV 90 mg/kg or IP 120 mg/kg demonstrating the greatest effect. Maximum tumor inhibition occurred on Day 5 for both route of administration.

Study # 07-137-M-SC/PO-PDI (#07-137-M- PO-EFI)

Figure 9- DOHH2 Tumor volume – time profiles in mice dosed once daily (QD) or twice daily (BID) with PCI-32765 by oral gavage

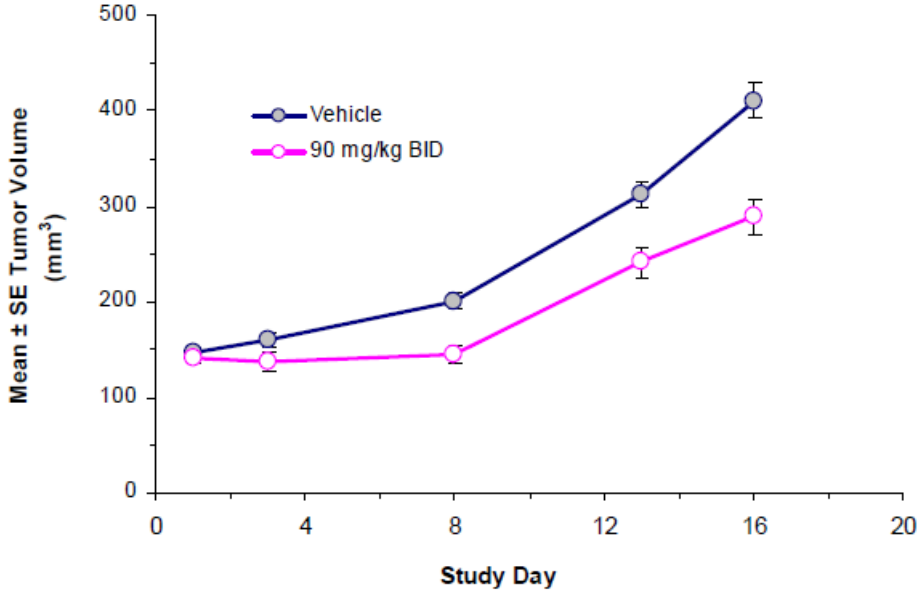


As noted, maximum inhibition of tumor growth occurred after 2 days of dosing (approximately 56% to 90% inhibition, on Day 3). Beginning on Day 10, greater tumor growth inhibition was observed when mice were administered PCI-32765 twice daily at

total daily dose of 50 mg/kg (43% to 54%) compared to the group receiving once daily dose of 50 mg/kg (18% to 38%).

Study #07-118-M-PO-EFI (#07-132-M-PO-PDI)

Figure 10- DLCL2 tumor volume – time profiles in mice dosed twice daily (BID) with



Tumor-to-control volume ratios were 0.77 to 0.70 on Days 8 through 16 at the 90 mg/kg dose level. Maximum inhibition response occurred at the 90 mg/kg dose level on Day 8 (~95% tumor inhibition).

- Efficacy of ibrutinib in mouse models of lymphoma and leukemia

Mantle cell lymphoma (MCL):
Study title: Efficacy study using the Mino mantle cell lymphoma tumor line in CB17 SCID mice administered ibrutinib continuously via medicated feed
(Study #12-035-M-PO-EFI) (Module 4, Section 4.2.1.1.)

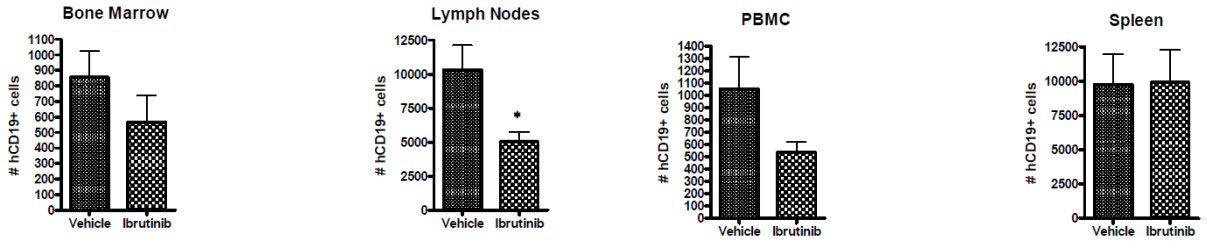
Female CB17 SCID mice were inoculated with the Mino mantle cell lymphoma cells ($4x10^7$ cells per 100 μL in PBS). The mice received feed containing ibrutinib (n=10/group) or vehicle (n=5/group) *ad libitum* at an estimated dose level of 0 (vehicle) or 12 mg/kg/day for 70 consecutive days. Inoculated mice were treated with an anti-IL-2 receptor β monoclonal antibody (TMβ1) preparation by intraperitoneal injection to deplete the murine natural killer cell population. Total white blood cell levels were monitored as an indicator of disease progression. On study Day 70, the spleen, liver, bone marrow and lymph nodes (inguinal, mesenteric, axillary, cervical and mandibular) were collected to determine levels of hCD19+ Mino-cell lymphocytes using a human-specific CD19 antibody and FACS analysis.

Results:

Treatment of ibrutinib in mantle cell inoculated mice mitigated disease conditions in comparison to vehicle-fed inoculated mice. The effect was manifested as the following findings:

- Clinical signs: less mice in the ibrutinib-fed group showed clinical signs of the disease which included bilateral neck masses, labored respiration, rough coat and/or kyphosis, than the control group (1/10 versus 5/10 affected mice).
- WBC counts: mice given control feed exhibited a 40% higher mean white blood cell (WBC) count at the last assessment time point (study day 56) compared to mice given ibrutinib-containing feed.
- Collective lymph node weights: although it did not reach statistical significance, mice administered ibrutinib had lower collective lymph node weights in comparison to Mino-cell bearing vehicle control mice.
- hCD19+ cell counts of bone marrow, PBMC and lymph nodes: substantively lower numbers of hCD19+ mantle cells were observed in tissues/preparations from ibrutinib-treated mice in comparison to tissues/preparations from vehicle-control mice (see figure below). Treatment with ibrutinib had no effect on hCD19+ cell counts in the spleen.

Figure 11- hCD19+ Mino mantle cell counts in tissues



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4.2 Secondary Pharmacology

***In vitro* studies:**

- *In vitro* receptor binding assays:

PCI-32765 was subjected to profiling/screening the inhibition of radioligand binding to 67 receptors and ion channels. For each receptor, the level of radioligand binding in the presence of PCI-32765 was determined. The results of studies #05-0383-V-X-RB, # 07-074-Hu-X-RB are summarized as followings.

Table 5- Inhibition of radioligand binding to receptors by PCI-32765 (10 μM)

Receptors (Channels)	% inhibition	IC_{50}
Dopamine transporter (DAT)	92%	0.995 nM (b)
Sodium channel (site 2)	57%	0.65 μM (a)
Tachykinin NK1 receptor	53%	50.9 nM (b)
Adenosine A2A receptor	52%	114 nM (a)
Serotonin (5-hydroxytryptamine) transporter (SERT)	44%	5.41 nM (b)
Norepinephrine transporter (NET)	43%	1.26 nM (a)
Adenosine A3 receptor	42%	0.717 nM (b)
Epidermal growth factor receptor	39%	4.38 nM (b)

(a) Study #05-0383-V-X-RB (b) Study # 07-074-Hu-X-RB

PCI-327665 inhibited BTK at an IC_{50} value of 0.46 nM. Therefore, it cannot be overlooked the potential of off-target adverse effect of PCI-32765 in humans via inhibition of non-BTK targets, to which PCI-32765 exhibited similar inhibitory effects (i.e., based on the IC_{50} values).

Reviewer's note:

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4.3 Safety Pharmacology

The safety pharmacology studies were reviewed by Dr. Margaret Brower.

Summary:
Safety pharmacology assessment included *in vitro* assessment of hERG channel activity and cardiac electrophysiology, and *in vivo* assessment of neurobehavior, respiratory function, and cardiovascular parameters following administration of Ibrutinib (PCI-32765).

PCI-32765 (parent drug) and PCI-45227 (metabolite M37) inhibited hERG channel activity in a concentration dependent manner in assays conducted in HEK293 cells; the IC_{50} values were 0.97 µM and 9.6 µM, respectively, suggesting that while ibrutinib is a low potency blocker of hERG channel, its metabolite showed little or no potential to block the hERG channel. However, ibrutinib had no effect on QT prolongation when studied in conscious dogs. Mean RR interval was prolonged but this seemed to be secondary to the depressed heart rates that occurred at the mid dose of 24 mg/kg (depressed up to 20%) and the high dose of 150 mg/kg (depressed up to 30%) from 1 to 6 hours postdose. Pulse pressure was increased up to 12% at the mid dose (MD) from 1 to 6 hours postdose, but was not similarly elevated at the high dose (HD). QTcV interval was shortened by 14 milliseconds at the HD peaking at 3 hours postdose.

There were no drug-related changes in CNS parameters. A tidal volume depression of 6% was observed in HD dogs up to 3 hours postdose, although this finding may have been the result of baseline variability. There were no other changes in respiratory parameters.

Study title: An oral (gavage) central nervous system pharmacological profile of PCI-32765 in rats	
Study no.:	06-024-R-PO-SP
Study report location:	Electronic submission , M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study report:	May 9, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765, lot # SCR-182-77, 97% pure

Key Study Findings:
- No drug related CNS effects exhibited in rats administered doses up to 150 mg/kg ibrutinib.

Methods	
Doses:	0, 2.5, 40, 150 mg/kg
Frequency of dosing:	Single doses
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% methylcellulose, 0.4% Cremophor and 0.1% sodium lauryl sulfate suspension
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	6 females/dose
Age:	8 weeks
Weight:	173 – 223 g
Satellite groups:	None
Unique study design:	• 20 mg/kg chlorpromazine hydrochloride used as positive control • Modified functional observational battery (FOB) and activity assessments performed prior to dosing, and at 0.5, 1.5, 2.5, 5 and 24 hours following dosing • Animals sacrificed following FOB evaluation without macroscopic or microscopic pathology

Results:
No drug related effects were observed on clinical observations or FOB parameters (home cage, handling, open field, sensory or physiological effects) at doses up to 150 mg/kg, the highest dose tested. Clorpromazine-treated animals exhibited neurological effects as expected.

Study title: Respiratory assessment of PCI-32765 administered by oral gavage to plethysmograph-restrained female Sprague Dawley rats	
Study no.:	07-077-R-PO-SP
Study report location:	Electronic submission , M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 5, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765, lot # SCR-182-77, 97% pure

Key Study Findings:

- Depressed tidal volume exhibited at 150 mg/kg from 16 to 165 minutes following dosing. This may have been a result of baseline variability of this parameter. There were no other changes in respiratory parameters.

Methods	
Doses:	0, 2.5, 40, 150 mg/kg
Frequency of dosing:	Single doses
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% methylcellulose, 0.4% Cremophor and 0.1% sodium lauryl sulfate suspension
Species/Strain:	Rat/Crl:CD(SD)
Number/Sex/Group:	8F/dose
Age:	13 weeks
Weight:	234 – 263 g
Satellite groups:	2F/dose as backup
Unique study design:	• Pneumotach used to detect respiratory function in animals restricted in head-out neck-sealed plethysmograph

Results:
There were no changes in respiratory rate or minute volume in PCI-32765-dosed animals compared to concurrent controls. A significantly lower tidal volume (~6%) was initially observed at the HD (150 mg/kg), remained depressed from 16 to 165 minutes post-dose. This finding may not be drug related due to baseline variability of this parameter.

Study title: Effects of PCI-32765 on cloned hERG potassium channels expressed in human embryonic kidney cells	
Study no.:	07-079-HEK-X (b) (4)
Study report location:	Electronic submission , M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 4, 2008
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	PCI-32765, lot # PIPR203, 95.9% pure

Key Study Findings:
- Ibrutinib (PCI-32765) inhibited hERG current in a concentration dependent manner with an IC_{50} of 0.97 µM and is considered a low-potency blocker.

Methods and Results:
hERG potassium channel inhibition was evaluated by the effect of parent compound PCI-32765 on the ion channel in the human embryonic kidney cell (HEK-293) transfected with hERG cDNA. Peak hERG tail current amplitude was measured prior to and following exposure to PCI-32765, vehicle (buffered physiological saline + 0.3% DMSO) or positive control (terfenadine).

PCI-32765 significantly inhibited hERG current in a concentration dependent manner with an IC_{50} of 0.97 μM (see table below). In comparison, 60 nM terfenadine, used as the positive control, inhibited hERG potassium current by 86.8%.

Table 6- Mean percent inhibition at PCI-32765 concentrations tested

Concentration (μM)	Mean	SD	SEM	n
0	0.4%	0.3%	0.2%	3
0.3	8.3%*	3.7%	2.1%	3
1	54.8%*	4.9%	2.9%	3
3	83.7%*	2.9%	1.7%	3
10	93.8%*	1.6%	0.9%	3

*Statistically ($P<0.05$) different than vehicle alone using ANOVA followed by Dunnett's test.

Table excerpted from Applicant's submission.

Figure 13- Concentration-response relationship and mean percent inhibition of hERG potassium current

Concentration-response relationship and mean percent inhibition of hERG potassium current following treatment of PCI-32765 (open circles, n = 3) fit to binding equation (solid line) with IC_{50} = 0.97 μM and Hill coefficient = 1.7

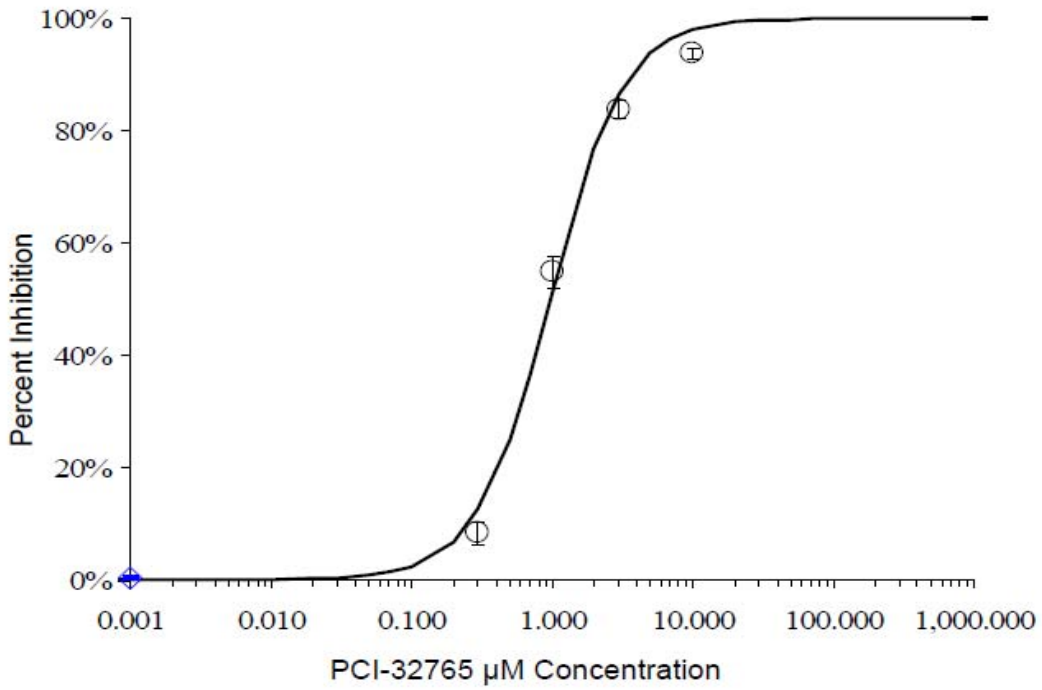


Figure excerpted from Applicant's submission.

Study title: Effect of PCI-45227 on cloned hERG potassium channels expressed in human embryonic kidney cells	
Study no.:	10-015-HEK-X-(b) (4)
Study report location:	Electronic submission , M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 22, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-45227, lot # 709-61, 98.3% pure

Key Study Findings:

- Metabolite PCI-45227 (M37) inhibited hERG current in a concentration dependent manner with an IC_{50} of 9.6 µM, 10 fold greater than the IC_{50} obtained with ibrutinib. M37 metabolite is considered a weak blocker of the Ikr current.

Methods and Results:

hERG potassium channel inhibition was evaluated by the effect of metabolite PCI-45227 on the ion channel in the human embryonic kidney cell (HEK-293) transfected with hERG cDNA. Peak hERG tail current amplitude was measured prior to and following exposure to PCI-45227, vehicle (buffered physiological saline + 0.3% DMSO) or positive control (terfenadine).

PCI-45227 inhibited hERG current in a concentration dependent manner with an IC_{50} of 9.6 µM (see table below). In comparison, 60 nM terfenadine, used as the positive control, inhibited hERG potassium current by 79.5%.

Table 7- Mean percent inhibition at PCI-45227 concentrations

Concentration (µM)	Mean	SD	SEM	N
0	0.4%	0.4%	0.2%	3
3	18.2%*	2.4%	1.4%	3
10	53.4%*	3.2%	1.8%	3
30	78.6%*	0.8%	0.4%	3
100	94.1%*	1.9%	0.9%	4

* Value is statistically different than vehicle alone.

Table excerpted from Applicant's submission

Figure 14- Concentration-response relationship and mean percent inhibition of hERG potassium current following treatment of PCI-45227

Concentration-response relationship and mean percent inhibition of hERG potassium current following treatment of PCI-45227 (open circles) fit to binding equation (solid line) with IC_{50} = 9.6 µM and Hill coefficient = 1.2. [The number of cells at each concentration is given in parentheses].

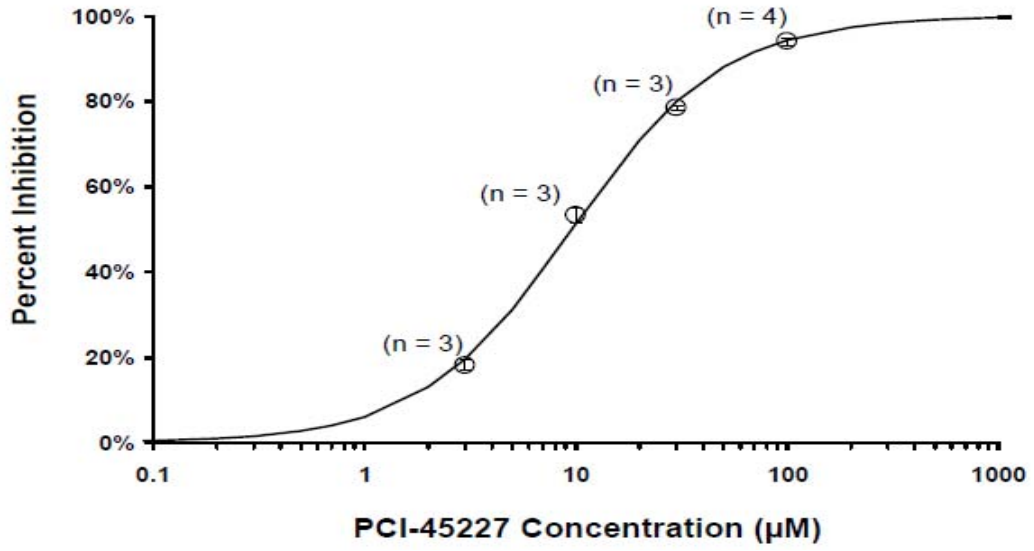


Figure excerpted from Applicant's submission

Study title: Cardiovascular assessment of orally administered PCI-32765 in conscious radiotelemetry-implanted male Beagle dogs	
Study no.:	06-026-D-PO-SP
Study report location:	Electronic submission , M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 29, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765, lot # SCR-182-77, 97.3% pure

Key Study Findings:

- Mean RR interval prolongation (up to 23% and 35% at 24 and 150 mg/kg, respectively) correlated with depressed heart rates observed from 1 to 6 hours postdose. Mean heart rates were depressed up to 20% and 30%, respectively, peaking at 5 hours postdose.
- Mean pulse pressure was increased up to 12% at 24 mg/kg from 1 to 6 hours postdose. Similar elevation in pulse pressure was not reflected at the HD.
- QTcV interval was slightly shortened (~14 milliseconds) at 150 mg/kg from 1 to 6 hours postdose, peaking at 3 hours. QT prolongation was not observed.

Methods	
Doses:	0, 1.5, 24, 150 mg/kg
Frequency of dosing:	Single doses
Route of administration:	Oral (gastric intubation)
Dose volume:	3 mL/kg
Formulation/Vehicle:	0.5% methylcellulose, 0.4% Cremophor, 0.1% sodium lauryl sulfate
Species/Strain:	Beagle dog
Number/Sex/Group:	4 males/dose
Age:	17-20 months
Weight:	9-11.5 kg
Satellite groups:	None
Unique study design:	• Study was performed in conscious dogs dosed on separate days with intervals of 3 days between doses according to a modified 4 x 4 Latin square crossover paradigm (see below). • Cardiovascular and ECG measurements obtained in conscious dogs using surgically implanted telemetry device (b) (4) • Following administration of each dose, parameters below recorded for 60-second interval every 10 min for ~24 hours. • Parameters measured: -Systolic, diastolic, and mean arterial BP -Heart rate -Pulse pressure -Body temperature -ECG intervals (RR, QRS, PR, QT, QTcV) • QTcV calculated from QT data according to equation QTcV = QT-0.087(RR-1) noted as (b) (4) correction.

	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5[a]
Male No. 3764	A	B	C	D	
Male No. 3762	B	A	D	C	A
Male No. 3643	C	D	B	A	
Male No. 2751	D	C	A	B	

[a] = Male no. 3762 had missing data at several time points overnight following the second dose. Therefore, the second dose was repeated as the fifth dose.

Table excerpted from study submission

Results:
Increases were observed in systolic, diastolic and mean arterial pressures (3 -5%) from 1 to 6 hours at 150 mg/kg, and pulse pressure at 24 and 150 mg/kg (12% and 6%, respectively) during the same time interval. Blood pressure changes were not considered to be of concern due to the small magnitude of change. Heart rates were depressed from 1 to 6 hours following dosing of 24 and 150 mg/kg PCI-32765 (12 and 19%, respectively); depression peaked at 5 hours (20 and 30%, respectively). Lowered heart rates were consistent with prolongation of RR intervals at these doses (see figures below excerpted from Applicant's submission).

Figure 15- Radiotelemetry data – Heart Rate

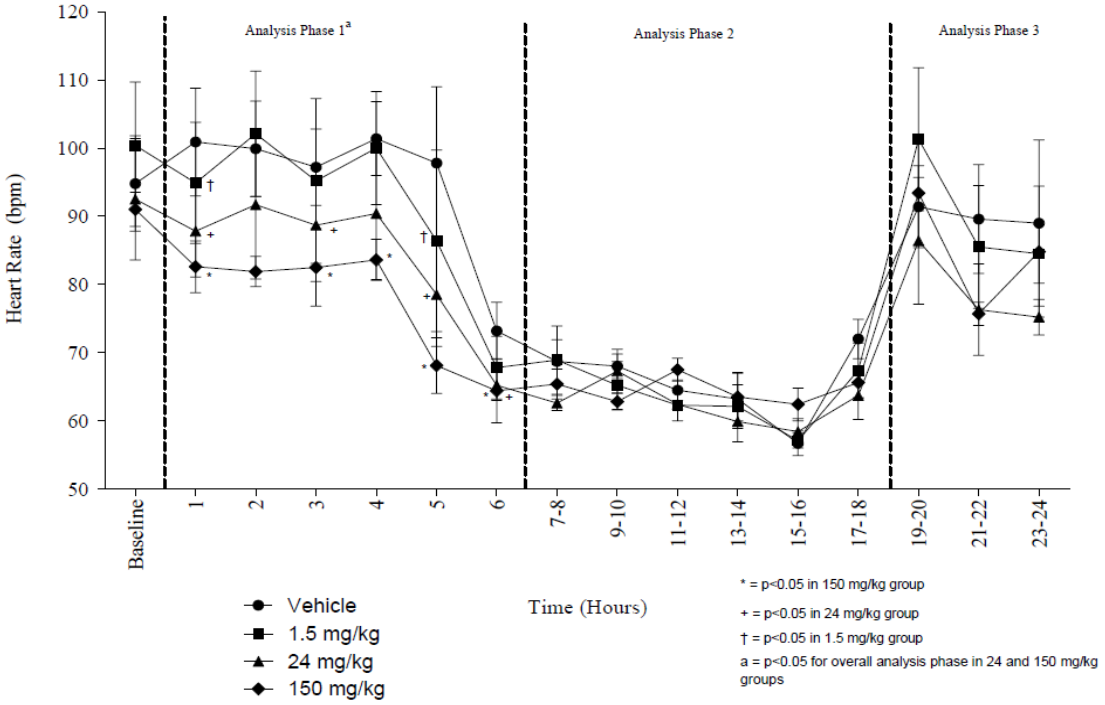
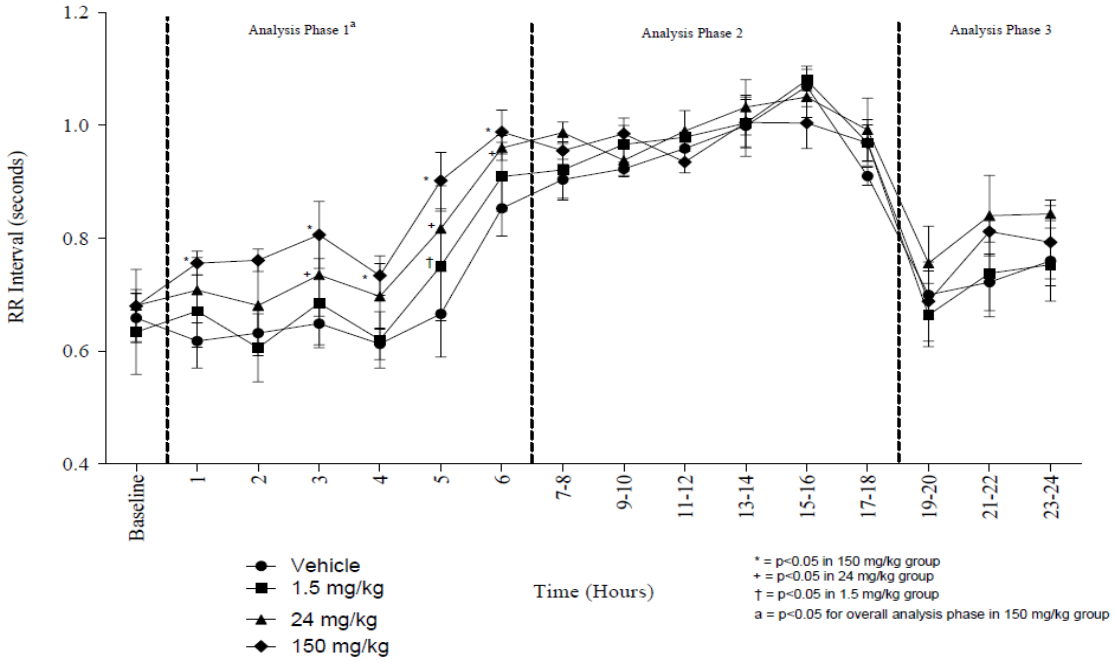
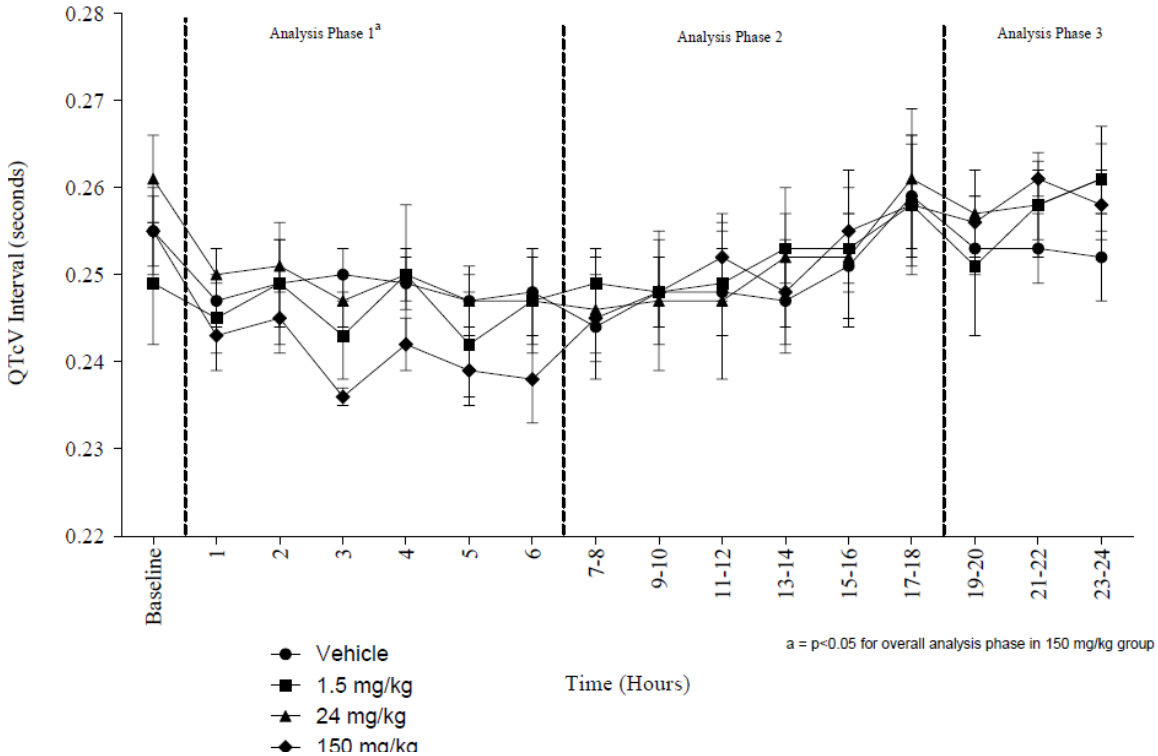


Figure 16- Radiotelemetry data – Heart Rate



PR interval was prolonged 5% to 9% at 150 mg/kg with a peak effect at 2 to 6 hours postdose. In addition, QTcV interval was slightly shortened at 150 mg/kg from 1 to 6 hours postdose with a peak at 3 hours (~14 milliseconds; see figure below excerpted from Applicant's submission).

Figure 17- Radiotelemetry data – QTcV Interval



Overall, primary cardiac changes at 24 mg/kg PCI-32765 included ~ ↑23% for RR interval, ↓20% for heart rate, and ↑12% for pulse pressure. Changes at the HD (150 mg/kg) included: ↑35% for RR interval, and ↓30% for heart rate, with ↓14 milliseconds for Heart Rate Corrected-QTcV Interval.

5 Pharmacokinetics/ADME/Toxicokinetics

Note: PK data were reviewed as part of the toxicology studies and results are reported with each toxicology study reviewed (see Section 6, General Toxicology, and Section 9, Reproductive and Developmental Toxicology). ADME studies have not been reviewed. The information presented in Section 5 is from the Applicant's submission.

Pharmacokinetics Summary:
Distribution studies were conducted in Long Evans rats. High concentrations of ^{14}C-PCI- 32765-derived-radioactivity was detected in the following tissues at 1h postdose following administration of 10 mg/kg radiolabeled ibrutinib: small intestine > esophagus > liver > urinary bladder > kidney. The percent of radioactivity associated with the cellular fraction of blood (predominated by RBC) ranged from 15.9% at 1hr postdose to 100% at the final sampling time of 504 hr postdose. PCI-32765 binds covalently to rat hemoglobin.

Binding of the drug to plasma protein ranged from 96 to 99% in samples from rodents, dogs, and humans. CYP3A4and 3A5 appear to be the major isozymes involved in the metabolism of PCI-32765. Biliary excretion was the major route of elimination of ^{14}C-PCI-32765 in male rats.

5.1 PK/ADME

Methods of analysis:
In brief, the determination of pharmacokinetic parameters and the quantitation of PCI-32765 in mouse, rat, and dog plasma and quantitation of metabolites in rat, dog and human plasma following oral or intravenous administration were performed by high-performance liquid chromatography/tandem mass spectrometer (HPLC/MS/MS) methods. Tissue distribution in rats, metabolism in mice, rats, dogs, and humans, and excretion in rats, dogs, and humans were conducted using ^{14}C-ibrutinib. Other methods used included quantitative whole body autoradiography (QWBA) in Long Evans rats, and blood-to-plasma partition in rats.

Absorption:
Oral biolavailability of ibrutinib was low to moderate in fasted rats and dogs, ranging from 18 to 23%% in rats and 7-11% in dogs.

In laboratory animals, significant first-pass metabolism and the hepatic and GI involvement in oral first-pass metabolism were supported by the evidence, such as low bioavailability via oral and intraperitoneal administration (<100% IP bioavailability) as well as lower metabolite (PCI-45227)-to-parent ratio following IV administration than the ratio following oral administration.

Distribution:

***In vitro* studies**

Blood-plasma partition:

Blood samples collected periodically from ^{14}C PCI-32765 treated (one single oral 10 mg/kg dose) Sprague Dawley or Long Evans (pigmented) rats. E*x vivo* blood-to-plasma partitioning was determined by analyzing the radioactivity in plasma and blood (following oxidation) via liquid scintillation counting (LSC) analysis.

Blood:plasma concentration ratios increased with time postdose. In Sprague Dawley (nonpigmented) rats, the percent of radioactivity associated with cellular fraction (mainly red blood cells) ranged from 25% at 1 hour postdose to 72% at 48 hours postdose. The corresponding blood:plasama concenraion ratios were 0.695 and 1.78, respectively. In Long Evans (pigmented) rats, the percent of radioactivity associated with cellular fraction was ~1% at 1 hour postdose, with a corresponding blood:plasma ratio of 0.6. At 24 to 72 hours postdose, blood:plasma concentration ratios were 1.20 to 1.94. By 168 hours post-dose, the blood:plasma concentration ratio increased to 9.43.

Table 8- Protein binding

		Fraction Unbound (fu)		Protein Binding (%)	
Species	Strain	0.0881 µg/mL	0.881 µg/mL	0.0881 µg/mL	0.881 µg/mL
Mouse	CD-1	0.00838	0.0152	99.2	98.5
Rat	Sprague-Dawley	0.00470	0.0267	99.5	97.3
Dog	Beagle	0.0381	0.0237	96.2	97.6
Human	Pooled	0.0249	0.0249	97.5	97.5

***In vivo* study:**

Study title: Distribution, metabolism, and excretion of ^{14}C-PCI-31523 following oral or intravenous administration to rats (Study #07-039-R-PO-ADME) (Section 4.2.2.3)

The distribution of radioactivity after a single oral administration of 10 mg/kg (100 µCi/kg) of a racemic mixture to Long Evans male rats was determined by whole-body autoradiography (QWBA). Blood samples from the animals were collected between 1 to 504 hours postdose, and tissues were collected at necropsy.

At one hour postdose, high concentrations of radioactivity were found in bile, suggesting a biliary excretion of ^{14}C-ibrutinib derived radioactivity. Radioactivity was not detected in tissues of the central nervous system with the exception of olfactory lobe. Excluding the organs of excretion, tissue:plasma concentration ratios were less than 1.0 for most tissues, with detectable levels of radioactivity present through 24 hours postdose. There was no relatively higher distribution of radioactivity to pigmented tissues, such as the skin or uveal tract.

Through 24 hours postdose, radioactivity presented more in plasma than in tissues, with tissue:plasma concentration ratios of less than 1.0 for most tissues, except for organs of excretion, Gi tract, liver and kidney. Elimination was nearly complete for most tissues by 168 hours postdose. By the final sampling time of 504 hours postdose, only the renal medulla, kidney, and blood had measurable concentrations of radioactivity. Overall, tissues with high concentrations of radioactivity were: small intestine, esophagus, liver, urinary bladder, renal medulla, kidney, and renal cortex.

	Animal Number (Euthanasia Time)					
Tissue	B06996 (1 Hour)	B06997 (4 Hours)	B06998 (24 Hours)	B06999 (72 Hours)	B07000 (168 Hours)	B07001 (504 Hours)
Adrenal gland	0.813	0.503	0.618	1.89	NA	NA
Bile	33.2	36.4	NA	NA	NA	NA
Blood	0.511	0.696	0.955	1.74	7.12	NA
Bone	NA	NA	NA	NA	NA	NA
Bone marrow	0.392	0.359	0.356	NA	NA	NA
Cecum	0.339	5.72	6.84	NA	NA	NA
Cecum contents	NA	NA	NA	NA	NA	NA
Cerebellum	NA	NA	NA	NA	NA	NA
Cerebrum	NA	NA	NA	NA	NA	NA
Diaphragm	0.514	0.276	0.479	NA	NA	NA
Epididymis	0.161	0.228	NA	NA	NA	NA
Esophagus contents	NA	NA	NA	NA	NA	NA
Esophagus	6.28	13.3	0.733	NA	NA	NA
Exorbital lacrimal gland	0.498	0.373	0.357	NA	NA	NA
Eye	0.196	NA	NA	NA	NA	NA
Eye (lens)	NA	NA	NA	NA	NA	NA
Fat (abdominal)	0.239	NA	NA	NA	NA	NA
Fat (brown)	0.324	0.291	NA	NA	NA	NA
Harderian gland	0.394	0.443	0.548	1.38	NA	NA
Intra-orbital lacrimal gland	0.522	0.357	NA	NA	NA	NA
Kidney	1.81	1.21	1.04	2.06	7.83	NA
Large intestinal contents	NA	NA	NA	NA	NA	NA
Large intestine	0.516	1.66	4.37	NA	NA	NA
Liver	6.87	6.47	5.25	7.36	11.9	NA
Lung	0.392	0.469	0.618	1.18	NA	NA
Lymph nodes	0.518	NA	0.397	NA	NA	NA
Medulla	NA	NA	NA	NA	NA	NA
Muscle	0.134	NA	NA	NA	NA	NA
Myocardium	0.440	0.440	0.515	1.20	NA	NA
Nasal turbinates	0.286	0.262	0.401	NA	NA	NA

	Animal Number (Euthanasia Time)					
Tissue	B06996 (1 Hour)	B06997 (4 Hours)	B06998 (24 Hours)	B06999 (72 Hours)	B07000 (168 Hours)	B07001 (504 Hours)
Olfactory lobe	0.098	NA	NA	NA	NA	NA
Pancreas	0.444	0.458	0.383	NA	NA	NA
Pituitary gland	0.376	0.443	0.467	2.14	NA	NA
Preputial gland	0.276	0.364	NA	NA	NA	NA
Prostate	0.202	0.279	NA	NA	NA	NA
Renal cortex	1.68	1.11	1.08	2.18	6.75	NA
Renal medulla	2.01	1.47	0.980	2.03	9.53	NA
Salivary gland	0.382	0.286	0.325	NA	NA	NA
Seminal vesicle	0.168	0.199	NA	NA	NA	NA
Skin (nonpigmented)	0.204	0.181	NA	NA	NA	NA
Skin (pigmented)	0.252	0.413	1.01	NA	NA	NA
Small intestine	26.6	15.6	1.54	NA	NA	NA
Spinal cord	NA	NA	NA	NA	NA	NA
Spleen	0.387	0.407	0.505	NA	NA	NA
Stomach	1.49	0.618	0.551	1.13	NA	NA
Testis	0.108	0.206	NA	NA	NA	NA
Thymus	0.213	0.179	NA	NA	NA	NA
Thyroid	0.652	0.752	1.03	2.22	NA	NA
Urinary bladder	0.883	4.16	0.865	NA	NA	NA
Uveal tract	0.507	0.699	NA	NA	NA	NA

B06996 through B07001 refer to animal numbers.

Metabolism:

Metabolism of PCI-32765 was investigated in rat, dog, and human liver microsomes and/or hepatocytes, and in recombinant CYP450 isozymes. Totally 41 metabolites of ibrutinib were identified in *in vitro* or *in vivo* staudies. In the *in vitro* studies, ibrutinib was metabolized mainly via CYP3A4/5-mediated pathways. In rats and human volunteers, following oral administration, low levels of unchanged ibrutinib in the bile or feces, while no unchanged drug was excreted in the urine, indicating that ibrutinib clearance is fully metabolic.

Following a single oral dose of ^{14}C-ibrutinib administered to rats and humans, the drug was extensively metabolized. The primary metabolic clearance pathways included: (1) hydroxylation (e.g., M35), (2) oxidative ring opening of the piperidine (e.g., M34 and M25) and (3) epoxidation of the ethylene on the acryloyl moiety (e.g., M37, also known as PCI-45227). Most remaining metabolites were formed by combinations of these main primary pathways or by further downstream secondary metabolism.

The main circulating entities in rat plasma were M15, M5 and M37 (PCI-45227). Following oral doses of ^{14}C ibrutinib in rats, up to 47% of the radioactivity was excreted in bile. There were no unchanged ibrutinib in the bile, and the main metabolites was M21 (a sulfate conjugate of M35). While very low radioactivity was found in urine (<2% of the dose), the main metabolites in feces were M34, M35 and M17 (approximately 16%, 14% and 8-12% of the dose, respectively). In comparison, the main circulating entities in humans were M21, M25, M34, PCI-45227 (dihydrodiol, M37), and unchanged drug.

The table below is the summary of *in vitro* metabolites of ^{14}C-ibrutinib in liver microsomes and hepatocytes of rat, rabbit, dog and human: Extent of metabolism (% injected) of ^{14}C-ibrutinib in liver microsomes and hepatocytes of rat, rabbit, dog and human is listed. The table is from the Applicant (Study #12-080-V-X-MT (FK10269) (eCTD Section 4.2.2.4))

Table 9- Summary of *in vitro* metabolites in humans and laboratory animals

Code	Retention time (min)	Proposed identity	m/z (ESI+)	Microsomes				S9 fractions		Hepatocytes			
				Rat	Rabbit	Dog	Human	Rat	Human	Rat	Rabbit	Dog	Human
M17	17.64	+34 (N ring open OH+O)	475.2094	-	-	-	-	X	-	-	-	-	-
M21	19.57	+96 (+O+SO3)	537.1556	-	-	-	-	-	-	X	-	X	X
M25	20.89	+32 (Ring open COOH)	473.1937	X	-	-	X	X	X	X	X	X	X
M26	21.77	+307 (+2H+GSH)	748.2877	-	-	-	-	-	-	-	-	X	-
M27	21.97	+305 (+GSH)	746.2721	-	-	-	-	-	-	X	X	X	-
M29	22.37	+34 (N ring open OH+O)	475.2094	-	-	-	-	X	-	-	-	-	-
M34	23.63	+18 (N ring open OH)	459.2145	-	-	-	-	-	-	X	X	X	X
M35	23.77	+16 (+O)	457.1988	-	-	-	-	-	-	X	X	X	X
M37	25.41	+34 (dihydrodiol)	475.2094	X	X	X	X	X	X	X	X	X	X
M38	26.40	+16 (+O)	457.1988	X	X	X	X	-	X	X	X	X	X
M39	28.06	+16 (+O)	457.1988	X	-	X	X	-	X	X	X	X	X
M40	28.75	+16 (+O)	457.1988	X	X	X	X	-	X	X	X	X	X
M41	31.73	-2 (-2H)	439.1882	X	X	X	X	-	X	X	X	-	X
UD	30.20	Unchanged ibrutinib	441.2039	X	X	X	X	-	X	X	X	X	X

X = observed

The inhibitory effect of ibrutinib on the following CYP45 isozymes was investigated: CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5.. While no inhibitory effects of ibrutinib on CYP1A2 and CYP2E1 were detected, ibrutinib exerted weak inhibition on the rest of the CYP450 isozymes tested (IC_{50} values ranged from 4 to 11 μg/mL). CYP3A4 and 3A5 were identified as the major CYP450 isozymes responsible for the metabolism of ibrutinib. .

Excretion:

The excretion of ibrutinib was evaluated in rats administered a 10 mg/kg dose of ^{14}C-ibrutinib (in a radiolabeled racemic mixture) by the oral route. It was found that over 80% of administered radioactivity was excreted in first 24 hours postdose. Excretion of ibrutinib-derived radioactivity via feces, mainly through biliary excretion, was the primary route of excretion. The overall mean recovery of radioactivity, including the radioactivity remaining in the carcasses, was approximately 92%.

No studies have been conducted to assess the excretion of ibrutinib into milk; however, given that ibrutinib is widely distributed into tissues, milk excretion of ibrutinib and its metabolites cannot be excluded.

5.2 Toxicokinetics

The TK data will be described in toxicology studies (see below).

6 General Toxicology

6.1 Single-Dose Toxicity

Not reviewed.

Single oral doses of ibrutinib were given to mice, rats and dogs. The summary of results is as follows:

Mice:
Animals survived doses up to 2000 mg/kg. While no clinical signs were observed at 500 or 1000 mg/kg, mild findings, such as inactivity, ptosis, and/or decreased body temperature and labored respiration, were reported in a few mice at the 2000 mg/kg dose.

Rats:
Ibrutinib doses up to 2000 mg/kg were administered. Deaths were noted within 7 days of dosing at 1000 mg/kg (females) and 2000 mg/kg (males). The findings included: clinical signs (GI related, skin and debility), weight loss, and gross pathological findings in the skin and GI tracts.

Dogs:
Dogs tolerated oral doses of ibrutinib up to 200 mg/kg, with mild ataxia and/or hypoactivity at 2 hours post-dosing. *Ex vivo* fluorescent-probe labeling of the active site of Btk in peripheral blood mononuclear cells showed that a single 100 mg/kg dose of ibrutinib resulted in complete active-site occupancy for at least 24 hours post-dosing.

6.2 Repeat-Dose Toxicity

Rats:
The following study was reviewed by Dr. Margret Brower.

Study title: A 28 day oral (gavage) toxicity study of PCI-32765 in rats with a 28-day recovery period	
Study no.:	06-017-R-PO-TX (b) (4) -622011
Study report location:	eCTD, Section 4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 10, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765, lot # SCR-182-77, 97.3% pure

Key Study Findings

- Elevated neutrophil and monocyte counts at HD reflect skin and GI inflammation
- Altered drug absorption at higher doses

Methods

Doses:	Males: 0 (control), 2.5, 40, 300 mg/kg/Day; Females: 0 (control), 2.5, 40, 150 mg/kg (as LD, MD and HD groups, respectively)
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5mL/kg
Formulation/Vehicle:	0.5% methylcellulose, 0.4% cremophor EL*, and 0.1% sodium lauryl sulfate
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	N=10, additionally, N=5 (Groups 1 & 4, recovery animals)
Age:	7 weeks
Weight:	Males: 214-272 g; Females:149-185 g
Satellite groups:	Toxicokinetic (TK) group: LD, MD, HD groups: 7/sex; vehicle control: 3/sex
Unique study design:	None
Deviation from study protocol:	Not remarkable

*** The formulation vehicle included 0.4% cremophor EL which is not included in the clinical formulation.**

Table 10- Observations and Results: 4-week study in rats

Observations	2.5mg/kg		40mg/kg		300m/k	150m/k
	M	F	M	F	M	F
Mortality (2x daily)					1(D16)	
Clinical observations (2x/d)	Decedent: (from D12)dried blood around nose/eyes, partial eye closure, soft feces, emaciation, dermal atonia, hypothermia, pale extremities HD: soft feces, dried blood around nose					
Body weight (predose, weekly)[a]					↓17 w2-4 ↓14 w8 rec	UR
Food consumption (predose, weekly)	↓14% in HDM compared to concurrent controls					
Ophthalmology (predose, prior to dose termination)	UR					
Hematology (following dosing and recovery)[b] Hgb/w4/w8 Hct/w4/w8 Reticulocyte/w4/w8 WBC/w4/w8 Platelets/w4/w8 Neutrophils/w4/w8 Monocyte/w4/w8 Coagulation (APTT/w4)					 ↓12/UR ↓9/UR ↑76/UR ↑14/↑12 ↑16/↑16 ↑4-fold/↑66 ↑4-fold/UR ↓13	 ↑47/UR ↑2.8fold/↑37 ↑60/UR
Clinical chemistry (following dosing and recovery)[c] ALT (w4) AST (w4) Triglycerides (w4)					 ↑2.3fold ↑1.3fold ↑33	
Urinalysis(following dosing and recovery)	UR					
Organ weights (D28/29) Thymus (absolute)[b]						
Gross pathology (D29/58)	Mandibular lymph node enlarged at HD					
Histopathology (D29/58)	All tissues listed in Histopathology Inventory following dosing (D28/29) following recovery (D58) (See following table)					
Toxicokinetics	Predose, 1, 2, 4, 8, 24h following dosing on D1 and 25. Control sampling conducted at 1h postdose on D1 and 25. (See following table)					

[a] Compared to concurrent controls
[b] Percent compared to concurrent controls; lower hgb and hct and higher reticulocytes consistent with anemia which normalized following recovery. Higher neutrophil and monocyte counts reflected skin and GI inflammation
[c] Percent compared to concurrent controls
[d].UR: unremarkable; MD, HD, HDM/HDF: mid dose, high dose, and high-dose male/high-dose female

Histopathology

Adequate Battery

Yes

Peer Review

Yes

Table 11- Histological Findings: 4-week study in rats

Tissue/Findings	2.5mg/kg		40m/k		300mg/kg	150mg/kg
	M	F	M	F	M	F
Mandibular lymph node/ hyperplasia /mild			1		5	3
/hyperplasia/moderate					1	2
Skin/exudate /mild					2	3
/exudate /moderate					1	
/inflammation /min-mild					2	2
/necrosis (mild)					1	1
Spleen/lymphoid depletion/ minimal					1	2
/lymphoid depletion/mild					8	5
Stomach/edema/mild					1	
/edema/moderate					1	
/hyperplasia/mild					2	
/ulceration/mild					1	
Thymus/necrosis/minimal					4	1
/necrosis/mild					1	1
Liver/necrosis/minimal					1	2
/necrosis/moderate				1		

Summary:
Histopathological changes in the mandibular lymph node, spleen and thymus normalized following recovery. Hepatic necrosis was observed in the decedent high dose males as well as other HD animals and a single mid dose female Increased ALT and AST in HD animals correlated with the incidence of hepatic necrosis.

Table 12- Toxicokinetics: 4-week study in rats

Parameter/	Males (mg/kg)			Females (mg/kg)		
Day 1	2.5	40	300	2.5	40	150
AUC last (ug.h/mL)	0.08	1	10.3	0.15	3.3	14.8
Normalized AUC	0.032	0.025	0.034	0.06	0.08	0.1
Cmax (ug/mL)	0.04	0.37	1.28	0.06	1.1	2.9
Normalized Cmax	0.02	0.01	0.004	0.02	0.03	0.02
t1/2 (h)	1.4	1.2	6	1.6	1.2	2.5
Day 24						
AUC last (ug.h/mL)	0.14	2.8	24	0.18	4	19
Normalized AUC	0.056	0.07	0.08	0.07	0.1	0.13
Cmax (ug/mL)	0.05	0.65	2	0.07	1.7	3.13
Normalized Cmax	0.02	0.02	0.01	0.03	0.04	0.02
t1/2 (h)	4	2	4	4.1	1.9	8.6

In general, systemic exposure increased with increasing dose. While AUC values increase slightly from Day 1 to Day 24 in females, exposures increased (~2 fold) in males on Day 24, indicating drug accumulation after repeated drug administration. Dose-normalized exposures were higher for females, as compared to males, on both days. The drug half-life ranged from 1.2 to 6 hr in males, and 1.2 to 8.6 hr in females.

Stability and Homogeneity

The last dosing formulation remained at 98% to 105% the initial concentrations indicating that PCI-32765 formulations stored at 2-8 °C were stable during the period of use.

The following study is reviewed by Dr. Chang.

Study title: A 13-week oral (gavage) toxicity study of PCI-32765 in rats with a 6-week recovery period

Study no.:	(b) (4)-622033 (10-068-R-PO-TX of Pharmacyclics, Inc.)
Study report location:	Module 4 Section 4.2.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 5, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765; Lot # 711-195; 97.63% (containing 2.09% of PCI-32769 (the S enantiomer of PC-32765))

Key Study Findings

- The dose level for the females at 300 mg/kg/day was reduced to 175 mg/kg/day on Day 8 due to severe body weight loss.
- Early mortalities occurred at 300 mg/kg/day groups in both genders.
- Drug-related toxicities were mainly at ≥ 175 mg/kg (females) or 300 mg/kg (males). The target organs were bone (thin cortical bone and fewer primary trabeculae), GI tract (inflammation and ulceration), lymphoid tissues/organs (depletion), pancreas (acinar atrophy), and skin (inflammation). Toxicities were reversible, or partially recovered at the end of recovery period.

Methods

Doses:	Males: 0, 30, 100, 300 mg/kg/day Females: 0, 30, 100, 300/175 mg/kg/day
Frequency of dosing:	Main-study groups: Daily for 90 days TK groups: Daily for 79 days
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% Methylcellulose (400 cps) and 0.1% sodium lauryl sulfate in water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	Main-study groups: 20/sex/group in main-study (exception: 15/sex/group for 30 mg/kg/day) TK groups: 10/sex/group
Age:	Approximately 2 months of age
Weight:	Males (main-study): 221-281 g at study start; Females (main-study): 151-202 g at study start.
Satellite groups:	None
Unique study design:	Peripheral blood immunophenotyping (TK groups)
Deviation from study protocol:	1. Starting from Day 8, dose for females in the 300 mg/kg/day group was reduced to 175 mg/kg/day, due to severe body weight losses. 2. Body weights were not collected at Week 13, due to scheduling error.

Observations and Results

Mortality

Early mortalities occurred at 300 mg/kg/day (8 males from Days 41-79 and 2 females on Day 1) and at 175 mg/kg/day (1 female on Day 90). The two females found dead on Day 1 at 300 mg/kg/day were replaced on Day 1. The possible causes of death (COD) were severe lymphoid depletion, as well as acute inflammation and ulceration of the intestinal tract.

The dose level for the females at 300 mg/kg/day was reduced to 175 mg/kg/day on Day 8 due to severe body weight loss.

Clinical Signs

Dose-related clinical signs of toxicity for both genders included thin body condition, partial closure of eye(s), clear and/or red material around the mouth, nose, other facial area, and forelimb(s), scabbing on various body surfaces, brown and yellow material on the urogenital and anogenital areas, hair loss, and soft feces. Findings were reversible.

Clinical signs of toxicity noted in early mortality animals of both genders included hypoactivity, dermal atonia, thin, pale and cool to the touch, rales, partial closure of eye(s), decreased defecation, soft feces, red material around the nose, facial area, and forelimb(s) and brown and yellow material on the urogenital and/or anogenital areas.

Body Weights

At the dosing phase, drug-related decrease in body weight was noted at 300 mg/kg/day for both males and females. The females at the reduced dose of 175 mg/kg/day had body weight gains comparable to control group, except for the last two weeks of dosing. At the recovery phase, body weight gain for both genders was comparable to the control animals. See tables and figures below.

Table 13- Body weight changes: 13-week study in rats

Group Mean Body Weight in Rats at the End of Dosing and Recovery Phases
Males

Body Weight (g) /Dose (mg/kg/day)	0	30	100	300
Week 13	543	523 (96)	531 (98)	508 (94)
Week 19	621	N/A	585 (94)	620

Week 13: End of dosing period; Week 19: End of recovery period.
Number in parenthesis indicates percent change compared to the control (100%).
N/A: not available, there were no recovery animals at 30 mg/kg/day.

Females (decreased at 175 mg/kg/day in Week13)

Body Weight (g) /Dose (mg/kg/day)	0	30	100	175
Week 13	307	308	300 (98)	282* (92) ↓
Week 19	314	N/A	330 (105)	320 (102)

Week 13: End of dosing period; Week 19: End of recovery period.
Dose was reduced from 300 mg/kg/day to 175 mg/kg/day on Day 8.
***: $p<0.05$ using Dunnett's test**
Number in parenthesis indicates percent change compared to the control.

Feed Consumption

At the dosing phase, drug-related decrease in body weight and food consumption was noted at 300 mg/kg/day for both males and females.

At the recovery phase, food consumption for both genders was comparable to the control animals.

Ophthalmoscopy

No drug-related ophthalmological findings were noted.

Hematology

Significant drug-related hematological changes were noted at 100 and 300 mg/kg/day in males and at 100 and 175 mg/kg/day in females and included: increases in reticulocyte and white blood cells (neutrophil and monocyte counts).

The higher mean absolute WBC, neutrophil, monocyte, and LUC counts were primarily due to values from animal #1136 (F). This animal had acute inflammation throughout the intestinal tract and ulceration of the mucosa in the rectum which likely resulted in the clinical pathology changes.

Table 14- Hematology parameters: 13-week study in rats

Males

Dose (mg/kg/day)	**0**	**30**	**100**	**300**
Reticulocyte (10^3/µL)				
Week 13	147.6	141.7	165.6 (112)	173.2* (117) ↑
Week 19	139.0	N/A	128.8 (92)	124.2 (89)
Leucocyte (103/µL)				
Week 13	9.12	8.62 (94)	9.14 (102)	11 (121)
Week 19	9.98	N/A	8.79 (88)	9.14 (92)
Neutrophil (103/µL)				
Week 13	1.38	1.19 (0.86)	1.94* (141) ↑	1.97 (143)
Week 19	1.29	N/A	1.30 (101)	1.34 (104)
Monocyte (103/µL)				
Week 13	0.24	0.26	0.33 (138)	0.38* (158) ↑
Week 19	0.22	N/A	0.23 (105)	0.25 (114)

Females

Dose (mg/kg/day)	**0**	**30**	**100**	**175**
Reticulocyte (10^3/µL)				
Week 13	129.1	133.3	161.2* (125)	177.6** (138) ↑
Week 19	173.6	N/A	135.1 (78)	115.9* (67)
RDW (%)				
Week 13	11.5	11.5	12.0* (104) ↑	13.2** (115) ↑
Week 19	10.9	N/A	11.4 (105)	11.3 (104)
HDW (g/dL)				
Week 13	2.27	2.31	2.35 (104)	2.44** (108) ↑
Week 19	2.14	N/A	2.02 (94)	2.03 (95)
MCV (fL)				
Week 13	53.0	52.7	52.9	50.4** (95) ↓
Week 19	52.8	N/A	53.7	51.7 (98)
MCH (pg)				
Week 13	18.1	17.9	18.1	16.9** (93) ↓
Week 19	19.1	N/A	19.4 (102)	18.7 (98)
MCHC (g/dL)				
Week 13	34.1	34.0	34.1	33.6* (99) ↓
Week 19	36.1	N/A	36.2	36.2

Leucocyte (103/µL)				
Week 13	6.90	7.18 (104)	8.10 (117)	10.56** (153)↑
Week 19	6.98	N/A	6.73 (94)	8.46 (121) ↑
Neutrophil (103/µL)				
Week 13	0.90	1.23 (137)	2.37 (263)	4.60** (511) ↑
Week 19	0.95	N/A	1.02 (107)	1.56 (164)
Monocyte (103/µL)				
Week 13	0.17	0.21 (124)	0.31 (182)	0.53** (312) ↑
Week 19	0.20	N/A	0.18 (90)	0.25 (125)
LUC (103/µL)				
Week 13	0.06	0.07	0.06	0.12 (200) ↑
Week 19	0.06	N/A	0.06	0.08 (133)

Week 13: End of dosing period; Week 19: End of recovery period.
Value in parentheses: Percent change compared to controls at the same time point.
*** p<0.05 using Dunnett's test; **: p<0.01 using Dunnett's test**

Clinical Chemistry

Significant drug-related changes, mainly noted at ≥ 100 mg/kg, in serum chemistry and included decrease in albumin, total protein, globulin, triglyceride, total bilirubin, glucose, calcium; increase in urea nitrogen, and phosphorus. Findings were reversible.

The findings were likely associated with general deterioration of physical conditions, especially in the females.

Table 15- Clinical chemistry parameters: 13-week study in rats

Males (Week 13):

Dose (mg/kg/day)	**0**	**30**	**100**	**300**
Albumin (g/dL)	4.3	4.2	4.1* (95) ↓	4.1* (95) ↓
T. Protein (g/dL)	6.8	6.8	6.5* (96) ↓	6.4** (94) ↓
Globulin (g/dL)	2.5	2.7	2.4 (96)	2.3* (92) ↓
Triglyceride (mg/dL)	6.8	6.8	6.5* (96) ↓	6.4** (94) ↓
Glucose (mg/dL)	116	118	117	108 (93)
T. Bilir. (mg/dL)	0.05	0.04	0.06 (120)	0.06 (120)
BUN (mg/dL)	13.6	15.1	15.2 (112)	16.7** (123) ↑
Calcium (mg/dL)	12.4	12.4	12.2 (98)	12.2 (98)
Potassium (mEq/dL)	4.74	4.38* (92)↓	4.33** (91) ↓	4.48 (95)
Phosphorus (mg/dL)	7.0	6.9	7.4	5.3 (76)

Week 13: End of dosing period.
Value in parentheses: percent change compared to controls at the same time point.
***: p<0.05 using Dunnett's test; **: p<0.01 using Dunnett's test**

Females:

Dose (mg/kg/day)	**0**	**30**	**100**	**175**
Albumin (g/dL)	5.2	5.0 (96)	4.6** (89) ↓	4.3** (83) ↓
T. Protein (g/dL)	7.5	7.3 (97)	6.5** (87) ↓	6.3** (84) ↓
Globulin (g/dL)	2.3	2.3	1.9** (83) ↓	2.0** (87) ↓
Triglyceride (mg/dL)	50	40 (80)	41 (82)	52 (104)
Glucose (mg/dL)	109	110 (101)	98* (90) ↓	99** (91) ↓
T. Bilir. (mg/dL)	0.05	0.04 (80)	0.06 (120)	0.06 (120)
BUN (mg/dL)	16.0	17.2 (108)	15.3 (96)	19.7* (123) ↑

Calcium (mg/dL)	12.8	12.6 (98)	12.3 (96)	12.1* (95) ↓
Potassium (mEq/dL)	4.33	4.08 (94)	4.05 (94)	4.06 (94)
Phosphorus (mg/dL)	6.1	6.1	6.5 (107)	6.9* (113) ↑

Week 13: End of dosing period.
Value in parentheses: Percent change compared to controls at the same time point.
***: p<0.05 using Dunnett's test; **: p<0.01 using Dunnett's test**

Urinalysis

No drug-related changes in urinalysis parameters were noted.

Gross Pathology

Drug-related gross pathology findings included a small thymus in females at 175 mg/kg/day.

For early mortality animals, the gross findings included small thymus and spleen, pale brain, kidney, pituitary and liver; distended stomach, cecum and jejunum; enlarged mandibular lymph node; lung not fully collapsed; red and brown material of skin; dark red areas of the stomach; and thick gray contents of the stomach.

Organ Weights

No significant drug-related changes in organ weight were noted at the end of dosing period or recovery period.

Histopathology

Drug-related toxicities noted at the scheduled sacrifice at the end of dosing phase and included severe lymphoid depletion in lymph nodes, spleen and thymus; atrophy of squamous epithelium with progression to ulceration in the nonglandular stomach, acute inflammation and ulceration of the intestinal tract, bone changes (thin cortical bone and fewer primary trabeculae) and acinar atrophy in the pancreas, cytoplasmic vacuolation in pituitary, and squamous atrophy of skin.

Most of those findings were absent at the end of the recovery period, except for acinar atrophy of the pancreas (males ≥100 mg/kg/day and females ≥ 100 mg/kg/day) and cytoplasmic vacuolation of pituitary (males ≥ 100 mg/kg/day) which had a partial recovery.

Table 16- Histopathological findings: 13-week study in rats

Selected Microscopic Findings in Rats at Week 13 (End of Dosing Phase)

	Males				Females			
Dosage (mg/kg/day):	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**175**
Spleen [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Depletion, Lymphoid	0	0	2	3	0	0	7	12
Minimal	-	-	2	2	-	-	7	3
Mild	-	-	0	1	-	-	0	8
Severe	-	-	0	0	-	-	0	1
Lymph Node, Axillary [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Depletion, Lymphoid	0	0	0	1	0	0	0	2
Minimal	-	-	-	1	-	-	-	0
Mild	-	-	-	0	-	-	-	1
Moderate	-	-	-	0	-	-	-	1
Lymph Node, Mandibular [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Depletion, Lymphoid, cortex	0	0	0	3	0	0	0	9
Minimal	-	-	-	1	-	-	-	3
Mild	-	-	-	2	-	-	-	6
Lymph Node, Mesenteric [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Depletion, Lymphoid	0	0	0	0	0	0	0	6
Minimal	-	-	-	-	-	-	-	4
Mild	-	-	-	-	-	-	-	1
Moderate	-	-	-	-	-	-	-	1
Thymus [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Depletion, lymphoid	0	0	0	0	0	0	0	1
Severe	-	-	-	-	-	-	-	1
Esophagus [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Atrophy, Squamous Epithelium	0	0	3	1	0	1	3	7
Minimal	-	0	3	1	-	1	3	6
Mild	-	-	0	0	-	0	0	1
Skin [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Atrophy, Squamous Epithelium	0	0	0	2	0	0	0	1
Minimal	-	-	-	2	-	-	-	1

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

	Males				Females			
Dosage (mg/kg/day):	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**175**
Stomach, Nonglandular [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Atrophy, Squamous Epithelium	0	0	0	0	0	0	1	3
Minimal	-	-	-	-	-	-	1	1
Mild	-	-	-	-	-	-	0	1
Moderate	-	-	-	-	-	-	0	1
Edema	0	0	0	0	0	0	0	3
Minimal	-	-	-	-	-	-	-	1
Mild	-	-	-	-	-	-	-	1
Moderate	-	-	-	-	-	-	-	1
Hyperplasia, Squamous Epithelium	0	1	0	0	0	0	0	1
Mild	-	1	-	-	-	-	-	0
Moderate	-	0	-	-	-	-	-	1
Ulceration	0	0	0	0	0	0	0	1
Minimal	-	-	-	-	-	-	-	1
Vagina [a]	**NA**	**NA**	**NA**	**NA**	**15**	**15**	**15**	**14**
Atrophy, Squamous Epithelium					0	0	0	1
Mild					-	-	-	1
Cecum [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Inflammation, Acute	0	0	0	1	1	0	0	4
Minimal	-	-	-	1	1	-	-	3
Mild	-	-	-	0	0	-	-	1
Colon [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Inflammation, Acute	0	0	0	0	0	0	0	2
Minimal	-	-	-	-	-	-	-	2
Duodenum [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Dilatation, Crypts	0	0	0	1	0	0	0	2
Minimal	-	-	-	1	-	-	-	2

[a] = Number of tissues examined from each group.
NA = Not applicable

(Excepted from the Applicant's submission)

	Males				Females			
Dosage (mg/kg/day):	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**175**
Rectum [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Inflammation, Acute	0	0	0	0	0	0	0	1
Minimal	-	-	-	-	-	-	-	1
Ulceration	0	0	0	0	0	0	0	1
Minimal	-	-	-	-	-	-	-	1
Femur [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Decreased Cortical and Trabecular Bone	0	0	0	0	0	0	2	2
Minimal	-	-	-	-	-	-	2	1
Mild	-	-	-	-	-	-	0	1
Sternum [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Decreased Cortical and Trabecular Bone	0	0	0	1	0	0	2	5
Minimal	-	-	-	1	-	-	2	3
Mild	-	-	-	0	-	-	0	2
Pancreas [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Atrophy, Acinar	0	15	13	8	0	5	4	1
Minimal	-	13	6	3	-	3	3	1
Mild	-	2	6	1	-	2	1	0
Moderate	-	0	1	4	-	0	0	0
Decreased Zymogen Granules	0	0	0	0	0	0	0	1
Mild	-	-	-	-	-	-	-	1
Adrenal Cortex [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Necrosis	0	-	2	0	0	0	0	1
Minimal	-	-	2	-	-	-	-	1
Pituitary [a]	**15**	**15**	**15**	**8**	**15**	**15**	**15**	**14**
Vacuolation, Cytoplasmic	1	9	7	8	0	0	0	0
Minimal	1	8	7	8	-	-	-	-
Mild	0	1	0	0	-	-	-	-

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

(Excepted from the Applicant's submission)

Selected Microscopic Findings in Rats in Week 19 (End of Recovery Phase)

	Males				Females			
Dosage (mg/kg/day):	**0**	**30**	**100**	**300**	**0**	**30**	**100**	**175**
Pancreas [a]	**5**	**NA**	**5**	**5**	**5**	**NA**	**5**	**5**
Atrophy, Acinar	0		4	4	0		1	1
Minimal	-		3	2	-		1	1
Mild	-		1	2	-		0	0
Pituitary [a]	**5**	**NA**	**5**	**5**	**5**	**NA**	**5**	**5**
Vacuolation, Cytoplasmic	1		3	2	0		0	0
Minimal	1		3	2	-		-	-

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

NA = Not applicable

(Excepted from the Applicant's submission)

Findings in the early deaths (data not shown):
For early mortality animals, the microscopic findings included widespread lymphoid depletion (axillary, mesenteric, and mandibular lymph nodes; spleen; Peyer's patches; and thymus), bone marrow depletion (sternum and femur); decrease in trabecular and cortical bone (femur and sternum), acute inflammation of the intestine (cecum, ileum, and rectum), dilatation of the intestinal crypts and glands (cecum, duodenum, and rectum), hemorrhage in the glandular stomach, cytoplasmic vacuolation of the pituitary, acinar atrophy of the pancreas, and decrease in zymogen granules in the pancreas.

Adequate Battery: Yes

Peer Review: Information was not provided:

Special Evaluation

Drug-related immunophenotyping findings included decrease in B-cell count and increase in T cell and natural killer cells in the peripheral blood.

Toxicokinetics

Table 17- Summary of toxicokinetic parameters in rats: PCI-32765 (ibrutinib) and metabolite PCI-45227

Toxicokinetic Summary Parameters for PCI-32765

Dosage	AUC_{last} (ng•hr/mL)		C_{max} (ng/mL)		T_{max} (hr)	
	Day 0	Day 78	Day 0	Day 78	Day 0	Day 78
Males						
30 mg/kg/day	1453	2480	546	618	0.5	0.5
100 mg/kg/day	3411	5506	627	666	2.0	1.0
300 mg/kg/day	10,192	21,732	989	1847	4.0	1.0
Females						
30 mg/kg/day	4409	19,712	1557	1413	1.0	0.5
100 mg/kg/day	14,609	20,661	3050	1923	1.0	1.0
175 mg/kg/day [a]	NA	51,549	NA	3970	NA	8.0
300 mg/kg/day [a]	43,237	NA	5263	NA	2.0	NA

[a] = The females were administered 300 mg/kg/day from study days 0-7 and 175 mg/kg/day from study day 8 until study day 78.
NA = Not applicable
(Excepted from the Applicant's submission)

Summary of toxicokinetic parameters in rats for PCI-45227 (metabolite of ibrutinib)

Dosage	AUC_{last} (ng•hr/mL)		C_{max} (ng/mL)		T_{max} (hr)		Metabolite/ Parent Ratio	
	Day 0	Day 78	Day 0	Day 78	Day 0	Day 78	Day 0	Day 78
Males								
30 mg/kg/day	1931	1536	499	283	1.0	2.0	1.2	0.57
100 mg/kg/day	2012	3698	435	405	2.0	2.0	0.5	0.62
300 mg/kg/day	12,452	17,547	1280	1322	4.0	8.0	1.1	0.75
Females								
30 mg/kg/day	4381	12,758	1006	744	1.0	24	0.92	0.60
100 mg/kg/day	4400	6872	640	736	2.0	1.0	0.28	0.31
175 mg/kg/day [a]	NA	13,055	NA	956	NA	1.0	NA	0.24
300 mg/kg/day [a]	12,576	NA	1503	NA	4.0	NA	0.27	NA

[a] = The females were administered 300 mg/kg/day from study days 0-7 and 175 mg/kg/day from study day 8 until study day 78.

NA = Not applicable

(Excepted from the Applicant's submission)

Dose-normalized AUC values and accumulation of PCI-32765 (ibrutinib) and PCI-45227 (metabolite):

Males:

	PCI-32765		PCI-45227	
Dose/Day	0	78	0	78
30	48.4	82.7 (171)	64.4	51.2 (80)
100	34.1	55.1 (162)	20.1	37 (184)
300	34	72.4 (213)	41.5	58.5 (141)

Females:

	PCI-32765		PCI-45227	
Dose/Day	0	78	0	78
30	147	657.1 (447)	146	424.9 (291)
100	146.1	206.6 (141)	44	68.7 (156)
175	NA	294.6 (204)*	NA	74.6 (178)*
300	144.1	NA (NA)	41.9	NA

***The ratio between AUC at 175 mg/kg (Day 78) and AUC value at 300 mg/kg (Day 0)**
Number in parenthesis indicates percent change compared to the control.

Summary:

- The exposure to oral PCI-32765 and its major metabolite, PCI-45227, increased as dose increased. The exposures were generally higher on Day 78 than Day 0 regardless of gender or dose, especially for PCI-32765, indicating accumulations of both parent drug and the metabolite.
- The mean exposures to PCI-32767 and to PCI-45227 were generally higher in females than males.

Dosing Solution Analysis

The dosing solutions were 101% to 108% of the target concentrations for PCI-32765; the formulations were determined to be homogeneous and stable for up to 10 days when stored refrigerated. The test article was not detected in the vehicle formulations.

Dogs

The following study is reviewed by Dr. Margret Brower.

Study title: A 28 day oral (gavage) toxicity study of PCI-32765 in dogs with a 28-day recovery period	
Study no.:	(b) (4) -622012
Study report location:	EDR Module 4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 11, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765, lot # SCR-182-77, 97.3% pure

Key Study Findings

- GI toxicities included inflammation/atrophy, and diarrhea/mucoid feces
- Unresolved corneal dystrophy at high dose

Methods	
Doses:	0 (control), 1.5, 24, 150 mg/kg/day (as LD, MD and HD groups, respectively)
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	5mL/kg
Formulation/Vehicle:	0.5% methylcellulose, 0.4% cremophor EL, and 0.1% sodium lauryl sulfate
Species/Strain:	Beagle dogs
Number/Sex/Group:	N=3; additionally N=2/sex/group as recovery animals (Groups 1 & 4, only)
Age:	7 weeks
Weight:	Males: 214-272 g; Females:149-185 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Not remarkable

Dose justification:

Based on a range-finding study in dogs (Study #06-015-D-S/POTXE) administered 100 mg/kg/Day for 8 days followed by 60 mg/kg/Day for 6 days. Hypoactivity, loose feces, , and ataxia were observed at 100mg/kg*; the incidence of these findings decreased with administration of the lower dose. At the completion of dosing, ALT was increased 2-fold, with an AUC of 4.3 μg·h/mL.

*Reviewer's note: Intolerance to the treatment at 100 mg/kg/day of ibrutinib in the DRF study may be possibly due to the intolerance to the vehicle which contained 28% (w/v)

HP-β-CD. The formulation was changed to a suspension free of HP-β-CD, and dose was reduced to 60 mg/kg/day in the subsequent 6 days of treatment.

Table 18- Observations and Results: 4-week study in dogs

Observations	1.5mg/kg		24mg/kg		150mg/kg	
	M	F	M	F	M	F
Mortality (2x daily)	None					
Clinical observations (2x/d)	HD: High incidence of diarrhea/soft feces (occasionally obs at lower doses).					
Body weight (predose, weekly)					↓9	↓17
Food consumption (predose, daily)	w1-2: MD/HDM ↓20% compared to concurrent controls w1: HDF ↓16% compared to concurrent controls					
Ophthalmology[a] (predose, d23, 27, 51) Corneal dystrophy/d23 /d27 /51					 1 2 2	 1 1 1
EKG (predose and 2h postdose on d2, 22)	UR					
Hematology (predose, d14, 25, 57)[b] WBC/D25 Platelets/D25 Neutrophils/D25	 ↑25	 ↑22		 ↑13	 ↑16 ↑24	 ↑36 ↑19 ↑24
Clinical chemistry (predose, d14, 25, 57)[c] ALP (D57) AST (D14/D25) Triglycerides (D14/25/57)		 UR/↑41			 ↑16/UR/UR	 ↑13 ↑19↑55 ↑30/35/53
Urinalysis	UR					
Organ weights (D28/29)	UR					
Gross pathology (D29/58)	UR					
Histopathology	All tissues listed in Histopathology Inventory following dosing (D28/29); small intestine, large intestine, gross lesions, and ocular sections following recovery (D58) (See following table)					
Toxicokinetics	Predose, 1, 2, 4, 8, 24h following dosing on D1 and 24. (See following table)					

[a] Corneal dystrophy varied in severity from slight to moderate and from focal to multifocal on D23, 27, and following recovery on D51. The finding did not resolve during the recovery period with the exception of bilateral to unilateral distribution in a single high dose (HD) female.
Note: Histopathology did not indicate the causative factor for the corneal dystrophy observed at the HD.

[b] Percent compared to concurrent controls. WBC and platelets normalized following recovery in HD dogs. Increases observed during dosing were consistent with histopathological findings of intestinal tract inflammation in 2 HD male and 1 HD female.

[c] Percent compared to concurrent controls. A number of liver indices were depressed at the HD during the dosing period compared to controls (e.g. total protein, albumin) indicating lower serum protein, which may be a result of the GI inflammation and atrophy. Parameters normalized following recovery with the exception of AST and triglycerides in HD females.

UR: unremarkable;
MD, HD, HDM/HDF: mid dose, high dose, high-dose male/high-dose female

Additional histopathology data:

Organ/finding	Males (mg/kg)			Females (mg/kg)		
	1.5	24	150	1.5	24	150
Cecum/chronic inflammation(mild)			1			
Colon/chronic inflammation(minimal)			1			
Ileum/atrophy(minimal)[a]						1
Rectum/chronic inflammation(min)			1			1

[a] Finding observed following recovery in 1of 2 HD males

Table 19- Toxicokinetic parameters: 4-week study in dogs

Parameter/	Males (mg/kg)			Females (mg/kg)		
Day 1	1.5	24	150	1.5	24	150
AUC last (ug.h/mL)	0.0053	1.78	2.02	0.0097	1.00	4.72
Normalized AUC	0.0035	0.074	0.014	0.0064	0.083	0.032
Cmax (ug/mL)	0.0035	0.95	0.75	0.0056	0.86	1.71
Normalized Cmax	0.0023	0.04	0.005	0.0037	0.036	0.011
t1/2 (h)	0.82	3.1	4.2	1.1	2.4	4.0
Day 24						
AUC last (ug.h/mL)	0.017	1.54	14.1	0.021	1.85	15.2
Normalized AUC	0.012	0.064	0.094	0.014	0.08	0.10
Cmax (ug/mL)	0.01	0.68	1.48	0.01	0.91	2.18
Normalized Cmax	0.007	0.03	0.01	0.007	0.038	0.015
t1/2 (h)	1	2.5	2.3	2.7	2.5	2.4

Summary:

- Following oral dosing on Day 1, systemic exposure to PCI-32765 increased with increasing dose from 1.5 to 24 mg/kg, but did not increase with dosing to 150 mg/kg. This may have been a result of altered absorption and shorter residence time of the drug in the GI tract of HD dogs due to the diarrhea and soft mucoid feces observed in these animals.
- Following dosing on Day 24, exposure, as measured by AUC, increased with increasing dose from 1.5 to 150 mg/kg.
- At steady state (Day 24), no gender differences were apparent.
- The drug half-life in plasma ranged from 0.8 to 4 hr in males, and 1 to 4 hr in females, with a trend for increased half-life with an increase in dose on Day 1.
- On Day 24, the T_{max} values increased in a dose related manner from 1 to 10 hr for males, compared to 1 to 3.4 hr for females.
- Plasma concentrations of the metabolite, PCI-48303, a glutathione conjugate, were below the LLOQ (10 ng/mL) in samples analyzed on Days 1 and 24.

The following study is reviewed by Dr. Chang.

Study title: A 13-week oral (gavage) toxicity study of PCI-32765 in Beagle dogs with a 13-week recovery period

Study no.:	10-069-D-PO-TX ([redacted]-622034)
Study report location:	Module 4, Section 4.2.3
Conducting laboratory and location:	[redacted]
Date of study initiation:	April 7, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765 (PCI-32765-00 [free base]), Lot No. 111131, 99.2% (active content by weight)

Key Study Findings

- Drug-related moribund sacrifices occurred at 80 and 220 mg/kg/day doses in males on Day 31. Subsequently, doses were reduced in Week 6.
- Drug-related findings included reduction in red-blood cell counts, serum albumin and serum γ-glutamyltransferase; decrease in heart rate; intestinal inflammation, lymphoid depletion of Peyer's patches, and gastric smooth muscle degeneration.
- Accumulated exposure of PCI-32765 and its metabolite PCI-45227 was observed.

Table 20- Study design of the 13-week study in dogs

Group Number	Treatment	Dosage Level (mg/kg/day)	Number of Animals [a] Males	Females
1	Vehicle [b]	0	6	6
2	PCI-32765	30	3	3
3	PCI-32765	80/60[c]	6	6
4	PCI-32765	220/120 [c]	6	6

[a] - 3 animals/sex/group were euthanized following up to 92 days of dose administration; the remaining animals in Groups 1, 3, and 4 were euthanized following a 13-week nondosing (recovery) period.

[b] - The vehicle was 0.5% methylcellulose (400 cps) and 0.1% sodium lauryl sulfate.

[c] - The dosage levels for Group 3 and Group 4 were lowered to 60 and 120 mg/kg/day, respectively, beginning on study day 42 (start of study week 6).

(Excerpted from the Applicant's submission)

Observations and Results

Mortality

Moribund sacrifice occurred on Day 31 for 1 male each in the 80 and 220 mg/kg/day groups. All other animals survived to scheduled sacrifice.

The cause of morbidity at 220 mg/kg/day dose was determined to be treatment-related enterocolitis and that at 80 mg/kg/day dose was due to severe acute lung inflammation and bacterial colonization of the lungs.

Clinical Signs

Drug-related clinical signs of toxicity, including abnormal excreta (soft feces and/or diarrhea), emesis, reddened or pale gums, raised redden or white area(s) on the gums, were primarily noted in the 80/60 and 220/120 mg/kg/day dose groups, throughout the dosing period. Adverse findings suggestive of effects on the central nervous system including continuous tremors, intermittent convulsion and rigid muscle tone were noted in the two animals at 200/120 mg/kg/day dose during the dosing phase; these signs were generally resolved during the recovery period.

The clinical signs noted in the two moribund sacrificed dogs included hypoactivity, dermal atonia, red nasal discharge, diarrhea, swollen facial area, and raised redden area(s) on the gums.

Body Weights

Group mean body weight:
The treated dogs had lower group mean body weight than the control, and such reductions in mean body weight, although not statistically significant, followed a dose-dependent trend. In Week 13, the reduction (compared to the control) at 220/120 mg/kg was up to 10% in males and 7% in females. There was a great inter-animal variation: At the end of recovery period, the body weights in the treated groups were comparable to those of the control animals.

Cumulative body weight changes (weight gains):
Male dogs in the 220/120 mg/kg group showed statistically significant reduction of weight gains, in comparison to the control.

Table 21- Cumulative weight changes (weight gains) in the 13-week study in dogs

Statistically significant cumulative weight changes (kg)* are summarized in the table below. NS: not statistically significant changes.

Dose	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
Control	0.6	0.7	0.	1	1.2	1.3	1.5	1.5	1.6	1.6	1.7
220/120 mg/kg	-0.3	-0.5 (NS)	-0.2 (NS)	-0.1	0.0	0.0	0.3	0.3	0.4	0.6	0.5

*** Expressed as changes during the period of pretreatment (0) and treatment week (e.g., 0-3: pretreatment to Week 3)**

Occasionally, statistically significant lower weight gains were reported in females treated with 220/120 mg/kg of PCI-32765. No remarkable differences of weight gains were observed in the recovery animals.

Feed Consumption

Dose-dependent decreases in food consumption were noted: up to 28% reduction at 220/120 mg/kg in comparison to the control (data not shown). Food consumption was comparable among all groups during the recovery period.

Ophthalmoscopy

No drug-related ophthalmological findings were noted.

ECG

Drug-related decreases in heart rates (with associated increased RR intervals) were noted at 80 mg/kg/day dose in Week 1 and at 60 and 120 mg/kg/day doses in Week 12. Also, mean RR interval was significantly increased in females at 30 mg/kg during Week 1. In all cases, the observations occurred 1 hour post-dosing. This finding was absent at the end of the recovery period.

Prolonged RR intervals (msec): expressed as mean ± standard error; numbers in the parentheses represent % increases from the control

	Males				**Females**			
Dose (mg/kg0	**Control**	**30**	**80/60**	**220/120**	**Control**	**30**	**80/60**	**220/120**
Week -2	**486 ± 41**	**521 ± 38 (7.2)**	**561 ± 37 (15)**	**534 ± 49 (10)**	**480 ± 31**	**572 ± 116 (19)**	**553 ± 33 (15)**	**472 ± 28 (-1.7)**
Week 0	**513 ± 25**	**642 ± 51 (25)**	**678 ± 31 (32)***	**579 ± 37 (13)**	**518 ± 33**	**752 ± 87 (45)***	**574 ± 41 (11)**	**530 ± 28 (2.3)**
Week 12	**519 ± 24**	**657 ± 99 (27)**	**683 ± 32 (32)***	**808 ± 48 (56)***	**558 ± 27**	**665 ± 96 (19)**	**628 ± 55 (13)**	**703 ± 56 (26)**
Week 25	**490 ± 56**	**N/A**	**496 ± 28 (1.2)**	**509 ± 60 (4)**	**570 ± 62**	**N/A**	**419 ± 19 (-27)**	**578 ± 100 (1.4)**

Heart rate (bpm): expressed as mean ± standard error: numbers in the parentheses represent % decreases from the control

	Males				Females			
Dose (mg/kg0	**Control**	**30**	**80/60**	**220/120**	**Control**	**30**	**80/60**	**220/120**
Week -2	**129 ± 10**	**120 ± 8 (-7)**	**111 ± 7 (-14)**	**119 ± 10 (-8)**	**130 ± 8**	**116 ± 20 (-11)**	**113 ± 6 (-13)**	**132 ± 7 (1.5)**
Week 0	**121 ± 6**	**102 ± 7 (-15)**	**92 ± 4 (-24)***	**109 ± 7 (-10)**	**121 ± 6**	**89 ± 12 (-26)**	**111 ± 8 (-8.3)**	**118 ± 7 (-2.5)**
Week 12	**118 ± 5**	**99 ± 12 (-16)**	**90 ± 4 (-24)***	**77 ± 5 (-35)***	**111 ± 6**	**99 ± 14 (-11)**	**101 ± 8 (-9)**	**91 ± 7 (-18)**
Week 25	**127 ± 16**	**N/A**	**122 ± 7 (-4)**	**120 ± 14 (-6)**	**111 ± 12**	**N/A**	**1449 ± 7 (30)**	**113 ± 18 (1.8)**

Similar findings of RR period prolongation accompanied with slower hear heart were reported in a safety pharmacology study, where dogs were administered a single oral dose at 24 or 150 mg/kg. The Applicant described the findings to be small magnitude in changes and the values within the normal range.

Hematology

Drug-related effects were evident at mid dose and high dose. Findings were reversible. See tables below for details.

The hematological findings noted in the 220 mg/kg/day moribund-sacrifice dog (#9066) included (in comparison to pretest values): increased white blood cell, absolute neutrophil and absolute lymphocyte counts; decrease in hemoglobin (HGB), mean corpuscular hemoglobin concentration (MCHC), and hematocrit (Hct); increase in mean corpuscular volume (MCV); and decrease in absolute reticulocyte count, as well as decrease in prothrombin time (PT), and increase in activated partial thromboplastin time (aPTT)

The hematological findings noted in the 80 mg/kg/day moribund-sacrifice dog (#9057) included (in comparison to pretest values): decrease in white blood cell and absolute neutrophil counts; increase in reticulocyte counts; slight increase in red blood cell counts, hemoglobin and hematocrit.

Table 22- Hematology parameters

Males

Dose (mg/kg/day)	**0**	**30**	**80/60**	**220/120**
RBC (mil/µL)				
Week -1	6.82	6.34	6.83	6.67
Week 5	6.46	6.10	5.88 (91)	5.28** (82) ↓
Week 12	6.62	6.09	5.89 (89)	5.64 (85)
Week 17	7.53	N/A	7.39 (98)	6.61 (88)
HGB (g/dL)				
Week -1	15.2	14.4	15.2	14.8

Week 5	15.0	14.6	14.0 (93)	12.3** (82) ↓
Week 12	14.8	14.0 (95)	13.5 (91)	12.8 (85)
Week 17	16.9	N/A	16.9	15.1 (87)
HCT (%)				
Week -1	46.3	44.6	47.6	45.3
Week 5	42.4	41.4	39.5 (93)	34.9** (82) ↓
Week 12	44.1	42.2	40.8 (93)	39.1 (89)
Week 17	50.0	N/A	50.1	45.6 (91)
RDW (%)				
Week -1	12.5	12.2	12.7	13.1
Week 5	12.4	12.5	13.5** (109) ↑	14.0** (113) ↑
Week 12	12.7	12.9	13.5 (106)	13.9** (110) ↑
Week 17	12.6	N/A	12.1	12.7
HDW (g/dL)				
Week -1	1.48	1.46	1.49	1.46
Week 5	1.70	1.73	1.80** (106) ↑	1.89** (111) ↑
Week 12	1.59	1.63	1.74 (109)	1.84** (116) ↑
Week 17	1.66	N/A	1.52 (92)	1.56 (94)

- **Week 12: 3 or 4 days prior to primary necropsy; Week 17: 4 weeks post-dosing (during recovery period); Week 26: on the day prior to recovery necropsy**
- **N/A: there were no recovery animals at the dose of 30 mg/kg/day.**
- **RDW: Red cell distribution width; HDW: Hemoglobin distribution width**
- **Value in parentheses: Percent change compared to controls at the same time point.**
- ****: p<0.01 using Dunnett's test**

Females

Dose (mg/kg/day)	0	30	80/60	220/120
RBC (mil/µL)				
Week -1	7.04	7.21	7.24	6.84 (97)
Week 5	6.33	6.62	6.19	5.83* (92) ↓
Week 12	6.38	6.89	6.52	5.94 (93)
Week 17	7.44	N/A	7.82	7.08 (95)
HGB (g/dL)				
Week -1	15.9	16.0	16.1 (101)	15.1 (95)
Week 5	14.9	15.4	14.5 (97)	13.6* (91) ↓
Week 12	14.5	15.6	14.8 (102)	13.3 (92)
Week 17	16.8	N/A	17.6 (105)	15.8 (94)
HCT (%)				
Week -1	48.6	48.6	49.6 (102)	46.4 (95)
Week 5	42.2	44.0 (104)	41.3 (98)	38.7* (92) ↓
Week 12	43.2	46.5 (108)	44.8 (104)	40.4 (94)
Week 17	49.8	N/A	52.8 (106)	47.3 (95)
RDW (%)				
Week -1	12.4	12.3 (99)	12.9 (104)	13.0 (105)
Week 5	12.7	13.0 (102)	13.1 (103)	13.5* (106) ↑
Week 12	12.9	12.6 (98)	13.2 (102)	13.4 (104)
Week 17	12.5	N/A	12.5	12.6 (101)
HDW (g/dL)				

Week -1	1.43	1.42 (99)	1.47 (103)	1.45 (101)
Week 5	1.67	1.73 (104)	1.76 (105)	1.81 (108)
Week 12	1.57	1.60 (102)	1.67 (106)	1.71* (109) ↑
Week 17	1.64	N/A	1.61 (98)	1.52 (93)
Platelet (10^3/µL)				
Week -1	350	336	340	405
Week 5	318	319	282 (89)	456* (143) ↑
Week 12	350	357	382 (109)	542** (155) ↑
Week 17	358	N/A	310 (87)	397 (111)
APTT (second)				
Week -1	10.8	10.8	11.1	11.3
Week 5	10.5	10.3	11.1 (106)	11.9* (113) ↑
Week 12	10.4	11.0	11.3* (109) ↑	11.7** (113) ↑
Week 17	N/A	N/A	N/A	N/A

- **Week 12: 3 or 4 days prior to primary necropsy; Week 17: 4 weeks post-dosing (during recovery period); Week 26: on the day prior to recovery necropsy**
- **N/A: there were no recovery animals at the dose of 30 mg/kg/day.**
- **RDW: Red cell distribution width; HDW: Hemoglobin distribution width; APTT: activated partial thromboplastin time**
- **Value in parentheses: Percent change compared to the control group at the same time point.**
- ****: p<0.01 using Dunnett's test**

Group Mean white blood cell counts
Males

Dose (mg/kg/day)	**0**	**30**	**80/60**	**220/120**
Neutrophil (%)				
Week -1	54.4	62.6	58.6	60.1
Week 5	55.9	61.8	55.3	64.9 (116)
Week 12	57.5	64.6 (112)	66.2 (115)	71.3** (124) ↑
Week 17	62.1	N/A	60.6	62.0
Lymphocyte (%)				
Week -1	35.8	27.2	31.5	31.4
Week 5	34.9	30.1 (86)	32.3 (93)	23.8 (68)
Week 12	33.4	26.7 (80)	23.9 (72)	19.8** (59) ↓
Week 17	28.9	N/A	29.1	29.7

- **Week 12: 3 or 4 days prior to primary necropsy; Week 17: 4 weeks post-dosing (during recovery period); Week 26: on the day prior to recovery necropsy**
- **N/A: there were no recovery animals at the dose of 30 mg/kg/day.**
- **Value in parentheses: percent change compared to controls at the same time point.**
- ****: p<0.01 using Dunnett's test**

Females

Dose (mg/kg/day)	0	30	80/60	220/120
Monocyte (%)				
Week -1	5.5	6.6	5.1	6.9 (125)
Week 5	5.3	5.4	8.7 (164)	10.0* (189) ↑
Week 12	4.2	4.4	6.0* (143)	5.8 (138)
Week 17	4.1	N/A	5.5 (134)	5.4 (132)
Monocyte (10^3/µL)				

Week -1	0.53	0.74	0.57	0.62
Week 5	0.56	0.65 (116)	1.00 (179)	1.36 (243)
Week 12	0.46	0.54 (117)	0.53 (115)	0.93* (202) ↑
Week 17	0.41	N/A	0.57 (139)	0.70* (170) ↑
Week 26 (recovery)	0.39	N/A	0.42 (108)	0.71* (182) ↑

- **Week 12: 3 or 4 days prior to primary necropsy; Week 17: 4 weeks post-dosing (during recovery period); Week 26: on the day prior to recovery necropsy**
- **N/A: there were no recovery animals at the dose of 30 mg/kg/day.**
- **Value in parentheses: Percent change compared to controls at the same time point.**
- ****: p<0.01 using Dunnett's test**

Comment:

- Slight perturbations of erythroid parameters, although statistically significant, during Weeks 5-12 at doses ≥ 80/60 mg/kg, did not have associated pathological evidences.
- The slightly elevated mean platelet counts fell within the (b) (4) historical control reference ranges for absolute platelet counts.
- Statistically significant increases in aPTT values were only observed in females.

Clinical Chemistry

Drug-related decreases in serum albumin and γ-glutamyltransferase (GGT) were noted at 80/60 mg/kg/day and 220/120 mg/kg/day doses. Findings were reversible. .

The serum chemistry findings noted in the 200 mg/kg/day moribund-sacrifice dog (#9066) included decrease in serum albumin, total protein, albumin/globulin ration; decrease in GGT; decrease in phosphorus and potassium; and increase in triglycerides and sorbitol dehydrogenase. Some of the findings may be the result of GI inflammation and poor physical condition.

Serum chemistry changes found in the preschedule sacrificed dog (#9057) included: decreased albumin and albumin/globulin ratio, increased total bilirubin, urea nitrogen, creatinine, aspartate aminotransferase, and alkaline phosphatase; increased cholesterol, triglycerides and phosphorus; and decreased potassium and calcium.

Table 23- Clinical chemistry parameters

Males

Dose (mg/kg/day)	**0**	**30**	**80/60**	**220/120**
ALT (U/L)				
Week -1	30	29	30	27
Week 5	40	32	35	21** (53) ↓
Week 12	42	32	35	23** (55) ↓
Week 17	49	N/A	46	41 (84)
GGT (U/L)				
Week -1	2.4	2.4	1.6	2.2
Week 5	2.8	2.6	1.3** (46) ↓	1.4** (50) ↓
Week 12	1.8	1.3 (72)	0.7 (39)	0.8 (44)

Week 17	1.3	N/A	1.1 (85)	0.9 (69)
Albumin (g/dL)				
Week -1	3.5	3.6	3.6	3.5
Week 5	3.4	3.0 (88)	2.9** (85) ↓	2.4** (71) ↓
Week 12	3.4	3.1 (91)	2.9** (85) ↓	2.7** (79) ↓
Week 17	3.5	N/A	3.6 (103)	3.2 (91)
Globulin (g/dL)				
Week -1	2.0	1.9	2.0	2.1
Week 5	2.3	2.7 (117)	2.6 (113)	3.0 (130)
Week 12	2.3	2.7 (117)	2.8 (122)	2.6 (93)
Week 17	2.2	N/A	2.4 (109)	2.5 (114)
A/G Ratio				
Week -1	1.76	1.85	1.88	1.74
Week 5	1.48	1.14 (77)	1.11** (75) ↓	0.83** (56) ↓
Week 12	1.45	1.16 (80)	1.10** (76) ↓	1.04** (72) ↓
Week 17	1.63	N/A	1.50	1.30 (80)
Glucose (mg/dL)				
Week -1	107	104	99	100
Week 5	105	101	95 (91)	89** (85) ↓
Week 12	98	94	91 (93)	85** (87) ↓
Week 17	99	N/A	92 (93)	93 (94)
Calcium (mg/dL)				
Week -1	12.2	12.4	12.6	12.4
Week 5	12.6	12.5	12.2	11.6** (92) ↓
Week 12	11.2	11.1	11.3	10.5 (94)
Week 17	11.5	N/A	11.3	11.8

- **Week 12: 3 or 4 days prior to primary necropsy; Week 17: 4 weeks post-dosing (during recovery period)**
- **N/A: there were no recovery animals at the dose of 30 mg/kg/day.**
- **Value in parentheses: Percent change compared to controls at the same time point.**
- ****: p<0.01 using Dunnett's test**

Females

Dose (mg/kg/day)	**0**	**30**	**80/60**	**220/120**
ALT (U/L)				
Week -1	29	26	31	27
Week 5	37	29	24 (65)	24** (65) ↓
Week 12	38	32	31 (82)	28** (74) ↓
Week 17	33	N/A	43 (130)	40 (121)
GGT (U/L)				
Week -1	1.7	1.6	1.7	1.3
Week 5	2.7	1.9 (70)	1.0** (37) ↓	1.3** (48) ↓
Week 12	1.8	0.2* (11)	0.4** (22)	0.6* (33)
Week 17	1.7	N/A	0.5 (29)	0.3 (18)
Albumin (g/dL)				
Week -1	3.7	3.7	3.7	3.5
Week 5	3.5	3.3 (94)	2.8** (80) ↓	2.6** (74) ↓
Week 12	3.4	3.4	3.0** (88) ↓	2.6** (76) ↓
Week 17	3.7	N/A	3.7	3.4
Globulin (g/dL)				

Week -1	1.9	2.0	2.0	1.9
Week 5	2.0	2.2	2.7** (135) ↑	2.9** (145) ↑
Week 12	2.0	2.4	2.5 (125)	3.0** (150) ↑
Week 17	2.0	N/A	2.9 (145)	2.7 (135)
A/G Ratio				
Week -1	1.95	1.87	1.89	1.86
Week 5	1.78	1.45 (82)	1.08** (61) ↓	0.90** (51) ↓
Week 12	1.77	1.42 (80)	1.25** (71) ↓	0.88** (50) ↓
Week 17	1.94	N/A	1.33 (69)	1.27 (66)
BUN (mg/dL)				
Week -1	12.0	10.7 (89)	11.7 (99)	11.1 (93)
Week 5	13.7	14.0 (99)	13.6 (99)	11.0 (80)
Week 12	16.3	14.6 (90)	12.9** (79) ↓	13.5* (83) ↓
Week 17	17.4	N/A	14.7 (84)	14.3 (82)
Calcium (mg/dL)				
Week -1	12.8	12.5	12.6	12.5
Week 5	13.0	12.4* (95) ↓	12.0** (92) ↓	11.8** (91) ↓
Week 12	11.4	11.1 (97)	11.0 (97)	10.6 (93)
Week 17	11.8	N/A	12.0	11.7

- **Week 12: 3 or 4 days prior to primary necropsy; Week 17: 4 weeks post-dosing (during recovery period)**
- **N/A: there were no recovery animals at the dose of 30 mg/kg/day.**
- **Value in parentheses: Percent change compared to controls at the same time point.**
- ****: p<0.01 using Dunnett's test**

All findings resolved at the end of recovery period (Week 26, data not shown).

Urinalysis

No drug-related urinalysis findings were noted.

Gross Pathology

Drug-related red areas in cecum and ileum were noted in a female at 220/120 mg/kg/day at the end of dosing phase; which correlated with the acute inflammation noted microscopically.

The gross findings for the 220 mg/kg/day moribund-sacrificed dog (#9066) included dark red areas in duodenum, jejunum, ileum and lung; brown areas in the stomach and brown discoloration of the teeth; enlarged mandibular and popliteal lymph nodes; depressed area of the ileum; white areas in the spleen; thickened gallbladder contents; and raised interdigital areas of the right hind paw.

The gross findings for the 80 mg/kg/day moribund-sacrificed dog (#9057) included dark red discoloration in the lung, bronchial and mediastinal lymph node, enlarged bronchial lymph node, lung not fully collapsed, and a small, rough-surfaced spleen.

Organ Weights

No drug-related organ weight changes were noted at the end of the dosing phase or the recovery phase.

Histopathology

Drug-related microscopic findings included acute inflammation in the intestine, lymphoid depletion, and gastric smooth muscle degeneration. Microscopic findings were absent at the end of the recovery phase.

Table 24- Histopathology

Selected Microscopic Findings - Interim Sacrifice (Week 13)

	Males				Females			
Dosage (mg/kg/day):	**0**	**30**	**80/60**	**220/120**	**0**	**30**	**80/60**	**220/120**
Peyer's Patches [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Deletion, lymphoid	0	0	1	2	0	0	1	3
Minimal	-	-	1	1	-	-	1	3
Mild	-	-	0	1	-	-	0	0
Ileum [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Inflammation, acute	0	0	0	0	0	0	0	1
Minimal	-	-	-	-	-	-	-	1
Cecum [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Inflammation, acute	0	0	0	0	0	0	0	1
Minimal	-	-	-	-	-	-	-	1
Stomach [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Degeneration, muscle	0	0	0	0	0	0	0	3
Minimal	-	-	-	-	-	-	-	2
Mild	-	-	-	-	-	-	-	1
Duodenum [a]	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Dilatation, glandular	0	1	1	0	0	0	3	2
Minimal	-	1	1	-	-	-	3	2

[a] = Number of tissues examined from each group.
(Excerpted from the Applicant's submission)

The microscopic findings for the 220 mg/kg/day moribund-sacrificed dog (#9066) included acute inflammation for gastro-intestinal tract and multiple lymph nodes; lymphoid depletion of Peyer's patches, thymus, and multiple lymph nodes; degeneration of testicular seminiferous tubules; adrenal cortical hypertrophy; bone marrow myeloid hyperplasia; extramedullary hematopoiesis in spleen and liver; and Kupffer cell hypertrophy in the liver.

The microscopic findings for the 80 mg/kg/day moribund-sacrificed dog (#9057) included acute inflammation and hemorrhage in the lung with bacterial colonies present;

acute inflammation in the heart (epicardium), larynx, trachea, and bronchial and mediastinal lymph nodes.

Testicular seminiferous tubule degeneration found in the moribund sacrificed dog (#9066) was considered to be a test article-related effect, possibly an indirect effect or related to the poor condition of the animal.

A 220/120 mg/kg/day group male (# 9049) was clinically diagnosed with minimal bilateral corneal dystrophy/degeneration (opacity of the cornea). However, microscopic evaluation did not demonstrate the characteristic corneal stromal lipid inclusions described by Roth *et al*, (Investigative Ophthalmology & Visual Science, 21(1), 95-106, 1981), and the corneas were considered to be normal microscopically.

Peer Review: Not specified

Toxicokinetics

Table 25- Toxicokinetics parameters of PCI-32765 and PCI-45227: 13-week study in dogs

Toxicokinetic Summary Parameters for PCI-32765

Gender	Males			Females		
PCI-32765						
Dosage (mg/kg/day):	**30**	**80**	**220**	**30**	**80**	**220**
Parameter (Units)			Study Day 0			
AUC_{last} (ng·h/mL)	263	1585	1294	261	2112	917
Dose Normalized AUC_{last}	8.77	19.8	5.88	8.69	26.4	4.17
C_{max} (ng/mL)	147	617	536	136	536	438
Dose Normalized C_{max}	4.91	7.72	2.44	4.53	6.70	1.99
T_{max} (h)	1.0	1.7	1.3	1.0	1.3	1.1
Dosage (mg/kg/day):	**30**	**80**	**220**	**30**	**80**	**220**
			Study Day 37			
AUC_{last} (ng·h/mL)	310	1056	17658	524	5808	5038
Dose Normalized AUC_{last}	10.3	13.2	80.3	17.5	72.6	22.9
C_{max} (ng/mL)	87.2	245	2144	271	1189	998
Dose Normalized C_{max}	2.91	3.06	9.76	9.03	14.9	4.54
T_{max} (h)	0.8	1.0	4.0	3.2	1.3	2.8
Accumulation Ratio	1.5	1.1	16	2.2	9.5	6.2
Dosage (mg/kg/day):	**30**	**60**	**120**	**30**	**60**	**120**
			Study Day 78			
AUC_{last} (ng·h/mL)	377	3414	12179	1683	2211	6628
Dose Normalized AUC_{last}	12.6	56.9	101	56.1	36.9	55.2
C_{max} (ng/mL)	151	1115	1859	451	842	1044
Dose Normalized C_{max}	5.01	18.6	15.5	15.0	14.0	8.70
T_{max} (h)	1.0	2.6	2.8	2.0	1.2	2.4

Units for D.N. AUC_{last} are (ng·h/mL)/(mg/kg); units for D.N. C_{max} are (ng/mL)/(mg/kg)

(Excerpted from the Applicant's submission)

Toxicokinetic Parameters for PCI-45227 in Dogs Dosed with PCI-32765

Gender	Males			Females		
PCI-32765						
Dosage (mg/kg/day):	**30**	**80**	**220**	**30**	**80**	**220**
Parameter (Units)			Study Day 0			
AUC_{last} (ng·h/mL)	224	1117	781	221	1298	474
Metabolite/Parent Ratio	1.1	0.83	0.55	0.84	0.65	0.50
Dose Normalized AUC_{last}	7.47	14.0	3.55	7.37	16.2	2.16
C_{max} (ng/mL)	74.7	165	167	70.6	200	140
Dose Normalized C_{max}	2.49	2.06	0.760	2.35	2.5	0.636
T_{max} (h)	1.3	2.0	1.8	1.0	2.7	1.2
Dosage (mg/kg/day):	**30**	**80**	**220**	**30**	**80**	**220**
			Study Day 37			
AUC_{last} (ng·h/mL)	290	757	4545	294	3121	1856
Metabolite/Parent Ratio	1.0	0.64	0.41	0.51	0.53	0.41
Dose Normalized AUC_{last}	9.66	9.46	20.7	9.80	39.0	8.43
C_{max} (ng/mL)	51.3	112	409	92.6	374	253
Dose Normalized C_{max}	1.71	1.39	1.86	3.09	4.68	1.15
T_{max} (h)	8.7	1.2	3.0	3.7	1.7	3.6
Accumulation Ratio	1.3	0.89	11	1.3	8.0	3.7
Dosage (mg/kg/day):	**30**	**60**	**120**	**30**	**60**	**120**
			Study Day 78			
AUC_{last} (ng·h/mL)	122	768	1488	347	352	1131
Metabolite/Parent Ratio	0.35	0.20	0.14	0.21	0.19	0.22
Dose Normalized AUC_{last}	4.08	12.8	12.4	11.6	5.86	9.43
C_{max} (ng/mL)	33.7	122	164	67.9	102	127
Dose Normalized C_{max}	1.12	2.03	1.30	2.26	1.70	1.05
T_{max} (h)	1.3	3.4	3.8	2.7	1.5	3.5

Units for D.N. AUC_{last} are (ng·h/mL)/(mg/kg); units for D.N. C_{max} are (ng/mL)/(mg/kg)

(Excerpted from the Applicant's submission)

Accumulation of PCI-32765 and metabolite PCI-45227:

Dose-normalized AUC values of the parent drug and the metabolite on Days 0 (start of dosing), 37 and 78 are compared. Numbers in the parentheses are ratios (%) of exposures compared to the corresponding vales on Day 0 (100%).

Males:

	PCI-32765			PCI-45227		
Day Dose	0	37	78	0	37	78
30	8.77	10.3 (117)	12.6 (147)	7.47	9.66 (129)	4.08 (55)
80/60	19.8	13.2 (67)	56.9 (287)	14	9.46 (68)	12.8 (91)
220/120	5.88	80.3 (1366)	101 (1718)	3.55	20.7 (5914)	12.4 (349)

Females:

	PCI-32765			PCI-45227		
Day Dose	0	37	78	0	37	78
30	8.69	17.5 (201)	56.1 (646)	7.37	9.8 (133)	11.6 (157)
80/60	26.4	72.6 (275)	36.9 (140)	16.2	39 (241)	5.86 (36)
220/120	4.17	22.9 (549)	55.2 (1324)	2.16	8.43 (390)	9.43 (437)

Summary:

- Large intra- and/or inter-animal variations in exposure were observed. Therefore, the half-lives were not calculated.
- Mean exposure to PCI-32765 and its metabolite PCI-45227 was generally similar between the genders on all evaluation days.
- On Day 0, the exposure at 220 mg/kg was substantially less compared to lower doses, indicating reduced absorption of PCI-32765. This may be at least partially contributed to severe GI toxicities in the dogs at the high dose (diarrhea, GI epithelial lesions).
- Maximum time to peak plasma level (T_{max}) for PCI-45227 ranged from 0.8 and 4.0 hours; T_{max} for PCI-45227 was from 1.0 and 8.7 hours.
- Accumulation of both the parent and the metabolite was observed after repeat dosing. Accumulation was substantial (for both PCI-32765 and PCI-45227) on Day 78. Accumulation was more evident for of the parent drug.

Dosing Solution Analysis

Analytical results indicated that the dosing formulations were at 100% to 106% of the target concentrations. No drug was detected in the vehicle formulation of vehicle control groups.

7 Genetic Toxicology

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

The following study is reviewed by Dr. Chiu.

Study title: Bacterial Mutation Assay	
Study no.:	06-027-Sal-X-MU
Study report location:	eCTD Section 4.2.3.3.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 28, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765 (free base), Lot No. SCR-182-77 (b) (4), 98.3% purity

Key Study Findings

In the *in vitro* bacterial reverse mutation assay with the plate incorporation method, ibrutinib (PCI-32765), did not produce genotoxic responses with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2 *uvr*A, in the absence or presence of S9 activation.

Methods

Strains:	*Salmonella typhimurium* strains TA98, TA1535, TA1537, TA100, and *Escherichia coli* strain WP2 *uvr*A
Concentrations in definitive study:	Plate incorporation method: 50 – 5000 µg/plate in the presence or absence of Aroclor 1254-induced rat liver S9 mix
Basis of concentration selection:	Concentrations of the test article for the definitive study were selected based on results from the preliminary toxicity assay.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	2-Nitrofluorene (2NF), sodium azide (SA), 9-aminoacridine (9AAD), methyl methanesulfonate (MMS)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Tester strains, S9 or sham mix, and vehicle or PCI-32765 were added to agar plates which were incubated for approximately 48 to 72 hours at 37±2˚C.

Study Validity

Selection of bacterial tester strains was adequate based upon ICH S2 guidance. Positive controls produced expected responses. Concentration selection for the plate incorporation method was adequate based upon use of a high concentration of 5000 µg/plate which showed some toxicity without precipitation. The S9 concentration was 10% which it is within acceptable limits.

Criteria for a valid test was provided in the study report and all of the following criteria must have been met for the assay to be considered valid:

- All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene.
- Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor.
- All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene.
- All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvr*A, 10 - 60.

- To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/mL.
- The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.
- A minimum of three non-toxic concentration levels is required to evaluate assay data.
- A concentration level is considered toxic if one or both of the following criteria are met: (1) A > 50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt concentration-dependent drop in the revertant count. (2) At least a moderate reduction in the background lawn (background code 3, 4 or 5).

Results

Solubility Test
The test article was soluble in DMSO at concentrations up to 500 mg/mL, the maximum concentration tested in the solubility test.

Sterility Results
No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions, and the S9 and sham mixes.

Preliminary Toxicity Assay (Experiment B1)
- A preliminary toxicity assay was performed for concentration selection. The concentration levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 μg/plate. Precipitation was observed beginning at 1500 μg/plate. No background lawn toxicity was observed but a decrease in revertant counts was observed at 5000 μg/plate with the tester strain TA1537 in the absence of S9 activation.
- No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.
- Based of the findings of the preliminary toxicity assay, the maximum concentration tested in the definitive mutagenicity assay was 5000 μg/plate.

Definitive Assay (Experiment B2)
Treatment with 50, 150, 500, 1500, and 5000 μg PCI-32765 did not cause any positive mutagenic response in any of the tester strains in either the presence or absence of S9 activation.
- Precipitate was observed beginning at 500 or 1500 μg/plate.
- No background lawn toxicity was observed but decreases in revertant counts were observed as follows: in the presence of S9 activation beginning at 1500 mg/plate with tester strain TA98, beginning at 500 mg/plate with tester strain TA1537, and at 5000 mg/plate with tester strains TA98 and TA1537.

- Because only two non-toxic concentration levels were obtained with tester strain TA1537 in the presence of S9 activation, this portion of the assay was re-tested in Experiment B3 with an adjustment in concentration levels.

Definitive Assay (Experiment B3)

Treatment with 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate PCI-32765 did not cause any positive mutagenic response in tester strain TA1537 in the presence of S9 activation.

- Precipitate was observed beginning at 1500 µg/plate.
- No toxicity was observed.

Table 26- Summary of results from definitive mutagenicity assays (Experiments B2 and B3) with PCI-32765 (Study No. 06-027-Sal-X-MU).

Test Article Id. : PCI-32765 (free base)
Study Number : AC07TT.503.BTL
Experiment Nos. : B2 and B3

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Vehicle	16 ± 1	106 ± 8	12 ± 2	8 ± 3	15 ± 3
50	10 ± 2	119 ± 5	12 ± 5	7 ± 0	16 ± 4
150	14 ± 2	115 ± 8	16 ± 5	7 ± 1	17 ± 7
500	13 ± 2	114 ± 19	13 ± 2	7 ± 2	14 ± 5
1500	9 ± 5	110 ± 8	14 ± 3	5 ± 2	16 ± 4
5000	5 ± 2	98 ± 6	10 ± 5	3 ± 1	12 ± 1
Positive	218 ± 25	694 ± 38	670 ± 22	905 ± 110	233 ± 3

Activation Condition : Rat Liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvr*A	TA1537[a]
Vehicle	21 ± 8	131 ± 16	18 ± 1	11 ± 7	22 ± 7	6 ± 3
5.0						10 ± 2
15						7 ± 3
50	22 ± 1	139 ± 13	12 ± 5	8 ± 2	21 ± 5	6 ± 4
150	15 ± 1	138 ± 19	11 ± 5	8 ± 2	29 ± 4	10 ± 3
500	21 ± 5	118 ± 7	12 ± 2	4 ± 3	16 ± 6	11 ± 2
1500	10 ± 4	115 ± 7	12 ± 1	5 ± 3	13 ± 5	8 ± 3
5000	8 ± 2	114 ± 3	9 ± 1	4 ± 1	10 ± 2	5 ± 3
Positive	676 ± 73	948 ± 27	136 ± 27	91 ± 19	211 ± 38	196 ± 25

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 50 µL
a = Data from Experiment B3

[Excerpted from Applicant's submission]

Formulation Analysis

- The actual concentration of the formulation used in Experiments B1, B2, and B3 were found to be within 87 to 95% of target.

- No test article was detected in the vehicle control formulation (LOQ = 0.01 mg/mL)
- Stability results indicate that PCI-32765 (free base) in DMSO was stable for 5 weeks when stored at ambient and -10°C.

7.2 *In Vitro* Assays in Mammalian Cells

The following study is reviewed by Dr. Chiu.

Study title: *In vitro* mammalian chromosome aberration test	
Study no.:	07-038-CHO-X-MU
Study report location:	eCTD Section 4.2.3.3.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 28, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765 (free base), Lot # SCR-182-77* ((b) (4); Sample 1; 97% purity) and 082032* (Sample 2; 99.7% purity)

Reviewer's note:
The impurity profiles of these two lots were different:

Lot number	Purity	Impurity profile	Comment
SCR-182-77	(b) (4)		An old lot, has not been used in nonclinical or clinical studies
082032			A lot used in a Phase 1 clinical trial

As noted, the impurities in Lot# 082032 were assessed to be not mutagenic. The impurities (b) (4) were not identified in the lots subsequently manufactured.

Key Study Findings

- Ibrutinib (PCI-32765: Lot No. SCR-182-77) was positive for the induction of structural chromosome aberrations and negative for the induction of numerical chromosome aberrations in CHO cells in both non-activated and S9-activate test systems, under the conditions tested.
- Ibrutinib (PCI-32765: Lot No. 082032) was negative for the induction of structural and numerical chromosome aberrations in CHO cells in the S9-activated test system, under the conditions tested.
- Based on the totality of data, Ibrutinib is considered negative for induction of chromosome aberration.

Methods	
Cell line:	Chinese hamster ovary (CHO-K1) cells
Concentrations in definitive studies:	Non-activate test system: 5 – 40 µg/mL S9-activated test system: 10 - 80 µg/mL
Basis of concentration selection:	Solubility, stability, pH, osmolarity, and preliminary concentration range finding assay
Negative control:	DMSO
Positive control:	Mitomycin C (MMC) for non-activated test system Cyclophosphamide (CP) for S9-activated test system
Formulation/Vehicle:	DMSO for test article and sterile distilled water for positive controls
Incubation & sampling time:	Cells were incubated with or without metabolic activation in a cell culture incubator at 37°C, 5% CO_2 in air for 16-24 hours.

Study Validity

The study was valid based on the following criteria:

- The frequency of cells with structural chromosome aberrations in the solvent control was within the range of the historical solvent control provided in the study report.
- The appropriate positive controls were employed and produced expected results.
- The appropriate number of cells was evaluated and two replicates of each test concentrations were tested which is in accordance with the current practice.
- Metaphase cells with 46 centromeres were examined under oil immersion. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations. The counting method was in compliance with the currently accepted procedure and therefore considered valid.
- According to the protocol a test article was considered to induce a positive response when the percentage of cells with aberrations (minus gaps) was increased in a concentration-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group. A reproducibly statistically significant increase at the high concentration only or one other concentration only with no concentration-response was considered positive. The criteria for the evaluation of the positive results were considered valid.
- The conditions of the assays were appropriate given the use of the limit concentration for 4 hr incubations and toxicity measured in the 20 hr incubation (FDA/CFSAN Redbook guidelines). The concentration selection based upon mitotic index was acceptable.

Results

Preliminary concentration range finding toxicity assay
A preliminary toxicity assay was performed for concentration selection. The concentrations tested were 0.4405 to 4405 μg/mL PCI-32765 [Lot No. SCR-182-77 (b) (4)) in the absence or presence of an S9 reaction mix.

- Visible precipitate was observed in treatment medium at concentration levels ≥ 132.15 μg/mL and concentration levels ≤ 44.05 μg/mL were visibly soluble in treatment medium at the beginning and conclusion of the treatment period.
- The osmolarity of the test article concentrations in the treatment medium [398 mmol/kg for 132.15 μg/mL (the lowest precipitating concentration) and 399 mmol/kg for 44.95 μg/mL (the highest visibly soluble concentration), respectively] did not exceed the osmolarity of DMSO (403 mmol/kg) by more than 20% and therefore were considered to be acceptable.
- The pH of the highest concentration of test article in the treatment medium was approximately 7.0.
- Significant toxicity (i.e. at least 50% cell growth inhibition relative to the solvent control) was observed at concentration levels ≥ 13.215 mg/mL in the non-activated 4-hour exposure group and at concentration levels ≥ 44.05 mg/mL in the S9-activated 4-hour and the non-activated 20-hour exposure groups.

Definitive chromosome aberration assays (1 – 4)
Assay 1

- PCI-32765 [Lot No. SCR-182-77 (b) (4)] was soluble in DMSO and in the treatment medium at allconcentrations at the beginning and conclusion of the treatment medium.
- The pH of the highest concentration of test article in treatment medium was approximately 7.0.
- The Applicant stated that due to inconsistent cytotoxic data, the initial chromosome aberration assay (Assay 1) was repeated. The data generated from this assay were included in the study file but were not reported and therefore were not reviewed by FDA.

Assay 2 ("First repeat assay")

- The concentrationconcentrations tested in this repeat chromosome aberration assay (Assay 2) were as follows:

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	10, 20, 25, 30, 32.5, 35, 37.5, 40
	20 hr	0 hr	5, 10, 12.5, 15, 17.5, 20, 25, 30
S9-activated*	4 hr	16 hr	10, 20, 25, 30, 32.5, 35, 37.5, 40

* Activated group not evaluated for chromosome aberrations due to inconsistent cytotoxicity results

[Excerpted from Applicant's submission]

- PCI-32765 [Lot No. SCR-182-77 (b) (4)] was soluble in DMSO and in the treatment medium at all concentration levels at the beginning and conclusion of the treatment medium.
- The pH of the highest concentration of test article in treatment medium was approximately 7.0.
- For the 4-hour non-activated group, toxicity of PCI-32765 (cell growth inhibition relative to the solvent control) in CHO cells was 52% at 37.5 µg/mL, the highest test concentration evaluated for chromosome aberrations. The mitotic index at the highest concentration level evaluated for chromosome aberrations, 37.5 mg/mL, was reduced 12% relative to the solvent control. The test concentrations selected for analysis of numerical and structural aberrations were 25, 32.5, and 37.5 µg/mL. The percentage of cells with numerical aberrations in the test article-treated group was not significantly increased relative to solvent control at any concentration levels ($p > 0.05$, Fisher's Exact test). The percentage of cells with structural aberrations was significantly different ($p \leq 0.01$, Fisher's Exact test) than controls at 32.5 and 37.5 µg/mL (5.5% and 8.5%, respectively). The Cochran-Armitage test was positive for a concentration response ($p \leq 0.05$). The positive control MMC significantly increased the percentage of cells with structural aberrations (18%) which was considered to be both statistically and biologically significant.
- For the 20-hour non-activated group, toxicity of PCI-32765 (cell growth inhibition relative to the solvent control) in CHO cells was 51% at 12.5 µg/mL, the highest test concentration evaluated for chromosome aberrations. No changes in the mitotic index were noted. The test concentrations selected for analysis of numerical and structural aberrations were 5, 10, and 12.5 µg/mL. The percentage of cells with structural aberrations was significantly different ($p \leq 0.01$, Fisher's Exact test) than controls at 10 and 12.5 µg/mL (4.0% and 7.0% for, respectively). The Cochran-Armitage test was positive for a concentration response ($p \leq 0.05$). However, the changes at 10 µg/mL were within the historical (2005 – 2007) solvent control range (0.0 – 5.0%) and therefore not considered to be biologically significant. The percentage of cells with numerical aberrations in the test article-treated group was not significantly increased relative to solvent control at any concentration level ($p > 0.05$, Fisher's Exact test). The positive control MMC significantly increased the percentage of cells with structural aberrations (17%) which was considered to be both statistically and biologically significant.

- For the 4-hour S9-activated group, no treatment groups were selected for microscopic analysis, due to inconsistent cytotoxic results.

Table 27- Summary of results from *in vitro* mammalian chromosomal aberration assay (Assay 2) with PCI-32765 [Lot No. SCR-182-77 (b) (4)]. (Study No. 07-038-CHO-X-MU).

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-S9	4	12.9	200	200	0.010	±0.100	2.0	1.0
PCI-32765 [Lot No. SCR-182-77 (b) (4)									
25	-S9	4	13.1	200	200	0.080	±0.725	3.0	3.5
32.5	-S9	4	12.5	200	200	0.055	±0.229	2.5	5.5**
37.5	-S9	4	11.4	200	200	0.100	±0.347	3.5	8.5**
MMC, 0.2	-S9	4	8.5	200	100	0.260	±0.691	0.0	18.0**
DMSO	-S9	20	11.2	200	200	0.000	±0.000	1.5	0.0
PCI-32765[Lot No. SCR-182-77 (b) (4)									
5	-S9	20	11.2	200	200	0.015	±0.122	0.5	1.5
10	-S9	20	11.1	200	200	0.090	±0.738	2.0	4.0**
12.5	-S9	20	11.2	200	200	0.075	±0.282	1.0	7.0**
MMC, 0.1	-S9	20	9.6	200	100	0.270	±0.679	0.0	17.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's Exact test.

[Excerpted from Applicant's submission]

Assay 3 ("Second repeat assay")

- The chromosome aberration assay was repeated to evaluate the test article (10 – 80 µg/mL) following 4-hour exposure in the S9-activated test system. All cells were harvested 20 hours after treatment initiation.
- PCI-32765 [Lot No. SCR-182-77 (b) (4)] was soluble in DMSO and in the treatment medium at all concentrations at the beginning and conclusion of the treatment medium.
- The pH of the highest concentration of test article in treatment medium was approximately 7.0.
- For the 4-hour activated group, toxicity of PCI-32765 (cell growth inhibition relative to the solvent control) in CHO cells was 55% at 37.5 µg/mL, the highest test

concentration evaluated for chromosome aberrations. The mitotic index at the highest concentration level evaluated for chromosome aberrations, 37.5 mg/mL, was reduced 6% relative to the solvent control. The test concentrations selected for analysis of numerical and structural aberrations were 10 and 37.5 µg/mL. The percentage of cells with numerical aberrations in the test article-treated group was not significantly increased relative to solvent control at any concentration levels ($p > 0.05$, Fisher's Exact test). The percentage of cells with structural aberrations was significantly different ($p \leq 0.01$, Fisher's Exact test) than controls at 10 and 37.5 µg/mL (4.0% and 11.0%, respectively). The Cochran-Armitage test was positive for a concentration response ($p \leq 0.05$). However, the changes at 10 µg/mL were within the historical (2005 – 2007) solvent control range (0.0 – 5.0%) and therefore not considered to be biologically significant. The positive control CP significantly increased the percentage of cells with structural aberrations (19%) which was considered to be both statistically and biologically significant.

Table 28- Summary of results from *in vitro* mammalian chromosomal aberration assay (Assay 3) with PCI-32765 [Lot No. SCR-182-77 (b) (4)]. (Study No. 07-038-CHO-X-MU).

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	+S9	4	10.9	200	200	0.005	±0.071	2.0	0.5
PCI-32765[Lot No. SCR-182-77 (b) (4)									
10	+S9	4	10.8	200	200	0.060	±0.370	2.5	4.0*
37.5	+S9	4	10.2	200	200	0.125	±0.374	4.0	11.0**
CP,	+S9	4	4.0	200	100	0.270	±0.664	0.0	19.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's Exact test.

[Excerpted from Applicant's submission]

Assay 4 ("Third repeat assay")

- The chromosome aberration assay was repeated to evaluate the test article, PCI-32765 (Lot No. 082032) following 4-hour exposure in the S9-activated test system.
- PCI-32765 (Lot No. 082032) was soluble in DMSO and in the treatment medium at all concentration levels at the beginning and conclusion of the treatment medium.
- The pH of the highest concentration of test article in treatment medium was approximately 7.0.
- For the 4-hour activated group, toxicity of PCI-32765 (cell growth inhibition relative to the solvent control) in CHO cells was 58% at 40 µg/mL, the highest test

concentration evaluated for chromosome aberrations. The mitotic index at the highest concentration level evaluated for chromosome aberrations, 40 µg/mL, was reduced 50% relative to the solvent control. The test concentrations selected for analysis of numerical and structural aberrations were 10, 20, and 40 µg/mL. The percentage of cells with numerical aberrations in the test article-treated group was not significantly increased relative to solvent control at any concentration levels ($p > 0.05$, Fisher's Exact test). The percentage of cells with numerical and structural aberrations was significantly different ($p \leq 0.01$, Fisher's Exact test) than controls at any test concentrations. The positive control CP significantly increased the percentage of cells with structural aberrations (21%) which was considered to be both statistically and biologically significant.

Table 29- Summary of results from *in vitro* mammalian chromosomal aberration assay (Assay 4) with PCI-32765 (Lot No. 082032). (Study No. 07-038-CHO-X-MU).

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	+S9	4	11.2	200	200	0.005	±0.071	0.0	0.5
PCI-32765 (Lot No. 082032)									
10	+S9	4	10.7	200	200	0.000	±0.000	0.5	0.0
20	+S9	4	8.2	200	200	0.010	±0.141	0.5	0.5
40	+S9	4	5.6	200	200	0.010	±0.100	1.5	1.0
CP,	+S9	4	4.2	200	100	0.300	±0.718	0.0	21.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using Fisher's Exact test.

[Excerpted from Applicant's submission]

Formulation Analysis

- The actual concentrations of the test article from Assay 1 (4 mg/mL), Assay 2 (4 mg/mL), Assay 3 (8 mg/mL), and Assay 4 (8 mg/mL) were 105%, 97%, 99%, and 107% of target, respectively.
- No test article was detected in vehicle control samples.
- PCI-32765 was stable for 5 weeks when stored at room temperature and at ≤ -10°C.

Study title: *In vitro* mammalian chromosome aberration test	
Study no.:	08-071-CHO-X-MU ((b) (4) #AC16MK.331.BTL)
Study report location:	eCTD, Module 4, Section 4.2.3.3.1

Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 10, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765 (ibrutinib); Lot# 082032, 99.7%

Key Study Findings

Under the condition of the study, ibrutinib (PCI-32765) (Lot #082032) was not clastogenic, in the absence of S9 activation

Methods	
Cell line:	Chinese hamster Ovary (CHO) cells
Concentrations in definitive study:	5, 10, 20 and 37.5 μg/mL
Basis of concentration selection:	See below
Negative control:	Vehicle control (dimethyl sulfoxide,DMSO)
Positive control:	See below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	See below

Background:
In a previous study ((b) (4) #AC07TT.331.BTL), PCI-32765 (Lot# 082032, up to 37.5 μg/mL) was found to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in the S9-activated test system. In contrast to this finding, another lot of PCI-32765 [Lot No. SCR-182-77 (b) (4)] was positive for the induction of structural chromosome aberrations and negative for the induction of numerical chromosome aberrations in CHO cells in both non-activated and S9-activated test systems. According to the Applicant, PCI-32765 (Lot No. 082032) was a lot with higher purity than the other lot. In the current study, same concentrations of PCI-32765 (lot #082032) were used to investigate the clastogenic effects of PCI-32765 in the absence of S9 activation.

Concentration selection criteria
Based on reduced cell growth (cytotoxicity studies) (the concentration with at least 50% reduction in cell growth and two lower concentrations) and on the depression of mitotic index during the chromosomal aberration test. A total of two experiments were performed.
Mitotic index: the percentage of cells in mitosis per 500 cells

Test agent stability: Stable in DMSO up to 6 mg/mL. Precipitation of test article was not observed at concentrations ≥ 60 μg/mL.
The osmolality in treatment medium of the highest concentration tested, 60 μg/mL, was 412 mmol/kg. The osmolality of the solvent (DMSO) in treatment medium was 418 mmol/kg. The osmolality of the test article concentration in treatment medium is acceptable because it did not exceed the osmolality of the solvent by more than 20%.

Test system:
CHO cells at 5 x 10^5 cells/25 cm^2 flask

Controls:
Vehicle: DMSO
Negative controls: vehicle (solvent) control.
Positive controls:
Mitomycin (MMC, 0.2 or 0.1 μg/mL), in the experiment of 4 hr or 20 hr exposure, respectively.

Table 30- Historical control data: chromosome aberration in CHO cells

Historical control data (2005-2007):
Structural aberration
Without S9 activation:

Historical Values	Solvent (%)	Positive Control[2] (%)
Mean	0.5	20.1
±SD[1]	0.7	6.0
Range	0.0-5.0	10.0-52.0

With S9 activation:

Historical Values	Solvent (%)	Positive Control[3] (%)
Mean	0.7	22.6
±SD[1]	0.9	9.9
Range	0.0-5.0	10.0-84.3

Combined numerical aberrations (polyploidy and endoreduplicated cells)
Without S9 activation:

Historical Values	Solvent (%)	Positive Control[2] (%)
Mean	1.7	1.6
±SD[1]	1.2	1.8
Range	0.0-6.5	0.0-24.0

With S9 activation:

Historical Values	Solvent (%)	Positive Control[3] (%)
Mean	2.6	2.0
±SD[1]	2.0	1.6
Range	0.0-12.5	0.0-6.5

1 SD = standard deviation.
2 Positive control for non-activated studies, Mitomycin C (MMC, 0.1-0.2 µg/mL).
3 Positive control for S9-activated studies, cyclophosphamide (CP, 10-20 µg/mL).

Exposure conditions:

Incubation and sampling times:

- Preliminary toxicity assay: in the absence of S9: Pulse treatment 4 hr and continued for 20 hr.
- Chromosome aberration assay: see table below

Concentrations used in the Experiments:

- Preliminary cytotoxicity assay: 2.5, 5, 10, 20, 30, 35, 37.5, 40, 50 and 60 µg/mL (in the 4 hr exposure group) and 0.5, 1, 2.5, 5, 10, 12.5, 15, 20, 25 and 30 µg/mL (in the 20 hr exposure group)
- Chromosome aberration assay: based on the preliminary toxicity assay (i.e., toxicity response, see below) the condition and concentrations in this assay were shown in the following table:

Treatment condition	Treatment time	Recovery time	Concentrations (µg/mL)
Without S9	4 hr	16 hr	10, 20, 37.5
	20 hr	0 hr	5, 10, 20

  - The highest concentration level selected was the concentration that induced at least 50% toxicity, as measured by mitotic inhibition, relative to the solvent control, with a sufficient number of scorable metaphase cells.
  - Two additional lower concentration levels were included in the treatment.
  - Duplicate samples for each concentration of test article and for the controls.

Evaluation of metaphase cells:

A minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations.

- Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements.
- Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings.
- Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange.
- Pulverized chromosome(s), pulverized cells and severely damaged cells (≥10 aberrations) also were recorded.
- Chromatid gaps and ispchromatid gaps were recorded but not included in the analysis.

- The mitotic index was recorded as the percentage of cells in mitosis per 500 cells counted.
- The percent polyploid and endoreduplicated cells was evaluated per 100 cells.

Evaluation of test result:

- The following parameters were calculated and reported: the number and types of aberration per cells, the percentage of structurally and numerically damaged cells (i.e., percent aberrant cells), and the frequency of structural aberrations per cell (i.e., mean aberrations per cell) in the total population of cells.
- Chromatid and isochromatid gaps were presented in the data but were not included in the total percentage of cells with ≥ one aberration or in the frequency of structural aberrations per cell.
- Statistical analysis of percent aberrant cells: Fisher's exact test, to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. Cochran-Armitage test was used to measure concentration-responsiveness for a positive result.
- The test article was considered to induce a positive response, when
  - the percentage of cells with aberration were increased in a concentration-responsive manner with ≥ one concentration being statistically elevated;
  - a reproducible significant increase at the high concentration only with no concentration response;
  - a reproducible significant increase at one concentration other than the high concentration with no concentration response.
- The test article was considered to be negative, when
  - Values that were statistically significant but did not exceed the range of historical solvent controls may be judged as not biologically significant.
  - Test articles not demonstrating a statistically significant increase in aberrations.

Study Validity

The study is considered valid, because:

- The percentage of cells with chromosome aberrations in the positive control had to be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control in each assay (structurally damaged cells: 20%)
- The vehicle control data were within the laboratory historical range.

Results

- Preliminary toxicity assay: in two exposure experiments.

Table 31- Preliminary toxicity assay

The table below is the summary (mean of the duplicate) of the preliminary cytotoxicity assay:

Experiment		Experiment 1		Experiment 3	
Condition*		4 hr treatment		20 hr treatment	
		Growth inhibition (%)	Mitotic index (%)	Growth inhibition (%)	Mitotic index (%)
DMSO	50 µL				4.7
PCI-32765	0.5 µg/mL			8	N/A
	1			23	N/A
	2.5	-7	N/A	23	N/A
	5	8	N/A	24	4.2
	10	7	3.8	30	3.8
	12.5			34	N/A
	15			40	N/A
	20	7	4.6	58	3.5
	25			76	N/A
	30	22	N/A	80	N/A
	35	36	N/A		
	37.5	50	4.4		
	40	53	N/A		
	50	122	N/A		
	60	161	N/A		
MMC	0.1	23	N/A	48	3.8
MMC	0.2	27	4.2	40	N/A

***Treatment: CHO cells were treated in the absence or presence of S9 for 4 or 20 hours at 37 ±1°C. Metaphase cells were collected 20 hr after initiating treatment.**
Mitotic Index= (cells in mitosis/500 cells scored) x 100
Percent change = (treatment mitotic index-control mitotic index)/control mitotic index, expressed as a percentage

Toxicity (mitotic inhibition) in excess of 50% (i.e., over 50% depression from the control) was observed at PCI-32765 concentrations of ≥ 37.5 µg/mL and 20 µg/mL in 4 hr and 20 hr exposures, respectively.

- Chromosome aberration assay: in two experiments.

The group means of the mitotic index determination and the cytogenetic analysis of two treatment conditions are summarized in the table below (from the Applicant; data of individual experiment are not shown). At the highest concentration evaluated microscopically for chromosome aberrations, 37.5 µg/mL (4 hr exposure) and 20 µg/mL (20 exposure), mitotic inhibition was 2% and 26%, respectively.

Table 32- Group means of mitotic index and cytogenetic analysis

4 hour exposure

4-HOUR TREATMENT, 16-HOUR RECOVERY PERIOD

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell
							Gaps	Chromatid		Chromosome				
			Numerical	Structural	Numerical	Structural		Br	Ex	Br	Dic	Ring		
DMSO	A	5.0	100	100	1	0	1	0	0	0	0	0	0	0.000
	B	4.0	100	100	0	0	0	0	0	0	0	0	0	0.000
PCI-32765-00														
10	A	3.8	100	100	4	1	0	0	0	0	1	0	0	0.010
	B	3.8	100	100	4	1	0	1	0	0	0	0	0	0.010
20	A	4.8	100	100	4	4	1	3	1	0	1	0	0	0.050
	B	4.4	100	100	4	3	0	2	2	0	0	0	0	0.040
37.5	A	4.2	100	100	4	3	3	2	2	0	1	0	0	0.050
	B	4.6	100	100	3	4	1	1	2	0	1	0	0	0.040
MMC,	A	4.0	100	25	1	20	1	4	1	0	1	0	0	0.240
0.2	B	4.4	100	25	1	20	1	1	3	0	1	1	0	0.240

20 hour exposure:

20-HOUR CONTINUOUS TREATMENT

Treatment (µg/mL)	Flask	Mitotic Index (%)	Cells Scored		% Aberrant Cells		Total Number of Structural Aberrations						Severely Damaged Cells	Average Aberrations Per Cell
							Gaps	Chromatid		Chromosome				
			Numerical	Structural	Numerical	Structural		Br	Ex	Br	Dic	Ring		
DMSO	A	4.8	100	100	2	0	0	0	0	0	0	0	0	0.000
	B	4.6	100	100	2	0	1	0	0	0	0	0	0	0.000
PCI-32765-00														
5	A	4.2	100	100	3	2	2	2	1	0	0	0	0	0.030
	B	4.2	100	100	4	3	1	2	0	0	1	0	0	0.030
10	A	3.6	100	100	3	4	1	2	1	0	1	0	0	0.040
	B	4.0	100	100	3	3	2	1	0	0	2	0	0	0.030
20	A	3.6	100	100	3	3	3	2	1	0	0	0	0	0.030
	B	3.4	100	100	4	4	1	3	0	0	2	0	0	0.050
MMC,	A	4.2	100	25	2	24	2	3	2	1	0	0	0	0.240
0.1	B	3.4	100	25	2	24	0	2	4	0	0	0	0	0.240

- **Treatment: CHO cells were treated for 20 hours at 37±1°C in the absence of an exogenous source of metabolic activation. Additional concentration levels of 0.5, 1, 2.5, 12,5 and 15 µg/mL were tested as a safeguard against excessive toxicity at higher concentration levels but were not required for microscopic examination. Concentration levels 25 and 30 µg/mL were not analyzed due to excessive toxicity.**
- **Mitotic index = number mitotic figures x 100/500 cells counted.**
- **% Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.**
- **Chromatid breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.**
- **Chromosome breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.**
- **Severely damaged cells: include cells with one or more pulverized chromosome and cells with 10 or more aberrations.**
- **Average aberrations per cell: severely damaged cells and pulverizations were counted as 10 aberrations.**

Summary of the two exposure conditions:

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-S9	4	4.5	200	200	0.000	±0.000	0.5	0.0
PCI-32765-00									
10	-S9	4	3.8	200	200	0.010	±0.100	4.0*	1.0
20	-S9	4	4.6	200	200	0.045	±0.252	4.0*	3.5**
37.5	-S9	4	4.4	200	200	0.045	±0.252	3.5*	3.5**
MMC, 0.2	-S9	4	4.2	200	50	0.240	±0.517	1.0	20.0**
DMSO	-S9	20	4.7	200	200	0.000	±0.000	2.0	0.0
PCI-32765-00									
5.0	-S9	20	4.2	200	200	0.030	±0.198	3.5	2.5*
10	-S9	20	3.8	200	200	0.035	±0.184	3.0	3.5**
20	-S9	20	3.5	200	200	0.040	±0.221	3.5	3.5**
MMC, 0.1	-S9	20	3.8	200	50	0.240	±0.431	2.0	24.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤ 0.05; **, p≤ 0.01; using Fisher's Exact test.

In summary:

<u>4-hour exposure:</u>

- The percentage of cells with structural aberrations was statistically increased relative to control at concentration levels 20 and 37.5 µg/mL (Fisher's Exact test) and the change was concentration-related (the Cochran-Armitage test). However, the percentage of cells with structural aberrations at concentration levels 20 and 37.5 µg/mL (3.5%) was within the historical solvent control range of 0.0% to 5.0%.
- The percentage of cells with numerical aberrations in the test article-treated group was statistically increased relative to solvent control at concentration levels 10, 20 and 37.5 µg/mL (Fisher's Exact test), but was negative in the Cochran-Armitage test for a concentration response. Also, the percentage of cells with numerical aberrations at concentration levels 10, 20 and 37.5 µg/mL (4.0%, 4.0% and 3.5%, respectively) was within the historical solvent control range of 0.0% to 6.5%.
- The percentage of structurally damaged cells in the MMC (positive control) treatment group (20.0%) was statistically significant.

<u>20-hour exposure:</u>

- The percentage of cells with structural aberrations was statistically increased relative to solvent control at concentration levels 5, 10 and 20 µg/mL (Fisher's Exact test). The Cochran-Armitage test was negative for a concentration response. However, the percentage of cells with structural aberrations at concentration levels 5, 10 and

20 µg/mL (2.5%, 3.5% and 3.5%, respectively) was within the historical solvent control range of 0.0% to 5.0%.

- The percentage of cells with numerical aberrations in the test article-treated group was not significantly increased relative to solvent control at any concentration level.
- The percentage of structurally damaged cells in the MMC (positive control) treatment group (24.0%) was statistically significant.

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

Study title: Mouse bone marrow erythrocyte micronucleus test following oral administration of PCI-32765	
Study no:	07-037-M-PO-MU [redacted] (b) (4) Study No. AC07TT.123M.BTL)
Study report location:	eCTD Section 4.2.3.3.2.
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	May 2, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765; Lot # SCR-182-77 [redacted] (b) (4); 97.3% purity

Key Study Findings

Under the conditions tested, a single oral administration of 500, 1000, or 2000 mg/kg PCI-32765 did not induce significant increases in the incidence of micronucleated polychromatic erythrocytes in the bone marrow of male ICR mice.

Methods

Concentrations in definitive study:	500, 1000, 2000 mg/kg
Frequency of dosing:	Single concentration
Route of administration:	Oral, gavage
Concentration volume:	20 mL/kg
Formulation/Vehicle:	0.5% methylcellulose, 0.4% Cremophor EL, and 0.1% sodium lauryl sulfate
Species/Strain:	Mouse/ICR [redacted] (b) (4)); 6 – 8 weeks old
Number/Sex/Group:	5 or 10 male mice/group
Satellite groups:	3 or 6 male mice/group for toxicokinetic evaluation
Basis of concentration selection:	Results from a previously conducted concentration-range finding study showed mild to moderate inactivity, ptosis, and/or decreased body temperature and labored respiration in male and female 2000 mg/kg group mice.
Negative control:	0.5% methylcellulose, 0.4% Cremophor EL, and 0.1% sodium lauryl sulfate
Positive control:	Cyclophosphamide monohydrate (CP); 50 mg/kg

Table 33- Summary of study design in Study No. 07-037-M-PO-MU.

Treatment (20 mL/kg)	Number of Male Mice Dosed	Number of Male Mice Used for Bone Marrow Collection at	
		24 hrs post-dose	48 hrs post-dose
Vehicle Control: 0.5% methylcellulose, 0.4% Cremophor EL, and 0.1% sodium lauryl sulfate	10 + 3*	5	5
Test Article: PCI-32765			
Low dose (500 mg/kg)	5 + 6**	5	-
Mid dose (1000 mg/kg)	5 + 6**	5	-
High dose (2000 mg/kg)	10 + 6*	5	5
Positive Control: CP (50 mg/kg)	5	5	-

*Satellite mice for 1-hour post-dose toxicokinetic assessment
**Satellite mice for 1- and 4-hour post-dose toxicokinetic assessments.

[Excerpted from Applicant's submission]

Reviewer's comments:
The concentration-range finding study in mice (Study No. 08-014-M-PO-ATI) used for concentration selection of this study was conducted previously by Pharmacyclics and not [redacted] (b) (4)*. However, the same drug lot, species, sex, and treatment regimen*

were used in the concentration-range finding and this in vivo micronucleus assay. The concentration-range finding also showed no apparent differences in toxicity between male and female mice. Therefore, the use of male mice only in this study is acceptable.

Study Validity

The study was deemed valid for the following reasons:

- Toxicokinetic evaluation demonstrated systemic exposure to the test article.
- Dosing appeared appropriate based on the results of the concentration-range finding study in mice and the recommended top concentration of 2000 mg/kg.
- The species and number of animals/sex/group were acceptable.
- Tissue sampling and analysis were acceptable.
- Positive controls exhibited appropriate responses.
- The proportion of immature erythrocytes among total erythrocytes was not less than 20% of the control value.

Criteria for a valid test were provided in the study report and all of the following criteria were also met:

- The incidence of micronucleated polychromatic erythrocytes (MPCEs) in the vehicle control group must not exceed the historical control range.
- The incidence of MPCEs in the positive control group must be significantly increased relative to the vehicle control group ($p \leq 0.05$, Kastenbaum-Bowman Tables).

Results

Micronucleus Study

- No mortality and clinical signs were noted.
- Non-concentration related reductions in PCEs/ECs ratio relative to the vehicle control groups of up to 10% were observed in the test article-treated groups at 24 hours after concentration administration. A reduction of 20 % in the PCEs/ECs ratio was observed in the test article-treated group at 48 hours after concentration administration.
- No statistically significant increase in the incidence of MPCEs in test article-treated groups, when compared to the vehicle control groups, was observed at 24 or 48 hours after concentration administration ($p \leq 0.05$, Kastenbaum-Bowman Tables).
- The positive control, cyclophosphamide, induced a statistically significant increase in the incidence of MPCEs ($p \leq 0.05$, Kastenbaum-Bowman Tables).
- The number of MPCEs in the vehicle control groups did not exceed the historical vehicle control range (2005 – 2007) provided in this submission.

Table 34- Summary of bone marrow micronucleus analysis following a single oral concentration of PCI-32765 in ICR mice in Study No. 07-037-M-PO-MU.

Treatment (20 mL/kg)	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MPCE/1000 PCE (Mean +/- SD)	Number of MPCE/PCE Scored
Vehicle	M	24	5	0.485 ± 0.05	---	0.2 ± 0.27	2 / 10000
PCI-32765							
500 mg/kg	M	24	5	0.477 ± 0.03	-2	0.1 ± 0.22	1 / 10000
1000 mg/kg	M	24	5	0.438 ± 0.05	-10	0.1 ± 0.22	1 / 10000
2000 mg/kg	M	24	5	0.451 ± 0.09	-7	0.0 ± 0.00	0 / 10000
Cyclophosphamide							
50 mg/kg	M	24	5	0.362 ± 0.05	-25	18.1 ± 4.52	*181 / 10000
Vehicle	M	48	5	0.550 ± 0.03	---	0.3 ± 0.45	3 / 10000
PCI-32765							
2000 mg/kg	M	48	4	0.440 ± 0.17	-20	0.3 ± 0.45	3 / 8000

*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)
Vehicle = 0.5% methylcellulose, 0.4% cremophor EL, and 0.1% sodium lauryl sulfate

[Excerpted from Applicant's submission]

Toxicokinetics

Toxicokinetic analysis indicated significant systemic exposure to PCI-32765 at all concentration levels at 1 and 4 hours post-dosing.

- Maximum plasma concentrations were observed at 4 hours post-concentration when compared to 1 hours post-concentration.
- Increases in plasma concentrations were generally concentration proportional.

Table 35- Summary of toxicokinetic data in Study No. 07-037-M-PO-MU.

Dosage (mg/kg)	C_{1h} (µg/mL)	C_{4h} (µg/mL)	C_{4h}/Dose (g/mL)
0 (vehicle)	BQL[1]	NA[2]	–
500	2.21 ± 0.39[3]	2.30 ± 0.95	0.0046 ± 0.0019
1000	2.12 ± 0.68	4.90 ± 1.16	0.0049 ± 0.0012
2000	3.74 ± 0.60	7.98 ± 1.68	0.0040 ± 0.0008

[1]BQL =Below Quantification Limit, LLOQ = 0.0003 µg/mL
[2]Samples not collected
[3]Mean ± SD, n=3

[Excerpted from Applicant's submission]

Dosing Formulation Analysis

- The actual concentrations of the dosing formulations were within 82 to 98% of the target concentrations.
- The dosing formulations were considered to be uniform based on the relative standard deviation (%RSD) values of ≤ 3% determined for samples taken at the top, middle, and bottom strata of each bulk suspension formulation.
- PCI-32765 concentrations in the post-concentration formulations were 95 to 109% of the pre-concentration concentrations, indicating that PCI-32765 stored at 2 – 8 °C were stable during the period of use.
- No test article was detected in vehicle samples (LOQ = 0.002 mg/mL).

7.4 Other Genetic Toxicity Studies

Genotoxicity studies of the impurity:
Background and summary of genotoxicity assessment of impurities in the drug substance of PCI-32765 (ibrutinib)

The Applicant stated that a total of 17 impurities of PCI-32765 (API) were established by spectrometry and/or NMR analysis. Impurities above the threshold of ICH Q3A/B were assessed for mutagenicity by Ames test (b) (4) or two SAR computational approaches (DEREK-Nexus and MultiCase analyses). Impurities tested were not mutagenic; see more information under Section 2.3.3 of this review.

Following is the review of the Ames studies for impurities (b) (4)

Studies #11-113-Sal-X-MU and 11-098-Sal-X-MU are reviewed by Dr. Chiu.

Study title: Bacterial Mutation Assay	
Study no.:	11-113-Sal-X-MU ((b) (4) Study No. AD36YT.503.BTL)
Study report location:	eCTD Section 4.2.3.3.1.
Conducting laboratory and location:	(b) (4) rive Rockville, MD 20850
Date of study initiation:	October 29, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Lot No. 745-59-4, (b) (4) wt/wt purity

Key Study Findings

In the *in vitro* bacterial reverse mutation assay with the plate incorporation method, impurity (b) (4), did not produce genotoxic responses with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2 *uvr*A, in the absence or presence of S9 activation.

Methods

Strains:	*Salmonella typhimurium* strains TA98, TA1535, TA1537, TA100, and *Escherichia coli* strain WP2 *uvr*A
Concentrations in definitive study:	Plate incorporation method: 50 – 5000 µg/plate in the presence or absence of Aroclor 1254-induced rat liver S9 mix
Basis of concentration selection:	Concentrations of the test article for the definitive study were selected based on results from the preliminary toxicity assay.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	2-Nitrofluorene (2NF), sodium azide (SA), 9-aminoacridine (9AAD), methyl methanesulfonate (MMS)
Formulation/Vehicle:	DMSO for (b) (4) and all positive control except (b) (4), which was diluted in water.
Incubation & sampling time:	Tester strains, S9 or sham mix, and vehicle or (b) (4) were added to molten selective top agar. After vortexing, the mixture was overlaid onto the surface of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for 48 to 72 hours at 37±2°C.

Study Validity

Selection of bacterial tester strains was adequate based upon ICH S2 guidance. Positive controls produced expected responses. Concentration selection for the plate incorporation method was adequate based upon use of a high concentration of 5000 µg/plate which showed no toxicity. The S9 concentration was 10% which it is within acceptable limits.

Additionally, criteria for a valid test was provided in the study report and all of the following criteria must have been met for the assay to be considered valid:

- All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene.

- Cultures of tester strains TA98 and TA100 must demonstrate the presence of the (b) (4).
- All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene.
- All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvr*A, 10 - 60.
- To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to 0.3×10^9 cells/mL.
- The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.
- A minimum of three non-toxic concentration levels is required to evaluate assay data.
- A concentration level is considered toxic if one or both of the following criteria are met: (1) A > 50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt concentration-dependent drop in the revertant count. (2) At least a moderate reduction in the background lawn (background code 3, 4 or 5).

Results

Solubility Test

Based on data from the Applicant, (b) (4) is soluble in DMSO at a concentration of up to 500 mg/mL.

Sterility Results

No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions, and the S9 and sham mixes. One contaminant colony was observed on the post-dosing sterility plate for the sham mix in the preliminary toxicity assay. However, since no contamination was observed on the assay plates, this observation was considered to have had no adverse impact on the integrity of the data or the validity of the study conclusion.

Preliminary Toxicity Assay

A preliminary toxicity assay was performed for concentration selection for the definitive study. The concentration levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate.

- Precipitation was observed beginning at 500 µg/plate.
- No toxicity was observed.

Based on the findings of the preliminary toxicity assay, the maximum concentration tested in the definitive mutagenicity assay was 5000 µg/plate.

Definitive Assay

Treatment with 50, 150, 500, 1500, or 5000 µg/plate did not cause any positive mutagenic response in any of the tester strains in either the presence or absence of S9 activation.

- Precipitate was observed beginning at 500 or 1500 µg/plate.
- No background lawn toxicity was observed. However, reductions in revertant counts were observed beginning at 1500 or 5000 µg/plate with a few conditions.

Table 36- Summary of results from Ames Assay with (b) (4) in the absence of S9 activation (Study No. 11-113-Sal-X-MU).

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
						(b) (4)
TA98	(b) (4)	5000 µg	*6*	*1*	*0.4*	
		1500 µg	*15*	*3*	*1.0*	
		500 µg	*16*	*6*	*1.1*	
		150 µg	*16*	*4*	*1.1*	
		50 µg	*22*	*6*	*1.5*	
	DMSO	100 µL	*15*	*3*		
TA100	(b) (4)	5000 µg	*80*	*10*	*0.8*	
		1500 µg	*91*	*22*	*0.9*	
		500 µg	*88*	*11*	*0.9*	
		150 µg	*87*	*11*	*0.9*	
		50 µg	*110*	*5*	*1.1*	
	DMSO	100 µL	*102*	*10*		
TA1535	(b) (4)	5000 µg	*10*	*3*	*0.8*	
		1500 µg	*9*	*3*	*0.8*	
		500 µg	*8*	*1*	*0.7*	
		150 µg	*8*	*2*	*0.7*	
		50 µg	*11*	*5*	*0.9*	
	DMSO	100 µL	*12*	*3*		

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	(b) (4)	5000 µg	*4*	*1*	*1.0*	(b) (4)
		1500 µg	*8*	*4*	*2.0*	
		500 µg	*8*	*2*	*2.0*	
		150 µg	*7*	*1*	*1.8*	
		50 µg	*4*	*2*	*1.0*	
	DMSO	100 µL	*4*	*1*		
WP2*uvr*A	(b) (4)	5000 µg	*8*	*3*	*0.3*	
		1500 µg	*12*	*4*	*0.4*	
		500 µg	*15*	*6*	*0.5*	
		150 µg	*20*	*6*	*0.7*	
		50 µg	*26*	*4*	*0.9*	
	DMSO	100 µL	*28*	*8*		
TA98	**2NF**	1.0 µg	*131*	*31*	*8.7*	
TA100	**SA**	1.0 µg	*739*	*58*	*7.2*	
TA1535	**SA**	1.0 µg	*510*	*13*	*42.5*	
TA1537	**9AAD**	75 µg	*177*	*89*	*44.3*	
WP2*uvr*A	**MMS**	1000 µg	*261*	*183*	*9.3*	

Key to Positive Controls

2NF	2-nitrofluorene
SA	sodium azide
9AAD	9-Aminoacridine
MMS	methyl methanesulfonate

Key to Plate Postfix Codes

(b) (4)

Key to Automatic & Manual Count Flags

[M]: Manual count [A]: Automatic count

[Excerpted from Applicant's submission]

Table 37- Summary of results from Ames Assay with (b) (4) in the presence of S9 activation (Study No. 11-113-Sal-X-MU).

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	(b) (4)	5000 µg	*18*	*8*	*1.1*	(b) (4)
		1500 µg	*22*	*2*	*1.3*	
		500 µg	*12*	*4*	*0.7*	
		150 µg	*21*	*5*	*1.2*	
		50 µg	*19*	*6*	*1.1*	
	DMSO	100 µL	*17*	*2*		
TA100	(b) (4)	5000 µg	*88*	*8*	*0.8*	
		1500 µg	*117*	*14*	*1.0*	
		500 µg	*113*	*7*	*1.0*	
		150 µg	*135*	*2*	*1.2*	
		50 µg	*112*	*7*	*1.0*	
	DMSO	100 µL	*117*	*17*		
TA1535	(b) (4)	5000 µg	*12*	*1*	*1.3*	
		1500 µg	*11*	*5*	*1.2*	
		500 µg	*13*	*9*	*1.4*	
		150 µg	*8*	*4*	*0.9*	
		50 µg	*12*	*3*	*1.3*	
	DMSO	100 µL	*9*	*3*		
TA1537	(b) (4)	5000 µg	*3*	*1*	*0.8*	
		1500 µg	*4*	*2*	*1.0*	
		500 µg	*4*	*2*	*1.0*	
		150 µg	*4*	*2*	*1.0*	
		50 µg	*6*	*2*	*1.5*	
	DMSO	100 µL	*4*	*1*		

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
WP2*uvr*A	(b) (4)	5000 µg	*10*	*2*	*0.4*	(b) (4)
		1500 µg	*14*	*3*	*0.5*	
		500 µg	*15*	*4*	*0.6*	
		150 µg	*29*	*7*	*1.1*	
		50 µg	*24*	*6*	*0.9*	
	DMSO	100 µL	*26*	*4*		
TA98	**2AA**	1.0 µg	*365*	*22*	*21.5*	
TA100	**2AA**	2.0 µg	*752*	*33*	*6.4*	
TA1535	**2AA**	1.0 µg	*70*	*10*	*7.8*	
TA1537	**2AA**	1.0 µg	*45*	*9*	*11.3*	
WP2*uvr*A	**2AA**	15 µg	*77*	*14*	*3.0*	

Key to Positive Controls

2AA 2-aminoanthracene

Key to Plate Postfix Codes

(b) (4)

Key to Automatic & Manual Count Flags

[M]: Manual count [A]: Automatic count

[Excerpted from Applicant's submission.]

Formulation Analysis

- The actual concentration of the target 0.015 and 50 mg/mL (b) (4) dosing formulations were (b) (4) and (b) (4) of the target concentrations, respectively, with (b) (4) relative standard deviation (RSD) and therefore met the acceptance criteria of (b) (4) of target concentration and (b) (4) RSD.
- Test article was detected in the vehicle control samples at concentrations (0.0032 mg/mL) slightly higher than the limit of quantitation (LOQ) concentration of 0.0025 mg/mL. However, the analytical test site determined that the contamination occurred at the analytical site and not at (b) (4) and, therefore, his observation was considered to have had no adverse impact on the integrity of the data or the validity of the study conclusion.

Study title: Bacterial Mutation Assay	
Study no.:	11-098-Sal-X-MU ((b) (4) Study No. AD36YU.503.BTL)
Study report location:	eCTD Section 4.2.3.3.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 2, 2011

GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Lot Nos. 758-47-2 (b) (4) wt/wt purity) and (b) (4) (b) (4) wt/wt purity)

Key Study Findings

In the *in vitro* bacterial reverse mutation assay with the plate incorporation method, the impurity (b) (4) did not produce genotoxic responses with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2 *uvr*A, in the absence or presence of S9 activation.

Methods	
Strains:	*Salmonella typhimurium* strains TA98, TA1535, TA1537, TA100, and *Escherichia coli* strain WP2 *uvr*A
Concentrations in definitive study:	Plate incorporation method: 50 – 5000 µg/plate in the presence or absence of Aroclor 1254-induced rat liver S9 mix
Basis of concentration selection:	5000 mg/plate was selected as the maximum concentration for all tester strains in the confirmatory mutagenicity assay based on results from the preliminary toxicity assay.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	2-aminoanthracene, 2-Nitrofluorene (2NF), sodium azide (SA), 9-aminoacridine (9AAD), methyl methanesulfonate (MMS)
Formulation/Vehicle:	DMSO for (b) (4) and all positive controls except (b) (4), which was diluted in water
Incubation & sampling time:	Tester strains, S9 or sham mix, and vehicle or (b) (4) were added to molten selective top agar. After vortexing, the mixture was overlaid onto the surface of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for 48 to 72 hours at 37°C.

Study Validity

Selection of bacterial tester strains was adequate based upon ICH S2. Positive controls produced expected responses. Concentration selection for the plate incorporation method was adequate based upon use of a high concentration of 5000 µg/plate which

showed some toxicity without precipitation. The S9 concentration was 10% which it is within acceptable limits.

Criteria for a valid test was provided in the study report and all of the following criteria must have been met for the assay to be considered valid:

- All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene.
- Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor.
- All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene.
- All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvr*A, 10 - 60.
- To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to 0.3×10^9 cells/mL.
- The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.
- A minimum of three non-toxic concentration levels is required to evaluate assay data.
- A concentration level is considered toxic if one or both of the following criteria are met: (1) A > 50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt concentration-dependent drop in the revertant count. (2) At least a moderate reduction in the background lawn (background code 3, 4 or 5).

Results

Solubility Test

Based on data from the Applicant (b) (4) is soluble in DMSO at a concentration of up to 500 mg/mL.

Sterility Results

No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions, the S9 and sham mixes, except for one contaminant colony on the pre-dosing sterility plate for S9 mix in the retest of the preliminary toxicity assay. However, no contamination was noted on the assay plates.

Preliminary Toxicity Assay

A preliminary toxicity assay was performed for concentration selection. The concentration levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg/plate (b) (4) (Lot No. 758-47-2).

- No mutagenic responses were observed with any of the *Salmonella* tester strains in either the absence or presence of S9 activation.
- Precipitation was observed beginning at 1500 µg/plate.

- No toxicity was observed.
- Due to unacceptable vehicle control values, (b) (4) (Lot 758-47-2), a retest of the preliminary toxicity assay was performed with *Escherichia coli* tester strain WP2 *uvr*A. In the retest, no mutagenic responses were observed in either the presence or absence of S9 activation, no toxicity was observed at a concentration of up to 5000 μg/plate, and precipitation was observed beginning at 1500 μg/plate.

Based on the findings of the preliminary toxicity assay, the maximum concentration tested in the definitive mutagenicity assay was 5000 μg/plate for all tester strains.

Definitive Assay (Experiments B3, B4, B5, and B6)
Experiment B3

- Treatment with 50, 150, 500, 1500, or 5000 μ/plate (b) (4) resulted in a concentration-related positive mutagenic response with tester strain TA1535 in the presence of S9 activation at concentrations of 500 to 5000 μg/plate (3.5-fold increase at 5000 μg/plate).
- No positive mutagenic responses were observed in any of the other tester strains in either the presence or absence of S9 activation.
- Precipitation was observed beginning at 1500 mg/plate.
- No toxicity was observed

Table 38- Summary of results from Ames Assay (Experiment B3) with (b) (4) (Lot No. 758-47-2) in the absence of S9 activation (Study No. 11-098-Sal-Mu).

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
						(b) (4)
TA98	(b) (4) **Lot 758-47-2**	5000 µg	*23*	*2*	*1.4*	
		1500 µg	*24*	*3*	*1.5*	
		500 µg	*24*	*3*	*1.5*	
		150 µg	*26*	*10*	*1.6*	
		50 µg	*17*	*4*	*1.1*	
	DMSO	100 µL	*16*	*4*		
TA100	(b) (4) **Lot 758-47-2**	5000 µg	*107*	*9*	*0.8*	
		1500 µg	*131*	*8*	*1.0*	
		500 µg	*123*	*10*	*0.9*	
		150 µg	*129*	*14*	*1.0*	
		50 µg	*109*	*11*	*0.8*	
	DMSO	100 µL	*132*	*25*		
TA1535	(b) (4) **Lot 758-47-2**	5000 µg	*15*	*2*	*0.9*	
		1500 µg	*17*	*5*	*1.0*	
		500 µg	*14*	*4*	*0.8*	
		150 µg	*17*	*5*	*1.0*	
		50 µg	*14*	*3*	*0.8*	
	DMSO	100 µL	*17*	*4*		

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	(b) (4) **Lot 758-47-2**	5000 µg	*6*	*3*	*0.8*	(b) (4)
		1500 µg	*7*	*1*	*0.9*	
		500 µg	*8*	*1*	*1.0*	
		150 µg	*7*	*2*	*0.9*	
		50 µg	*5*	*2*	*0.6*	
	DMSO	100 µL	*8*	*1*		
WP2*uvr*A	(b) (4) **Lot 758-47-2**	5000 µg	*35*	*4*	*0.9*	
		1500 µg	*31*	*8*	*0.8*	
		500 µg	*32*	*3*	*0.8*	
		150 µg	*38*	*9*	*0.9*	
		50 µg	*32*	*7*	*0.8*	
	DMSO	100 µL	*41*	*4*		
TA98	**2NF**	1.0 µg	*114*	*17*	*7.1*	
TA100	**SA**	1.0 µg	*706*	*6*	*5.3*	
TA1535	**SA**	1.0 µg	*597*	*47*	*35.1*	
TA1537	**9AAD**	75 µg	*212*	*69*	*26.5*	
WP2*uvr*A	**MMS**	1000 µg	*601*	*7*	*14.7*	

Key to Positive Controls

2NF	2-nitrofluorene
SA	sodium azide
9AAD	9-Aminoacridine
MMS	methyl methanesulfonate

Key to Plate Postfix Codes

(b) (4)

Key to Automatic & Manual Count Flags

M: Manual count A: Automatic count

[Excerpted from Applicant' submission]

Table 39- Summary of results from Ames Assay (Experiment B3) with (b) (4) (Lot No. 758-47-2) in the presence of S9 activation (Study No. 11-098-Sal-Mu).

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	(b) (4) **Lot 758-47-2**	5000 µg	*32*	*6*	*1.5*	(b) (4)
		1500 µg	*35*	*4*	*1.6*	
		500 µg	*30*	*3*	*1.4*	
		150 µg	*30*	*8*	*1.4*	
		50 µg	*28*	*5*	*1.3*	
	DMSO	100 µL	*22*	*2*		
TA100	(b) (4) **Lot 758-47-2**	5000 µg	*146*	*9*	*1.1*	
		1500 µg	*145*	*6*	*1.1*	
		500 µg	*160*	*15*	*1.3*	
		150 µg	*154*	*18*	*1.2*	
		50 µg	*128*	*18*	*1.0*	
	DMSO	100 µL	*127*	*7*		
TA1535	(b) (4) **Lot 758-47-2**	5000 µg	*39*	*7*	*3.5*	
		1500 µg	*19*	*5*	*1.7*	
		500 µg	*19*	*6*	*1.7*	
		150 µg	*15*	*6*	*1.4*	
		50 µg	*10*	*1*	*0.9*	
	DMSO	100 µL	*11*	*1*		

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1537	(b) (4) Lot 758-47-2	5000 µg	4	1	0.6	(b) (4)
		1500 µg	6	3	0.9	
		500 µg	8	3	1.1	
		150 µg	4	2	0.6	
		50 µg	8	4	1.1	
	DMSO	100 µL	7	2		
WP2*uvr*A	(b) (4) Lot 758-47-2	5000 µg	35	4	1.1	
		1500 µg	30	2	0.9	
		500 µg	28	2	0.8	
		150 µg	34	5	1.0	
		50 µg	30	4	0.9	
	DMSO	100 µL	33	5		
TA98	2AA	1.0 µg	494	103	22.5	
TA100	2AA	2.0 µg	1104	67	8.7	
TA1535	2AA	1.0 µg	165	104	15.0	
TA1537	2AA	1.0 µg	68	16	9.7	
WP2*uvr*A	2AA	15 µg	181	28	5.5	

Key to Positive Controls

2AA 2-aminoanthracene

Key to Plate Postfix Codes

(b) (4)

Key to Automatic & Manual Count Flags

M: Manual count A: Automatic count

[Excerpted from Applicant's submission]

Experiments B4

A retest of the definitive assay was conducted to confirm the positive mutagenic response with tester strain TA1535 observed in Experiment B3.

- Treatment with 50, 150, 500, 1500, 2500, or 5000 µ/plate (b) (4) (Lot no. 758-47-2) resulted in a 2.4-fold increase in revertant counts with strain TA1535 in the presence of S9 activation at a concentration of 500 mg/plate.
- Precipitation was observed beginning at 1500 mg/plate.
- No toxicity was observed

The results of the retest did not meet the criteria for a positive response with tester strain TA1535 in the presence of S9 activation.

Table 40- Summary of results from Ames Assay (Experiment B4) with (b) (4) (Lot No. 758-47-2) in the presence of S9 activation (Study No. 11-098-Sal-Mu).

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
	(b) (4)					(b) (4)
TA1535	**Lot 758-47-2**	5000 µg	*44*	*4*	*2.4*	
		2500 µg	*22*	*5*	*1.2*	
		1500 µg	*20*	*6*	*1.1*	
		500 µg	*12*	*2*	*0.7*	
		150 µg	*15*	*3*	*0.8*	
		50 µg	*17*	*2*	*0.9*	
	DMSO	100 µL	*18*	*2*		
TA1535	**2AA**	1.0 µg	*81*	*8*	*4.5*	(b) (4)

Key to Positive Controls

2AA 2-aminoanthracene

Key to Plate Postfix Codes

(b) (4)

Key to Automatic & Manual Count Flags

[M]: Manual count [A]: Automatic count

[Excerpted from Applicant's submission]

Experiments B5 and B6

At request of the Applicant, additional retests of the definitive assay was conducted to evaluate the equivocal mutagenic responses observed in Experiments B3 and B4, by using a more highly purified lot of the test article (Lot No. RM758-188-1).

- Using two independent cultures of TA1535, no positive mutagenic responses were observed with tester strain TA1535 in the presence of S9 activation, at concentration levels of 50, 150, 500, 1500, 2500, or 5000 µg/plate.
- Precipitation was observed beginning at 1500 mg/plate.
- No toxicity was observed

Table 41- Summary of results from Ames Assay (Experiment B5) with (b) (4) (Lot No. RM758-188-1) in the presence of S9 activation (Study No. 11-098-Sal-Mu).

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1535	(b) (4) **Lot RM758-188-1**	5000 µg	*9*	*1*	*1.0*	(b) (4)
		2500 µg	*10*	*4*	*1.1*	
		1500 µg	*6*	*2*	*0.7*	
		500 µg	*6*	*2*	*0.7*	
		150 µg	*7*	*2*	*0.8*	
		50 µg	*14*	*3*	*1.6*	
	DMSO	100 µL	*9*	*2*		
TA1535	**2AA**	1.0 µg	*56*	*20*	*6.2*	

Key to Positive Controls

2AA 2-aminoanthracene

Key to Plate Postfix Codes

(b) (4)

Key to Automatic & Manual Count Flags

M: Manual count A: Automatic count

[Excerpted from Applicant's submission]

Table 42- Summary of results from Ames Assay (Experiment B6) with (b) (4) (Lot No. RM758-188-1) in the presence of S9 activation (Study No. 11-098-Sal-Mu).

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA1535	(b) (4) Lot RM758-188-1	5000 µg	*14*	*1*	*1.4*	(b) (4)
		2500 µg	*13*	*7*	*1.3*	
		1500 µg	*11*	*3*	*1.1*	
		500 µg	*9*	*6*	*0.9*	
		150 µg	*13*	*3*	*1.3*	
		50 µg	*14*	*4*	*1.4*	
	DMSO	100 µL	*10*	*5*		
TA1535	2AA	1.0 µg	*88*	*19*	*8.8*	

Key to Positive Controls		Key to Plate Postfix Codes
2AA	2-aminoanthracene	(b) (4)
Key to Automatic & Manual Count Flags		

[M]: Manual count [A]: Automatic count

[Excerpted from Applicant's submission]

Formulation Analysis

- The actual concentration of the target 0.015 and 50 mg/mL (b) (4) (Lot No. 758-47-2) dosing formulations were 99% and 102 - 119% of the target concentrations, respectively, with ≤ 3.5% relative standard deviation (RSD). Although the actual concentration of the low concentration level in the retest of the definitive mutagenicity assay with (b) (4) (Lot No. 758-47-2) was higher than expected, the critical top concentration level was within (b) (4) % of target concentration and therefore met the acceptance criteria of (b) (4) % of target concentration and (b) (4) (b) (4) RSD.
- The actual concentration of the target 0.50 and 50 mg/mL (b) (4) (Lot No. RM758-188-1) dosing formulations were 90% of the target concentrations and therefore met the acceptance criteria of (b) (4) % of target concentration.
- Test article was detected in the vehicle control samples at concentrations (< 0.005 mg/mL) slightly higher than the limit of quantitation (LOQ) concentration of 0.0025 mg/mL. However, the analytical test site determined that the contamination occurred at the analytical site and not at (b) (4) and, therefore, his observation was considered to have had no adverse impact on the integrity of the data or the validity of the study conclusion.

Study title: Bacterial Reverse Mutation Assay of (b) (4)

Study no.:	12-170-Sal-X-MU-IMP ((b) (4) Study # AD70NX.502ICH.BTL)
Study report location:	eCTD, Module4, Section 4.2.3.3.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 12, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) Lot #815-190-5, purity (b) (4) (concentration factor 1.03)

Key Study Findings

Impurity (b) (4) was negative in the Ames assay.

Methods

Strains:	*Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvrA*
Concentrations in definitive study:	50, 150, 500, 1500 and 5000 µg per plate. One additional concentration, 3000 µg per plate was used for TA100 in the presence of S9 activation.
Basis of concentration selection:	5000 µg/plate is the highest recommended concentration based on ICH S2
Negative control:	Vehicle control (dimethyl sulfoxide, DMSO)
Positive control:	See below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	See below

Test agent stability: Stable in dimethyl sulfoxide (DMSO) under frozen conditions for 6 days

Metabolic activation system: Aroclor 1254 induced rat liver microsome S-9 mix

Controls:

Vehicle: DMSO (50 µL/plate)

Negative controls: vehicle control

Positive controls: all dissolved in DMSO except for sodium azide, which was diluted with water.

With S-9: 2-aminoanthracene: TA98, TA1535 and TA1537: (1 µg/plate), TA100 (2 µg/plate) and WP2 *uvr*A (15 µg/plate).

Without S-9: TA98: 2-nitrofluorene (1.0 µg/plate), TA 1537: 9-aminoacridine (75 µg/plate), TA100 and TA1535: sodium azide (1.0 µg/plate), WP2 *uvr*A: methyl methanesulfate (1000 µg/plate).

Study design:

The assay was performed in two phases, using the plate incorporation methods. In the first phase, the preliminary toxicity assay was used to establish the range of concentrations for the mutagenicity assay. The second phase, the mutagenicity assay (initial and confirmatory assays), was used to evaluate the mutagenic potential of the test article.

Positive mutagenicity: the following is excerpted from the Applicant's report
For the test article to be evaluated positive, it must cause a concentration-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2.0-times the mean vehicle control value.

Study Validity
The study is considered valid, because:

- Tester strain integrity was documented in the report.
- All tester strain culture titers were greater or equal to 0.3 $x10^9$ cells/mL, to ensure that appropriate numbers of bacteria were plated.
- Both negative (vehicle) and positive control data were within the laboratory historical range.
- The mean positive control value (± S9-mix) exhibited at least three fold increase over the respective mean vehicle control value for each tester strain.
- There was a minimum of three nontoxic concentrations. A concentration level was considered toxic if one or both of the following criteria were met: 1) ≥ 50% reduction in mean number of revertants/plate relative to the mean vehicle control value; or 2) at least a moderate reduction in the background lawn, i.e., background lawn code 3-5) in each tester strain, both in the absence and presence of S9-mix.

Exposure conditions:
Incubation and sampling times: 48-72 hours

Concentrations used:

- Preliminary assay (Experiment A1): 6.7, 10, 34, 67, 101, 168, and 252 μg per plate
- Mutagenicity assay (Experiment B1): 6.7, 10, 33, 67, 100, 167 and 250 μg/plate

Results:

- No background lawn toxicity was observed up to 250 μg/plate.
- Up to 250 μg/plate, no test article precipitate was observed.
- (b) (4), Lot #815-190-5, purity (b) (4) was negative in the Ames assay.

Study title: Bacterial Reverse Mutation Assay

Study no.:	11-099-Sal-X-MU ([redacted] (b) (4) Study AD38UL.503.BTL)
Study report location:	eCTD, Module 4, Section 4.2.3.3.1.
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	October 13, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	[redacted] (b) (4) Lot #758-5-R1, purity [redacted] (b) (4) (a correction factor of [redacted] (b) (4) was used in concentration formulation preparations to compensate for purity.)

Key Study Findings

- Impurity [redacted] (b) (4) was negative in the Ames assay.

Methods

Strains:	TA98, TA1535, TA1537, TA100, and WP2 *uvr*A
Concentrations in definitive study:	50, 150, 500, 1500, and 5000 μg/plate
Basis of concentration selection:	No significant toxicity to exclude 5000 μg/plate
Negative control:	DMSO
Positive control:	With S9: 2-aminaoanthracene for TA98, TA100, TA1535, TA1537 and WP2 *uvr*A. Without S9: 2-nitrofluorene for TA98; sodium azide for TA100 and TA1535; 9-aminoacridine for TA1537; Methyl methanesulfonate for WP2*uvr*A
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48 to 72 hours & stored at 2-8°C
Criteria for positive and negative:	Positive: • Concentration-related increase over 2 increasing concentrations (≥3 fold for TA1535 and TA1537; ≥2 fold for TA98, TA100 and Wp2 uvrA (pKM101) in the mean revertants per plate compared to mean vehicle control. Equivocal: • A biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. Negative: • Neither positive nor equivocal.

Study validity

The following criteria were provided:

- Strain genotype integrity: All *S. typhimuriam* strains must demonstrate sensitivity to UV light and crystal violet stain, TA98 and TA100 resistance to ampicillin.
- Demonstration of strain specific characteristic mean number of revertants (TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 uvrA, 10 – 60).
- Strain titers must be ≥0.3×10^9 cells/mL.
- Positive controls must exhibit 3-fold increase in the mean of revertants compared to mean vehicle control value.
- A minimum of 3 non-toxic concentration levels are required to evaluate the data.
- For toxicity: Reduction in the mean number of revertants per plate must >50% in the test article concentration as compared to the mean vehicle control value.

Results:

- No background lawn toxicity was observed; however, reductions in revertant counts were observed at 5000 μg/plate.

- Test article precipitate was observed ≥1500 µg/plate.
- [redacted] (b) (4), Lot #758-5-R1, purity [redacted] (b) (4) was negative in the Ames assay.

8 Carcinogenicity

Not conducted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not conducted.

9.2 Embryonic Fetal Development

Rats:

Study title: An oral (gavage) embryo fetal development study of PCI-32765 in rats	
Study no.:	11-132-R-PO-TT ([redacted] (b) (4)-622042)
Study report location:	eCTD, Module 4, Section 4.2.3.5.2
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	September 10, 2012
GLP compliance:	Yes (also OECD Principles of GLP)
QA statement:	Yes
Drug, lot #, and % purity:	PCI-32765, Lot #111131, 99.4%

Key Study Findings

- Adverse uterine effects (increased post-implantation loss and increased resorption) were evident at the high dose of 80 mg/kg ibrutinib.
- Fetal toxicities (visceral malformations and variations, and skeletal variations) were most evident at the high dose of 80 mg/kg.
- Reduced fetal weight was seen at mid dose (40 mg/kg) and high dose (80 mg/kg).
- Maternal toxicities were observed mainly at 80 mg/kg.
- The AUC in animals at ibrutinib dose of 80 mg/kg is approximately 14 times the AUC in patients with MCL administered the dose of 560 mg/day [redacted] (b) (4) [redacted] The human AUCs used for the conversion are 933 ng·h/mL for MCL [redacted] (b) (4)
- The AUC in animals at ibrutinib dose of 40 mg/kg is approximately 6 times the AUC in patients with MCL administered the dose of 560 mg/day [redacted] (b) (4) [redacted]

Methods	
Concentrations:	0 (control), 10, 40 and 80 mg/kg (as Groups 1, 2, 3 and 4, respectively)
Frequency of dosing:	Daily for 12 days (GD 6-17)
Concentration volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% methylcellulose and 0.1% sodium lauryl sulfate in water for injection
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	The embryo-fetal development phase (main study groups) females N=25/group (Groups 1-4)*
Satellite groups:	None. A toxicokinetic evaluation (Study (b) (4)-622042T) was conducted (n=12* for Groups 2-4, and n=4 in Group 1)
Study design:	See below
Deviation from study protocol:	Not remarkable

*** The number of animals/group was expected to obtain a sample size of 16-20 litters at termination, and to obtain at least 3 samples per time point for toxicokinetic evaluation.**

Study design:

- Age of animals: approximately 13 weeks old.
- Body weights: group means ~255 g.
- Time-mated females were treated during gestation days (GDs) 6-17. The animals in the main study were sacrificed on GD 21, while the TK animals on GD 15 or GD 16.

Observations

Clinical signs: Mortality and moribundity (twice daily, between GD 0 and GD 20), clinical signs (during concentration administration and 1-2 post dosing, between GD 0 and GD 20). Data from the TK animals (between GDs 0-18).

Body weights: Maternal body weight for the embryo-fetal development phase (main study groups): on GDs 0, 6-18 (daily) and on GD 20; TK animals on GDs 0 and 6-17. Mean body weight changes were calculated for each corresponding interval and also for GDs 6-9, 9-12, 12-18, and 6-18 for the main study groups.

Food consumption: Main study animals on GDs 0, 6-18 (daily), and 20; not for TK animals. Data were reported as g/animal/day and g/kg/day for the corresponding BW change intervals.

Gross pathology: At scheduled necropsy (GD 20): major viscera of all main study animals including gross evaluation of placentae

Laparo-histopathology: <u>All organs/tissues were considered normal unless otherwise indicated</u>

Toxicokinetics: On gestation days (GD) 6 and 17 at 0 (pre-concentration), 0.5, 1, 2, 4, 8, 12 and/or 24 hr postconcentration; n=4/group (~0.3 mL blood samples were collected from a lateral tail vein). TK animals were euthanized on GD 18, and pregnancy status was determined for each female.

Cesarean section: GD 20

Reproductive parameters: Dams**: gravid uterine weight, uterine site description (live fetus, early or late resorption), number, type, and position of implantation sites, corpora lutea (main study animals)

Fetal examination (live fetuses): data from all fetuses included weights, sexes, external findings, visceral and skeletal examination. Heads from approximately 50% of the fetuses in each group were sectioned for soft tissue examination, while the rest of heads were examined by a midcoronal slice. Fetal kidneys were examined and graded for renal papillae development (Woo and Hoar, Teratology 6: 191-196, 1972)

** Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss.

Statistical analyses: group means were compared against controls by employing the following methods:

- Maternal body weights and weight gains, food consumption and were analyzed by a parametric One-way ANOVA. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group.
- Hysterotomy parameters (litter size, embryo/fetal mortality) (in terms of number per litter and proportion per Litter) and postmortem observations (fetal sex, weight, external, palatal and Skeletal or visceral anomalies) were analyzed the Kruskal-Wallis nonparametric ANOVA test (Kruskal and Wallis, 1952). The test was used to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunn's test was used to compare the test article-treated groups to the control group.
- Data collected from non-pregnant females were excluded from statistical analyses.

Mortality

None

Clinical Signs

Not remarkable.

Body Weight

Group mean body weights:
Statistically significant reduction in daily group mean body weight at 80 mg/kg was noted starting on GD 15. The % reductions from the control at 80 mg/kg were approximately 3.5, 4.5, 7, 10 and 14% on GDs 15, 16, 17, 18, 19 and 20, respectively (data not shown)

Group mean body weight change: body weight gains, net weight changes and gravid uterine weights
Statistically significant reductions in weight gains were seen starting on GDs 14-15. However, the reduced body weight gains were secondary to the reduction in gravid uterine weights; there were no noteworthy differences in the net body weight changes. The table below is the summary of statistically significant group mean weight changes (g) in the control and group 4 (80 mg/kg). The percent reduction from the control is presented in the parenthesis.

Table 43- Group mean body weight change in dams:

		Weight Gain (g) (Daily changes) (change from control)				
Concentration	**N**	**GD 14-15**	**GD 15-16**	**GD 16-17**	**GD 17-18**	**GD 18-20**
0 (Control)	25	9	11	16	17	37
80 mg/kg	24	4 **(-56%)**	6 **(-45%)**	5 **(-69%)**	7 **(-59%)**	16 **(-57%)**

		Periodic changes in weight gain (g) (change from control)			
Concentration	**N**	**GD 6-9**	**GD 9-12**	**GD 12-18**	**GD 6-18**
0 (Control)	25	11	18	62	91
80 mg/kg	24	6 **(-45%)**	15 **(NS)**	33 **(-47%)**	54 **(-41%)**

		Additional weights (g) (change from control)				
Concentration	N	GD 0	GD 20	Gravid uterine weight	Net body weight	Net BW change
0 (Control)	25	255	419	88.3	330.3	75.3
80 mg/kg	24	254 **(NS)**	361 **(-14%)**	27.7 **(-69%)**	332.8 **(NS)**	78.4 **(NS)**

Net body weight: the GD 20 dam BW excluding the weight of the uterus and its content.
Net BW change: the GD 20 BW change which excludes the weight of the uterus and its content.
NS: not significant.

Feed Consumption

Treatment of PCI32765 at 80 mg/kg occasionally resulted in statistically significant reductions in food consumption.

Statistically significant decreases in food consumption are summarized in the table below. Food consumption was expressed as g/animal/day as well as g/kg/day.

			Daily changes (g/animal/day)	Daily changes (g/kg/day)	Periodic changes (g/kg/day)
Group	Concentration	N	GD 9-10	GD 9-10	GD 18-20
1	Control	25	24	80	72
4	80 mg/kg	24	22 (-8%)	73 (-9%)	77 (+ 7%)

Toxicokinetics

All females in the toxicokinetic groups survived to the scheduled euthanasia. Two and 1 females in the 10 and 80 mg/kg/day groups, respectively, were not pregnant. All other toxicokinetic phase females were gravid.

Table 44- Toxicokinetic parameters

The table below is the summary of PK parameters of PCI-32765 and its dihydrodiol metabolite (PCI-45227) on GDs 6 and 17, respectively (from the Applicant)

Analyte	PCI-32765			PCI-45227		
Dose (mg/kg)	10	40	80	10	40	80
Gestation day 6						
C_{max} (ng/ml)	462	1590	2290	299	1026	1147
T_{max} (h)	1	2	2	1	2	2
$AUC_{0\text{-}inf}$ (ng.h/ml)	1398	4983	11603	1271	4703	8227
Gestation day 17						
C_{max} (ng/ml)	466	1310	2627	261	726	1665
T_{max} (h)	0.51	1	1	2	1	2
$AUC_{0\text{-}24\,h}$ (ng.h/ml)	1278	5348	13729	1203	5110	12027

Comment:

- AUC values of both the parent drug and its metabolite increased with increased concentrations in an approximately concentration-proportional fashion. This finding was reported on both GD 6 and GD 17, indicating less likely accumulations following repeated administration of PCI-32765.
- In comparison, increases in C_{max} values in both API and the metabolite were less than concentration proportionality on both GD 6 and 17.

Stability and Homogeneity

The test article and control article were acceptable for use. The stability and homogeneity of dosing formulation were acceptable, based on concentration (strength) and pH values. According to the investigator, homogeneity was demonstrated previously at test article concentration ranges of 6 mg/mL to 60 mg/mL (Kirkpatrick, 2012, (b) (4)-622033*) and 12 mg/mL to 88 mg/mL.
* A 13-Week Oral (Gavage) Toxicity Study of PCI-32765 in Rats with a 6-Week Recovery Period (Study No. (b) (4)-622033). (b) (4)

Necropsy

Not remarkable upon maternal macroscopic examinations.

Cesarean Section Data

Table 45- Terminal and necroscopic evaluations, Dams

	Control	10 mg/kg	40 mg/kg	80 mg/kg
Females mated	25	25	25	25
Number of females pregnant (%)	25 (100%)	23 (92%)	25 (100%)	24 (96%)
Pregnant at C-section	25	23	25	24
Dams with viable fetuses	25 (100%)	23 (100%)	25 (100%)	16 (**67%**)
Dams with all resorption	0	0	0	8 (**33%**)
Corpora lutea				
Total	424	378	452	403
Average/animal (mean)	17	16.4	18.1	16.9
Implantation sites				
Total	403	364	406	376
Average/animal (mean)	16.1	15.8	16.2	15.7
Preimplantation loss				
Total	21	14	46	27
(%) (total/corpora lutea x 100%)	5%	3.7%	10.2%	6.7%
Average/animal (mean)	0.8	0.6	1.8	1.1
Postimplantation loss)§				
Total	27	16	29	**278**
(%) (total/corpora lutea x 100%)	6.4%	4.2%	6.4%	**69%**
Average/animal (mean)	1.1	0.7	1.2	**11.6**
Dead fetuses	0	0	0	0
Total resorptions (early + late resorptions)				
Total	27	16	29	**278**
% (resorptions/implantation sites x 100%)	6.7%	4.4	7.1%	**73.9%**
Average/animal (mean)	1.1	0.7	1.2	**11.6**
Early resorptions				
Total	27	16	28	**277**
% (resorptions/implantation sites x 100%)	6.7%	4.4%	6.9%	**73.7%***
Average/animal (mean)	1.1	0.7	1.1	**11.5**
Late resorptions				
Total	0	0	1	1
% (resorptions/implantation sites x 100%)	0	0	0.2%	0.3%
Average/animal (mean)	0	0	0	0
Viable fetuses				
Total	376	348	377	**98**
% (viable/implantation sites x 100%)	93.3%	95.6%	92.9	**26.1%***
Average/animal (mean)	15	15.1	15.1	**4.1***
Males: total (per dam)	190 (7.6)	182 (7.9)	189 (7.6)	**45 (1.9)**
Females: total (per dam)	186 (7.4)	166 (7.2)	188 (7.5)	**53 (2.2)**
Viable male fetuses (%)	190 (50.5)	182 (52.2)	189 (50.1)	**45 (45.9)**
Live fetal body weight (g) (mean): absolute†	3.8	3.7	**3.4* (↓10.5%)**	**3.1* (↓18.4%)**
Mean male fetal weight (g)	3.9	3.8	**3.6* (↓7.7%)**	**3.2* (↓17.9%)**
Mean female fetal weight (g)	3.7	3.6	**3.4* (↓8.1%)**	**3.0* (↓18.9%)**

Absolute fetal weight: average fetal weights based on average numbers of viable fetuses
*** Statistically significant changes**

§ The 16 dams with viable fetuses at 80 mg/kg had 12.5% to 94.1% postimplantation loss (primarily early resorption, only one with late resorption).
† The (b) (4) historical control data of fetal body weights were 3.4 g to 3.5 g.

Offspring

The incidence of fetal external/visceral and skeletal malformations and variations was shown in the tables below.

Table 46- Offspring: external, visceral and skeletal variations

- External malformations and variations: no drug-related effects were noted; findings were not concentration-dependent or statistically significant

The table below is adapted from the Applicant's submission.

	Fetus				Litter			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Concentration (mg/kg)	**0**	**10**	**40**	**80**	**0**	**10**	**40**	**80**
Number evaluated	376	348	377	98	25	23	25	16
Malformations								
Mandibular micrognathia								
Total (mean %)	0 (0)	2 (0.6)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)
Enlarged urogenital opening								
Total (mean %)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)
Total incidence (%)	0 (0)	2 (0.6)	1 (0.3)	0 (0)	0 (0)	1 (4.3)	1 (4)	0 (0)

The malformations were mainly in two dams (one each at 10 mg/kg and 40 mg/kg, respectively): #66775 and 66784, respectively.

- Visceral malformations and variations: most evident at the high concentration of 80 mg/kg

The table below is adapted from the Applicant's submission.

	Fetus				Litter			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Concentration (mg/kg)	**0**	**10**	**40**	**80**	**0**	**10**	**40**	**80**
Number evaluated	376	348	377	98	25	23	25	16
Malformations								
Dextrocardia (%) Total (%per litter)	0 (0)	0 (0)	0 (0	**1 (1.3)**	0 (0)	0 (0)	0 (0)	**1 (6)**
Retroesophageal aortic arch Total (%per litter)	0 (0)	0 (0)	0 (0)	**1 (0.7)**	0 (0)	0 (0)	0 (0)	**1 (6)**
Persistent truncus arteriosus Total (%per litter)	0 (0)	0 (0)	0 (0)	**1 (3.1)**	0 (0)	0 (0)	0 (0)	**1 (6)**
Right-sided aortic arch Total (% per litter)	0 (0)	0 (0)	0 (0)	**1 (1)**	0 (0)	0 (0)	0 (0)	1 (6)
Interrupted aortic arch Total (% per litter)	0 (0)	0 (0)	1 (0.3) 1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)
Total incidence (%)	0 (0)	0 (0)		**4 (6.1)**	0 (0)	0 (0)	**1 (4)**	**4 (24)**
Variations								
Hemorrhagic ring around the iris								

Total (% per litter)	0 (0)	0 (0)	0 (0)	**1 (3.1)**	0 (0)	0 (0)	0 (0)	**1 (6)**
Liver accessory lobules								
Total (% per litter)	1 (0.3)	0 (0)	0 (0)	**1 (0.4)**	1 (4)	0 (0)	0 (0)	**1 (6)**
Liver pale								
Total (% per litter)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)
Major blood vessel variation								
Total (% per litter)	0 (0)	0 (0)	**1 (0.3)**	**1 (0.7)**	0 (0)	0 (0)	**1 (4)**	**1 (6)**
Renal papilla(e) not developed and/or distended ureter								
Total (% per litter)	3 (0.8)	3 (0.9)	4 (1.3)	0 (0)	3 (12)	2 (8.7)	2 (8)	0 (0)
Total incidence (%)	4 (1.0)	4 (1.2)	5 (1.6)	3 (4.3)	4 (16)	3 (13)	3 (12)	3 (18)

Soft tissue malformations were noted in 1 and 4 dams in the 40 and 80 mg/kg/day groups, respectively. The description is as follows:

<u>80 mg/kg:</u>

- Dextrocardia (heart and great and major blood vessels laterally transposed): fetus #66739-12.
- Persistent truncus arteriosus (pulmonary arteries that arose from the ascending aorta, the right pulmonary artery coursed retroesophageal) and interventricular septal defect (a 1 mm opening in the anterior portion of the septum): fetus #66763-16
- Retroesophageal aortic arch (the aortic arch coursed retroesophageal immediately following the left carotid artery and returned to the normal position adjacent to the ductus arteriosus): fetus #66809-11
- Right-sided aortic arch (the aortic arch and descending aorta coursed to the right of the vertebral column and the right carotid and subclavian arteries arose independently from the aortic arch [no brachiocephalic trunk], and the left carotid and subclavian arteries arose from a common vessel from the aortic arch): fetus #66814-05

The mean % per litter values for the soft tissue malformations at 80 mg/kg were higher than the concurrent control group. In addition, the mean % per litter value of retroesophageal aortic arch was above the (b) (4) historical control data range. The visceral malformations noted in this group were considered to be of similar developmental origin (all involved the heart and major blood vessels). Based on the similar developmental origin and on other evidence of developmental toxicity (such as a lower mean number of viable fetuses and lower mean fetal body weights) in the 80 mg/kg/day group, the visceral malformations were considered related to treatment of dams with PCI-32765.

<u>40 mg/kg:</u>

Interrupted aortic arch (the brachiocephalic trunk and the left carotid artery arose from the ascending aorta, the left subclavian artery arose from the descending aorta, and the ductus arteriosus communicated with the descending aorta): fetus #66784-13.
The mean % per litter value of an interrupted aortic arch (0.3% per litter) was within the (b) (4) historical control data range (0.0% to 0.3% per litter) and it was noted in a single fetus; therefore it was not attributed to PCI-32765 administration.

- Skeletal malformations and variations: there were no clear drug-related skeletal malformations. Skeletal variations were mainly at the high concentration of 80 mg/kg.

The table below is adapted from the Applicant's submission.

	Fetus				Litter			
Group	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Concentration (mg/kg)	**0**	**10**	**40**	**80**	**0**	**10**	**40**	**80**
Number evaluated	376	348	377	98	25	23	25	16
Malformations								
Vertebral centra anomaly								
Total (%per litter)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)
Sternoschisis								
Total (%per litter)	0 (0)	0 (0)	0 (0)	**1 (0.5)**	0 (0)	0 (0)	0 (0)	**1 (6)**
Vertebral anomaly								
Total (%per litter)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)
Total incidence (%)	0 (0)	2 (0.6)	0 (0)	1 (0.5)	0 (0)	2 (8.7)	0 (0)	1 (6)
Variations								
Cervical centrum #1 ossified								
Total (% per litter)	72 (19.7)	103 (30.1)	132 (35.1)	12 (8.5)	21 (84)	22 (96)	23 (92)	6 (37.5)
14th rudimentary rib(s)								
Total (% per litter)	23 (6.3)	20 (5.8)	3 (0.8)	4 (3.9)	9 (36)	5 (21.7)	3 (12)	2 (12.5)
Bent rib(s)								
Total (% per litter)	1 (0.2)	0 (0)	2 (0.5)	0 (0)	1 (4)	0 (0)	2 (8.7)	0 (0)
Reduced ossification of the vertebral arches								
Total (% per litter)	3 (o.7)	0 (0)	0 (0)	0 (0)	2 (8)	0 (4.2)	0 (4)	0 (29.2)
Sternebra(e) #5/#6 unossified								
Total (% per litter)	23 (5.4)	14 (3.9)	**40 (11)**	**32 (30.2)**	7 (28)	4 (17.4)	**16 (64)**	**12 (75)**
Hyoid unossified								
Total (% per litter)	7 (1.6)	4 (1.1)	4 (1)	1 (0.4)	3 (12)	3 (13)	4 (16)	1 (8)
Reduced ossification of the 13th rib(s)								
Total (% per litter)	1 (0.3)	2 (0.6)	7 (1.9)	7 (17.4)	1 (4)	1 (4.3)	4 (16)	6 (37.5)
Sternebra(e) malaligned (slight ormoderate)								
Total (% per litter)	2 (0.7)	3 (0.9)	0 (0)	2 (1.2)	2 (8)	3 (13)	0 (0)	2 (12.5)
25 Presacral vertebrae								
Total (% per litter)	2 (0.5)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)
14th Full rib(s)								
Total (% per litter)	1 (0.3)	1 (0.3)	0 (0)	0 (0)	1 (4)	1 (4.3)	0 (0)	0 (0)
7th Cervical rib(s)								
Total (% per litter)	2 (0.5)	1 (0.3)	0 (0)	0 (0)	1 (4)	1 (4.3)	0 (0)	0 (0)
Reduced ossification of the skull								
Total (% per litter)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)
Reduced ossification of the rib(s)								
Total (% per litter)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)	0 (0)
General reduced ossification of the entire skeleton								
Total (% per litter)	0 (0)	2 (0.6)	0 (0)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)
Total incidence (%)	139 (33.2)	150 (41.2)	188 (43.9)	**58 (51.4)**	51 (2)	41 (1.8)	52 (2.1)	29 (1.8)

Bold print numbers: statistically significant changes from the control.

PCI-32765-related skeletal variations were mainly in the 80 mg/kg/day group and consisted of higher mean litter proportions of sternebra(a) #5 and/or #6 unossified and

reduced ossification of the 13th rib(s), and a lower mean litter proportion of cervical centrum #1 ossified. As a result, the mean % per litter values of total malformations and variations in the 80 mg/kg/day group (6.6% and 52.5% per litter, respectively) were higher than the concurrent control group (0.0% and 33.8% per litter, respectively) and higher than the maximum mean value in the (b) (4) historical control data (1.6% and 51.1% per litter, respectively); the difference was significant for variations.

Rabbits:

The following concentration range finding (DRF) study is reviewed. Results are summarized below.

Study title: An oral (gavage) concentration range-finding study of the effects of PCI-32765 on embryo/fetal development in rabbits (Study (b) (4)-622040; Applicant #10-064-B-PO-TTE) (non-GLP) (Module 4)

Key Study Findings:

- Oral administration of PCI-32765 (GD 7-19) in time mated rabbits elicited maternal toxicities, including mortality and abortions at 100 mg/kg, and clinical signs (reduced defecation, small feces), reduction in body weight and food consumption at ≥ 30 mg/kg.
- Embryonic toxicity (increases in resorption and implantation loss, decreases in viable fetuses and fetal body weights) were observed at 100 mg/kg. There were no changes in net body weights, net body weight gains and gravid uterine weights.
- No apparent teratogenic effects were observed.

- Agents: PCI-32765 (Lot #111131, estimated purity: 99.5%); vehicle: 0.5% methylcellulose and 0.1% sodium lauryl sulfate in sterile water for injection.
- Study design: time mated female New Zealand white rabbits (n=6/group) (approximately 5 months old; 2901-3431 g) were administered orally by gavage (5 mL/kg) with PCI-32765 at 0 (control), 10, 30 and 100 mg/kg/day (as Groups 1-4, respectively) for 13 days starting on gestation day (GD) 7 through GD 19. Surviving animals were euthanized (cesarean section) on GD 29. The following evaluations were conducted: Maternal mortality, clinical observations, body weight, food consumption, gravid uterine weight, laparohysterotomy findings on GD 29 (corpora lutea, litter size, embryo/fetal mortality), and postmortem observations; fetal weight, and external and palatal anomalies, and placental appearance. Plasma toxicokinetics of PCI-32765 were also evaluated: blood samples collected (via ear artery or saphenous vein) at 0.5, 1, 2, 4, 8 and 24 hours after dosing on GDs 7 and 19.

Table 47-Summary of study result: embryo-fetal development study in rabbits (concentration range finding study)

<table>
<tr><th>Evaluation</th><th>Outcomes</th></tr>
<tr><td colspan="2">Maternal findings (Dams)</td></tr>
<tr><td>TK (C_{max} and AUC)</td><td>TK parameters of PCI-32765 On GD7 and GD19 (table from the Applicant)
<table>
<tr><th>Gestation Day</th><th>Dosage (mg/kg/ day)</th><th>AUC[a] (ng.h/mL)</th><th>AUC/dose (ng.h/mL)/ (mg/kg)</th><th>C_{max} (ng/mL)</th><th>C_{max}/dose (ng/mL)/ (mg/kg)</th><th>T_{max} (h)</th><th>$T_{1/2}$ (h)</th></tr>
<tr><td>7</td><td>10[b]</td><td>448 (155)</td><td>44.8 (15.5)</td><td>71.5 (26.6)</td><td>7.15 (2.66)</td><td>3.6 (0.9)</td><td>4.9 (1.6)</td></tr>
<tr><td>7</td><td>30[c]</td><td>1050 (635)</td><td>35.2 (21.2)</td><td>193 (137)</td><td>6.43 (4.57)</td><td>1.8 (1.3)</td><td>4.6 (1.1)</td></tr>
<tr><td>7</td><td>100[c]</td><td>4970 (1170)</td><td>49.7 (11.7)</td><td>911 (453)</td><td>9.11 (4.53)</td><td>2.8 (1.5)</td><td>4.0 (1.0)</td></tr>
<tr><td>19</td><td>10[b]</td><td>565 (443)</td><td>56.5 (44.3)</td><td>142 (154)</td><td>14.2 (15.4)</td><td>0.5 (0)</td><td>3.0 (0.8)</td></tr>
<tr><td>19</td><td>30[c]</td><td>1310 (478)</td><td>43.7 (15.9)</td><td>311 (287)</td><td>10.4 (9.6)</td><td>1.0 (0.8)</td><td>4.9 (1.8)</td></tr>
<tr><td>19</td><td>100[c]</td><td>21000 (10600)</td><td>210 (106)</td><td>1830 (553)</td><td>18.3 (5.5)</td><td>2.8 (1.5)</td><td>10.0 (6.4)</td></tr>
</table>
TK parameters of metabolite PCI-45227 on GDs 7 and 19 (table from the Applicant)
<table>
<tr><th>Gestation Day</th><th>PCI-32765 Dosage (mg/kg/day)</th><th>AUC[a] (ng.h/mL)</th><th>Percent Relative to Parent AUC[b]</th><th>C_{max} (ng/mL)</th><th>T_{max} (h)</th><th>$T_{1/2}$ (h)</th></tr>
<tr><td>7</td><td>10[c]</td><td>1300 (290)</td><td>269</td><td>155 (46.7)</td><td>3.6 (0.9)</td><td>7.3 (1.4)</td></tr>
<tr><td>7</td><td>30[d]</td><td>5910 (697)</td><td>523</td><td>800 (236)</td><td>2.2 (1.5)</td><td>6.6 (1.5)</td></tr>
<tr><td>7</td><td>100[d]</td><td>26700 (4770)</td><td>499</td><td>3520 (700)</td><td>3.3 (1.0)</td><td>5.5 (0.8)</td></tr>
<tr><td>19</td><td>10[c]</td><td>1670 (200)</td><td>274</td><td>300 (75.2)</td><td>1.2 (1.6)</td><td>5.6 (1.3)</td></tr>
<tr><td>19</td><td>30[d]</td><td>8820 (2020)</td><td>625</td><td>1250 (515)</td><td>1.6 (1.4)</td><td>7.4 (2.9)</td></tr>
<tr><td>19</td><td>100[d]</td><td>38500 (10800)</td><td>170</td><td>3060 (1540)</td><td>4.0 (2.2)</td><td>11.0 (6.2)</td></tr>
</table>
• On GD 7 exposures to PCI-32765 and its metabolite were approximately concentration proportional. However, a greater than unity in AUC values was noted on GD 19 at 100 mg/kg, which also suggested accumulation of PCI-32765.
• The AUC for metabolite PCI-45227 relative to parent AUC ranged from 170% to 625% across all concentrations and gestation days. This data suggests extensive metabolism of PCI-32765 to PCI-45227 in rabbits.</td></tr>
<tr><td>Mortality</td><td>4/6 dams at 100 mg/kg aborted and necropsied on GD 22 and 23 due to:
• Clinical signs, starting on GD12: decreased and/or small feces, red material in the cage pan (in the three aborted females)
• Weight loss; reduced absolute body weight, starting on GD 8, up to 16% reduction compared to the control.
• Reduction in food consumption: up to ↓ 110 grams/day
• Reduced food consumption and associated weight loss.

One animal in the control group was euthanized on GD 23 due to decreased defecation (GD 18-23), weight loss and reduced food consumption (GD 13-14).</td></tr>
<tr><td>Clinical sign</td><td>Mainly at ≥30 mg/kg: decreased and small feces between GDs 12-22,</td></tr>
</table>

Group mean body weights and group mean and weight gains

Mean group body weights:
Reductions of group mean BW at 100 mg/kg were observed starting on GD15. Between GD 18-GD 20, the reduction reached statistical significance. These reductions were 12%, 13%, 15% On GDs 18, 19 and 20, respectively.
Mean weight changes (weight gain):
Reductions in daily weight change were noted, starting GD 8-9. Occasionally the changes reached statistical significance.
Daily weight gains (g): numbers in the parentheses are % changes from the control

Group	Concentration	N	GD 10-11	GD 14-15	GD 16-17	GD 17-18
1	Control	6	48	58	19	24
4	100 mg/kg	6	-18 (-138%)	-31 (-153%)	-63 (-432%)	-43 (-279%)

Periodic weight gains (g): numbers in the parentheses are %% changes from the control

Group	Concentration	N	GD 10-13	GD 13-20	GD 7-20
1	Control	6	81	95	231
4	100 mg/kg	6	-49 (-160%)	-297 (-413%)	-284 (-223%)

Other weight changes (g): numbers in the parentheses are %% changes from the control

Group	Concentration	N	GD 0	GD 20	Gravid uterine weight	Net body weight	Net BW change
1	0 (Control)	6	3127	3727	498.7	3227.9	100.5
4	100 mg/kg	6	33074 **(NS)**	3580 (**NS)**	340.4 (**NS)**	3239.7 **(NS)**	165.7 **(NS)**

- There were no treatment-related effects on net BW or net BW changes at 100 mg/kg. However, 1 of the two surviving dams had a lower gravid uterine weight on GD 29, which corresponded to decreased fetal growth in this female.
- At 30 mg/kg, there were transient and non-significant mean BW losses during GDs 16-20, and a lower weight gain during GDs 13-20, respectively. However, the overall weight gain (Days 7-20) in this group was comparable to the control group.

Food consumption (g/animal/day) (g/kg/day)

Mainly at 100 mg/kg.
Statistically significant reductions as g/animal/day

Concentration	N	GD 10-11	GD 12-13	GD 13-14	GD 10-13	GD 7-20
0	6	191	165	145	176	159
100 mg/kg	6	95 (-50%)	82 (-49%)	62 (-57%)	89 (-49%)	80 (-50%)

Statistically significant reductions as g/kg/day

Concentration	N	GD 9-10	GD 10-11	GD 11-12	GD 12-13	GD 13-14
0	6	50	56	48	48	42
100 mg/kg	6	35 (-30%)	29 (-48%)	27 (-44%)	24 (-50%)	18 (-57%)
Concentration	N	GD 19-20	10-13	13-20	7-20	20-29
0	6	37	52	41	46	35
100 mg/kg	2	12 (-68%)	27 (-48%)	13 (-68%)	24 (-48%)	44 (NS)

- Reduction in food consumption recovered partially after cessation of dosing (i.e., GD 20-29).
-

Maternal necropsy; Description of findings in aborted dams

Three aborted females: #66421, 66426 and 66438:
- No remarkable macroscopic findings.
- #66421 aborted 1 dead fetus with no apparent external malformations. The female also had 8 former implantation sites and 2 early resorptions and 3 late resorptions.
- #66426 aborted 1 late resorption that was found partially cannibalized. The female was with 6 former implantation sites, in utero.
- #66438 aborted 8 early resorptions and was 10 former implantation sites in utero.

<table>
<tr><td></td><td><u>Animal euthanized:</u>
#66437 (100 mg/kg) was euthanized on GD 22 and was noted with 4 live fetuses with no apparent external malformations and 6 early resorptions in utero.
#66434 (control group) was euthanized on GD 23; the female was internally normal with 10 live fetuses in utero.</td></tr>
<tr><td>Laparohysterotomy findings (% per litter) (↓ or ↑ % from the control)</td><td>The following is adapted from Applicant's submission:
<table>
<tr><td>Group (F0)</td><td>Group 1</td><td>Group 2</td><td>Group 3</td><td>Group 4</td></tr>
<tr><td>Concentration (mg/kg)</td><td>Control</td><td>10</td><td>30</td><td>100</td></tr>
<tr><td>N (number of does)</td><td>5</td><td>5(a)</td><td>6</td><td>2</td></tr>
<tr><td>Number of corpora lutea</td><td>10</td><td>9.8</td><td>8.8</td><td>10.5</td></tr>
<tr><td>Total implantation sites</td><td>9.2</td><td>9.6</td><td>8.2</td><td>9.0</td></tr>
<tr><td>Preimplantation loss (%)</td><td>7.7</td><td>1.8</td><td>8.5</td><td>15.3 (↑99%)</td></tr>
<tr><td>Post implantation loss (%)</td><td>7.5</td><td>2</td><td>5.5</td><td>22.8 (↑204%)</td></tr>
<tr><td>Total resorption (%)</td><td>7.5</td><td>2</td><td>5.5</td><td>22.8 (↑204%)</td></tr>
<tr><td>Early resorption (%)</td><td>5</td><td>2</td><td>3.4</td><td>9.1 (↑81%)</td></tr>
<tr><td>Late resorption (%)</td><td>2.5</td><td>0</td><td>2.1</td><td>13.7 (↑448%)</td></tr>
<tr><td>Dead fetuses (%)</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Viable fetuses (%)</td><td>92.5</td><td>98</td><td>94.6</td><td>77.3 (↓16%)</td></tr>
<tr><td>Body weight of all live fetuses (g)</td><td>41.3</td><td>39.7</td><td>42.8</td><td>33.8 (↓18%)</td></tr>
</table>
(a): #66430 (10 mg/kg) was not gravid, so totally 5 females were examined in the group.
Due to only 2 survived females at 100 mg/kg, the comparison of control and Group 4 was not subjected to statistical analysis.</td></tr>
<tr><td colspan="2">Feto-embryonic findings (postmortem observations)</td></tr>
<tr><td>External morphology</td><td>Not remarkable.</td></tr>
</table>

***For rabbits that consumed less than 10 g of feed per day for 3 or more consecutive days, the basal diet was supplemented (supplemented on the fourth day) with non-certified kale. Dietary supplementation for these animals was discontinued on the third day of consuming ≥35 g of feed/day. In addition, the amount of kale consumed daily was documented for each rabbit.**

9.3 Prenatal and Postnatal Development

Not conducted.

10 Special Toxicology Studies

None

Integrated Summary and Safety Evaluation

A full battery of toxicology studies that supported the safety evaluation of ibrutinib for the current indications were conducted in *in vitro* systems as well as in mice, rats, rabbits, and dogs. The target organs of ibrutinib are GI tract and lymphoid tissues. The general toxicology studies were conducted in appropriate animal species, following administration route and dosing regimens that adequately addressed safety concerns in human usage. In general, the toxicity profile was similar in rodent and nonrodent species. See Executive Summary, Section 1.2, for a discussion of nonclinical findings.

Of note, one common adverse effect in patients treated with ibrutinib, thrombocytopenia, was not observed non-clinically. Contrarily, increased platelet counts were seen in rats and dogs, along with increased fibrinogen levels. Both findings in animals may be secondary to the GI bleeding and inflammation.

Dose-dependent RR interval prolongation and decreased heart rate was reported in dogs. *In vitro* receptor binding assays revealed that ibrutinib can bind to dopamine transport (DAT). Ibrutinib inhibited the binding of the radioligand to DAT receptor with an IC_{50} of 0.995 nM (in comparison to the inhibition of Btk with an IC_{50} = 0.46 nM). Whether RR interval prolongation and decreased heart rate was an off-target effect via dopamine inhibition is not certain.

Minimum to mild decreases in cortical and trabecular bones were found in rats treated at ≥100 mg/kg for 13 weeks. The mechanism underlying the bone findings was not determined. It may be a direct ibrutinib effect, or possibly associated with acute inflammation. Inflammation-mediated osteopenia (IMO) during acute inflammation in growing rats, due to a transient inhibition of bone formation, was reported (Pfeilschifter *et al.*, Calcif Tissue Int. 41: 321-325, 1987). However, the link between inflammation and mild bone loss in fully grown rats remained unknown.

See Section 1.2 for discussion of genetic toxicity and reproductive and developmental toxicities of PCI-32765.

12 Appendix/Attachments

None

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

/s/

SHWU LUAN LEE
08/20/2013

HALEH SABER
08/20/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

NDA Number: 205552 **Applicant: Pharmacyclics, Inc.** **Stamp Date: June 28, 2013**

Drug Name: Ibrutinib **NDA Type: 505 (b) (1)**

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

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

File name: Pharmacology_Toxicology Filing Checklist for NDA 08082013

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		Comparisons of human exposures to animal exposures are expressed as safety factors in terms of AUC.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		An ongoing toxicology study is dedicated to qualify the proposed specifications for impurities (b) (4) and the data are expected to be submitted in December 2013. The Agency concurred with the plan in a pre-NDA meeting (4/6/2013).
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.

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

If the NDA/BLA 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.

Shwu-Luan Lee — August 7, 2013

Reviewing Pharmacologist — Date

Team Leader/Supervisor — Date

File name: Pharmacology_Toxicology Filing Checklist for NDA 08082013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA

APPEARS THIS WAY ON ORIGINAL

File name: Pharmacology_Toxicology Filing Checklist for NDA 08082013

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/s/

SHWU LUAN LEE
08/08/2013

HALEH SABER
08/08/2013