CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207997Orig1s000
207997Orig2s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Date: March 9, 2017
From: Christopher Sheth, PhD
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 207997
Drug: RYDAPT (midostaurin)
Indications: 1.1 Newly diagnosed acute myeloid leukemia (AML) that is FLT3 mutation-positive as detected by an FDA-approved test, in combination with standard cytarabine and daunorubicin induction and cytarabine consolidation.
1.2 Aggressive systemic mastocytosis (ASM), systemic mastocytosis with associated hematological neoplasm (SM-AHN), or mast cell leukemia (MCL).
Applicant: Novartis Pharmaceuticals Corp.

Midostaurin is a small molecule inhibitor of fms-like tyrosine kinase-3 (FLT3) and tyrosine kinase KIT (KIT). Midostaurin also inhibits many other kinases with low nanomolar potency. The antiproliferative and/or proapoptotic effects of midostaurin were demonstrated in mast cell lines and in mast cells from a patient with systemic mastocytosis. In addition, apoptosis and/or cell cycle arrest was observed in acute myeloid leukemia (AML) cells expressing wild-type FLT3 and in cells overexpressing FLT-3 internal tandem duplication mutant (FLT3-ITD). Anticancer activity of midostaurin was demonstrated in vitro using different types of cells and in xenograft models of AML. The established pharmacological class for midostaurin is kinase inhibitor.

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 reflected the intended clinical use. Rats and dogs exhibited midostaurin-related toxicities in the heart, lungs, GI tract, liver, lymph nodes, spleen, thymus, bone marrow, kidney, and glandular tissues. The pancreas, uterus, and ovaries were also adversely affected by midostaurin in that rat, and dogs exhibited inflammation in the brain. Midostaurin crossed the blood-brain barrier in tissue distribution studies. Midostaurin is not a potent inhibitor of the hERG channel. Other adverse findings in animal safety pharmacology studies include dose-related decreases in mean arterial pressures and heart rates in addition to severe hypotension and respiratory arrest. A juvenile toxicology study in rats was also conducted at lower doses and exposures than those associated with major reproductive toxicity in rats and the observed organ toxicities were consistent with those expected from midostaurin administration.

Midostaurin was not genotoxic in the battery of genetic toxicology studies conducted. Midostaurin may impair male and female fertility. When male and female rats were

administered midostaurin during a fertility study, midostaurin treatment was associated with testicular degeneration and atrophy, reduced sperm count and motility, and decreased reproductive organ weights in males. In addition, females exhibited increased resorptions (including total resorption of litter), decreased pregnancy rates, and decreased numbers of implants and live embryos. Based on this information the recommendation that females use effective contraception during treatment and for 4 months following the last dose of midostaurin was deemed acceptable for the RYDAPT label.

Pharmacokinetic studies in pregnant rats and rabbits also demonstrated that midostaurin can be transferred to the fetus in utero and in milk during lactation. The effects of midostaurin treatment on reproductive and developmental toxicology study endpoints included embryolethality and fetotoxicity in rats and rabbits, at dose levels that were generally maternally toxic, but notably at dose levels corresponding to exposures well below human exposures at the recommended human doses of 50 and 100 mg BID. In an embryo-fetal development study in rats, late embryofetal death, reduced fetal weight with effects on fetal growth including dilated brain ventricles, severe renal pelvic cavitation, reduced skeletal ossifications, malpositioned ovary and uterus, extra rib, and widened anterior fontanelle were observed in the absence of maternal toxicity. Also, in a pre- and postnatal development study in rats, midostaurin was maternally toxic (decreased body weight and body weight gain) with adverse effects on maternal performance (dystocia and decreases in number of live offspring). The specific hazard is that the intended patient populations for the drug may include pregnant females that receive chemotherapy regimens for their disease. Because of the risks identified in the animal reproductive and developmental toxicology studies, midostaurin cannot be safely added to those regimens. While the developmental toxicology studies in animals revealed fetal variations but not malformations, clinical practices are such that the review team concluded that a boxed warning for embryo-fetal toxicity is warranted.

It is noteworthy that the two predominant active metabolites observed in humans following midostaurin administration (CGP52421 and CGP62221) were not assessed in dedicated GLP-compliant genotoxicity or reproductive and developmental toxicity studies. The general toxicology of the metabolites were partially characterized in animal species that produce one or both of the metabolites, namely the rat (CGP52421) and dog (CGP52421 and CGP62221). While the AML indication being sought is an advanced cancer, the ASM indication is not; however, the principles of ICH S9 may be used since the prognosis of ASM is poor and some subtypes involve risk of leukemic transformation.. Thus additional studies to assess the genotoxicity of the metabolites were not considered necessary.

The risk for reproductive and developmental toxicities arising from exposure to the CGP52421 metabolite were characterized in the rat and rabbit, as both species produce appreciable amounts of the metabolite (relative to humans) in vivo. There was inadequate exposure to the CGP62221 metabolite in the rat and rabbit studies. Pharmacology/Toxicology ultimately determined that the reproductive and developmental studies provided for review were adequate given 1) the indications being currently sought and 2) the toxicities identified with the parent molecule (midostaurin) itself as described above. In silico modeling also suggested the parent and metabolites have similar genotoxicity profiles. If additional

indications are sought at a later date, additional investigations of the potential reproductive and developmental risks arising from exposure to the CGP62221 metabolite may be needed.

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

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

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/s/

CHRISTOPHER M SHETH
03/09/2017

MEMORANDUM

RYDAPT (midostaurin)

Date: March 9, 2017
To: File for NDA 207997
From: John K. Leighton, PhD
Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Rydapt conducted by Dr. Simpson, and secondary memorandum and labeling provided by Dr. Sheth. I concur with Dr. Sheth's conclusion that Rydapt may be approved for the proposed indications and that no additional nonclinical studies are needed at this time.

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/s/

JOHN K LEIGHTON
03/09/2017

MEMORANDUM

Date:	February 7, 2017
From:	Christopher Sheth, PhD Pharmacology/Toxicology Supervisor Division of Hematology Oncology Toxicology (DHOT) Office of Hematology and Oncology Products (OHOP)
Re:	Approvability for Pharmacology and Toxicology
NDA:	207997
Drug:	RYDAPT (midostaurin)
Indications:	Advanced systemic mastocytosis and FLT3 mutation positive acute myeloid leukemia
Applicant:	Novartis Pharmaceuticals Corp.

Midostaurin is a small molecule inhibitor of fms-like tyrosine kinase-3 (FLT3) and tyrosine kinase KIT (KIT). Midostaurin also inhibits many other kinases with low nanomolar potency. The antiproliferative and/or proapoptotic effects of midostaurin were demonstrated in mast cell lines and in mast cells from a patient with systemic mastocytosis. In addition, apoptosis and/or cell cycle arrest was observed in acute myeloid leukemia (AML) cells expressing wild-type FLT3 and in cells overexpressing FLT-3 internal tandem duplication mutant (FLT3-ITD). Anticancer activity of midostaurin was demonstrated in vitro using different types of cells and in xenograft models of AML. The established pharmacological class for midostaurin is kinase inhibitor.

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 reflected the intended clinical use. Rats and dogs exhibited midostaurin-related toxicities in the heart, lungs, GI tract, liver, lymph nodes, spleen, thymus, bone marrow, kidney, and glandular tissues. The pancreas, uterus, and ovaries were also adversely affected by midostaurin in that rat, and dogs exhibited inflammation in the brain. Midostaurin and/or its metabolites crossed the blood-brain barrier in tissue distribution studies. Midostaurin is not a potent inhibitor of the hERG channel. Other adverse findings in animal safety pharmacology studies include dose-related decreases in mean arterial pressures and heart rates in addition to severe hypotension and respiratory arrest. A juvenile toxicology study in rats was also conducted at lower doses and exposures than those associated with major reproductive toxicity in rats and the observed organ toxicities were consistent with those expected from midostaurin administration.

Midostaurin was not genotoxic in the battery of genetic toxicology studies conducted. Midostaurin may impair male and female fertility. When male and female rats were administered midostaurin during a fertility study, midostaurin treatment was associated with testicular degeneration and atrophy, reduced sperm count and motility, and decreased reproductive organ weights in males. In addition, females exhibited increased resorptions

(including total resorption of litter), decreased pregnancy rates, and decreased numbers of implants and live embryos. Based on this information the recommendation that females use effective contraception during treatment and for 4 months following the last dose of midostaurin was deemed acceptable for the RYDAPT label.

The midostaurin review team decided to include a boxed warning on the RYDAPT label to highlight the risks of embro-fetal toxicity. While the developmental toxicology studies in animals revealed fetal variations but not malformations, clinical practices are such that a boxed warning is warranted. Pharmacokinetic studies in pregnant rats and rabbits also demonstrated that midostaurin can be transferred to the fetus in utero and in milk during lactation. The specific hazard is that the intended patient populations for the drug include pregnant females that sometimes receive chemotherapy regimens for their disease. Because of the risks identified in the animal reproductive and developmental toxicology studies, midostaurin cannot be safely added to those regimens.

The effects of midostaurin treatment on reproductive and developmental toxicology study endpoints included embryolethality and fetotoxicity in rats and rabbits, at dose levels that were generally maternally toxic, but notably at dose levels corresponding to exposures well below human exposures at the recommended human doses of 50 and 100 mg BID. In an embryo-fetal development study in rats, late embryofetal death, reduced fetal weight with effects on fetal growth including dilated brain ventricles, severe renal pelvic cavitation, reduced skeletal ossifications, and widened anterior fontanelle were observed in the absence of maternal toxicity. Also, in a pre- and postnatal development study in rats, midostaurin was maternally toxic (decreased body weight and body weight gain) with adverse effects on maternal performance (dystocia and decreases in number of live offspring).

The nonclinical studies were reviewed by Dr. Natalie Simpson. The nonclinical findings are summarized in the “Executive Summary” and “Integrated Summary” of the NDA review and reflected in the product label.

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

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/s/

CHRISTOPHER M SHETH
02/07/2017

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:	207997
Supporting document/s:	SDN 3 and 4
Applicant's letter date:	April 5, 2016 (Part 1 Rolling); May 26, 2016 (Part 2 Rolling); July 25, 2016 (Part 3 Rolling); August 29, 2016 (Part 4 Rolling)
CDER stamp date:	August 29, 2016
Product:	RYDAPT (midostaurin, PKC412)
Indication:	Advanced systemic mastocytosis and FLT3 mutation positive acute myeloid leukemia
Applicant:	Novartis Pharmaceuticals Corp.
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)
Reviewer:	Natalie E Simpson, PhD
Supervisor/Team Leader:	Christopher M Sheth, PhD
Division Director:	John Leighton, PhD (DHOT) Ann Farrell, MD (DHP)
Project Manager:	Kimberly Scott, RN, BSN, OCN

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of NDA 207997 are owned by Novartis Pharmaceuticals Corp. or are data for which Novartis Pharmaceuticals Corp. has obtained a written right of reference. Any information or data necessary for approval of NDA 207997 that Novartis Pharmaceuticals Corp. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 207997.

Reference ID: 4051584

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1 Executive Summary

1.1 Introduction

NDA 207997 has been submitted for midostaurin for the treatment of patients with advanced systemic mastocytosis (ASM) and FLT3 mutation positive acute myeloid leukemia (AML). Midostaurin is a multi-kinase inhibitor with most characterized activity against fms-like tyrosine kinase-3, FLT3 (wild-type, internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutants), the cellular homolog of the feline sarcoma viral oncogene v-kit, c-KIT (KIT) mutant kinase, platelet derived growth factor receptor (PDGFR), and members of the serine/threonine kinase PKC (protein kinase C) family. The proposed clinical dose of 50 or 100 mg is to be administered orally as a soft capsule twice a day (BID) in patients with ASM and AML, respectively. Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of midostaurin for the proposed indications.

1.2 Brief Discussion of Nonclinical Findings

In in vitro biochemical and/or cellular assays, midostaurin (RYDAPT, PKC412, CGP41251, NVP-PKC412) and its predominant metabolites CGP62221 and CGP52421, inhibited many kinases in the therapeutically relevant nanomolar range, including FLT3 (wild-type, ITD and TKD mutants), KIT (D816V mutant), spleen tyrosine kinase (SYK), colony-stimulation factor -1 (CSF-1R, c-FMS), PDGFRα, PDGFRβ, proto-oncogene tyrosine-protein kinase ROS1 (specifically inhibited by CGP62221), fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor 2 (VEGFR2, KDR), as well as members of the PKC family. Inhibitory activity of midostaurin against the FLT3-ITD and D835Y TKD mutants was more potent than FLT3 wild-type; more potent for KIT (D816V mutant) kinase than wild-type KIT; and, more potent for PDGFR than insulin-like growth factor receptor (IGF-1-R), epidermal growth factor receptor (EGF-R), insulin receptor (Ins-R), KIT wild-type, VEGFR2, and PKC kinases. The potency of CGP52421 was lower for FLT3, PDGFRα, and PKC enzymatic inhibition compared to midostaurin, as well as in in vitro cellular assays. Despite this difference, CGP52421 may also to contribute to PKC412's therapeutic activity in AML since trough levels in patients for the metabolite are higher. While targeting of these kinases by midostaurin has demonstrated antitumor activity in AML cell lines, mast cells (primary cells and cell lines), and in vivo mouse xenograft models for AML, the flip side is that inhibition of these kinases is also associated with many of the adverse events observed with midostaurin treatment: hypertension, proteinuria, edema, gastrointestinal (GI) toxicity, left ventricular ejection fraction (LVEF) dysfunction, and neutropenia with susceptibility for infections.[1]

Safety pharmacology studies suggest a low potential for midostaurin or its predominant metabolites to inhibit the human Ether-à-go-go-Related Gene (hERG) potassium channel. Studies in rats indicate midostaurin has diuretic potential and vasodilatory properties. Midostaurin also demonstrated some potential to cross the blood: brain

[1] https://jzliu.shinyapps.io/KINASE

barrier in tissue distribution studies and can induce dopamine, serotonin, noradrenaline, and reduce GABA release, without majorly affecting neurotransmitter uptake. In mice, single administrations of midostaurin caused ataxia at therapeutically relevant doses.

Multiple tissue distribution studies in rats and rabbits demonstrate midostaurin distributes to most organs, with the GI tract, liver, kidneys, and glandular tissue (including pancreas) having the highest concentrations of midostaurin following oral administration. Midostaurin is absorbed slower in animal models than humans, but overall midostaurin has comparable pharmacokinetics across species (e.g., CGP52421 is a predominant metabolite and has a longer half-life than the parent, excretion is hepatobiliary, with little detected in urine, and concentration versus time profiles are biphasic with a long terminal half-life). An exception is that CGP62221 is not present in the rat and is only present at low levels in the rabbit, and additionally targets ROS1 kinase, which may under predict potential reproductive and developmental toxicity. Pharmacokinetic studies in pregnant rats and rabbits also demonstrated that midostaurin can be transferred to the fetus in utero and in milk during lactation.

In single dose toxicology studies, toxicities observed in the mouse were predominately neurological in nature, but GI toxicities were more pronounced in rats and dogs. The primary target organs following repeated dosing of midostaurin in rats and dogs were comparable, with toxicity to the GI tract, liver, kidney, immune system, heart (prolongation in P-Q interval), lung (bronchopneumonia, pneumonitis), male and female reproductive systems, and glands (pancreas, thyroid, adrenal, lacrimal, salivary and submaxillary). Pancreatic toxicity in rats mainly occurred in doses that resulted in lethality, except in juvenile rats. In the general toxicology studies, most findings were reversible once dosing stopped, except uterine dilation in rats, decreases in serum albumin and urine specific gravity in rats, and anemia in rats, which were still present after 1 month of recovery. Given that the doses administered to rats and dogs resulted in AUC exposures that are much lower than those observed in patients at clinical doses of 50 and 100 mg BID (~166 µmol.hr/L), the aforementioned toxicities may be clinically relevant.

Midostaurin was not mutagenic in vitro in the bacterial reverse mutation assay (Ames test) or in Chinese hamster V97 cells. Midostaurin increased the frequency of polyploidy cells in an in vitro chromosomal aberrations assay in Chinese hamster ovary cells, but was not clastogenic in an in vivo rat bone marrow micronucleus assay when tested up to the maximum tolerated dose (MTD) of 200 mg/kg (1200 mg/m2). This dose was approximately 20-fold the recommended human dose, based on body surface area. A GLP Ames test was not conducted to determine whether the midostaurin metabolites are mutagenic, however, non-GLP Ames tests in two bacterial strains and in silico modeling did not reveal any genotoxic potential for CGP62221 or CGP52421. In a GLP Ames test and an in vitro chromosomal aberrations assay in human peripheral blood lymphocytes, the degradation product (b) (4) demonstrated a comparable aneugenic profile as midostaurin, without demonstrating mutagenic or clastogenic potential.

An extensive reproductive toxicology package was submitted with the midostaurin application that demonstrated midostaurin to be embryo-lethal and fetotoxic, with effects on fertility parameters and maternal performance. In a male and female fertility study in rats, reproductive toxicity was observed in males, including testicular degeneration/atrophy, reduced sperm count and motility, and decreased male reproductive organ weights. In females, increased resorptions (including total resorption of litter), decreased pregnancy rate, and decreased number of implants and live embryos were observed. In an embryo-fetal development study in rats, late embryofetal death (increased late resorptions), reduced fetal weight with effects on fetal growth including dilated brain ventricles, severe renal pelvic cavitation, reduced skeletal ossifications, and widened anterior fontanelle were observed in the absence of maternal toxicity. In an embryo-fetal development study in rabbits, midostaurin was maternally toxic (reduced body weight (BW) gain and food consumption, dose-related increase in spontaneous abortions) and fetotoxic (decreased fetal weight, with reduced gall bladder size and reduced skeletal ossifications). In a pre- and postnatal development study in rats, midostaurin was maternally toxic (decreased body weight and body weight gain) with adverse effects upon maternal performance (dystocia and decrease in the number of live offspring). Midostaurin was fetotoxic with irreversible effects of reduced body weight still present at postnatal day 78 and impaired fertility when male and female offspring were mated (decreased number of implantation sites and live fetuses/litter). The doses in which these adverse effects of reproduction and development occurred corresponded to exposures well below human exposure at the recommended human doses of 50 and 100 mg BID. A juvenile toxicology study in rats was also conducted at lower doses and exposures than those associated with major reproductive toxicity in rats and the observed organ toxicities were consistent with those expected from midostaurin administration.

Based on internal discussions amongst the midostaurin review team, it has been decided to include a boxed warning on the label to highlight the risks of embryo-fetal toxicity. The considerations leading to inclusion of the boxed warning were that: 1) the clinical practice is such that the intended populations include females and males of reproductive potential in addition to pregnant females that sometimes receive chemotherapy regimens for their disease and 2) this drug cannot be safely added to those regimens because of the risks identified in the animal reproductive and developmental toxicology studies.

1.3 Recommendations

1.3.1 Approvability

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

1.3.2 Additional Non Clinical Recommendations

None

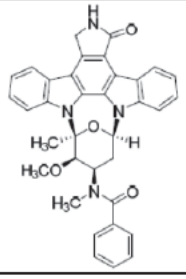
Reference ID: 4051584

1.3.3 Labeling

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

2 Drug Information

2.1 Drug

CAS Registry Number	120685-11-2
Generic Name	Midostaurin
Code Name	PKC412, CGP41251, NVP-PKC412
Chemical Name	(N-[(9S,10R,11R,13R)-N-(2,3,10,11,12,13-hexahydro-10-methoxy-9-methyl-1-oxo-9,13-epoxy-1H,9H-diindolo[1,2,3-gh:3',2',1'-lm]pyrrolo[3,4-j][1,7]benzodiazonin-11-yl)-N-methylbenzamide)
Molecular Formula/ Molecular Weight	$C_{35}H_{30}N_4O_4$/ 570.64
Structure or Biochemical Description	
Pharmacologic class	Kinase Inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

The IND 57120 was the original submission for PKC412 by Novartis Pharmaceuticals.

2.3 Drug Formulation

The drug product is a soft capsule containing 25 mg of midostaurin and the excipients [(b) (4)]/polyoxyl 40 hydrogenated castor oil, gelatin, [(b) (4)]/polyethylene glycol 400, [(b) (4)] [(b) (4)]/dehydrated alcohol, corn oil-mono-di-triglycerides, titanium dioxide ([(b) (4)]/Vitamin E, [(b) (4)] yellow/ferric oxide [(b) (4)], [(b) (4)] red/ferric oxide [(b) (4)], carmine [(b) (4)], hypromellose 2910, propylene glycol and purified water.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

Table 1 Summary of Proposed Limits and Justifications of Impurities for Midostaurin

Chemical	Proposed Limit	Acceptable Y/N	Justification
(b) (4)			

2.6 Proposed Clinical Population and Dosing Regimen

Midostaurin at 50 or 100 mg is to be administered orally as a soft capsule BID in patients with ASM and AML, respectively.

(b) (4)

2.7 Regulatory Background

Novartis Pharmaceuticals submitted their original application for PKC412 under IND 57120 in 1998, (b) (4) The NDA 207997 was received from Novartis as a rolling submission between April and August of 2016, with the PDUFA goal date based on the date of the last submission, August 29, 2016.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

Study#	Title	Module
PKF-98-02545	In Vitro Enzymatic and Cellular Profile of CGP41251, a Derivative of Staurosporine (Biology Report IT 55/93. 1993)	4.2.1.1.
PKF-98-02546	Kinetic Properties and Pharmacological Profile of CGP 41251 (Biology Report 90/89, Annex to BBB/IT 10/89)	4.2.1.1.
PKF-98-02547	In Vitro Antiproliferative Activity of CGP41251:The NCI and In-House Antiproliferative Screens	4.2.1.1.
PKF-98-02553	Effect of Plasma (and Components) on the CGP41251 Mediated Inhibition of PKC-α and In Vitro Antiproliferative Activities	4.2.1.1.
RD-2004-01878	Antiproliferative Activity of PKC412 in Various Human AML Cell Lines	4.2.1.1.
RD-2008-01357	Biological Profile of PKC412 and Two Metabolites	4.2.1.1.
RD-2014-00163	Dose Dependent Anti-tumor Efficacy of Midostaurin in an AML Xenograft Model	4.2.1.1.

Secondary Pharmacology

Study #	Title	Module
RD-2016-00522-v2	CGP052421-NX-1: Secondary Pharmacology Profile	4.2.1.2.
RD-2016-00523-v2	CGP062221-NX-1: Secondary Pharmacology Profile	4.2.1.2.
RD2016-00531	CGP041251-NX-1: Secondary Pharmacology Profile	4.2.1.2.

Safety Pharmacology

Study #	Title	Module
PCS-R0250111	Electrophysiological Safety Measurements of hERG Currents in Stably Transfected HEK293 Cells (not GLP)	4.2.1.3.
PCS-R0770504	Effects of 512-03 (Hydroxy-PKC412) on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	4.2.1.3.
PCS-R0770505	Effects of Desmethyl-PKC412 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells	4.2.1.3.
PKF-90-02620	Effects of CGP 41251 (Protein Kinase C Inhibitor) on Neurotransmitter release and uptake and on Phosphatidylinositol Turnover (Biology Report 18/90)	4.2.1.3.
PKF-90-02621	CNS Evaluation of CGP41251 (Protein Kinase C Inhibitor) (Biology Report 36/90)	4.2.1.3.
PKF-90-02622	Evaluation of Cardiovascular and Renal Effects of CGP 41251 (PKC412) (Protein Kinase C Inhibitor; Antitumor Agent) (Biology Report 43/90)	4.2.1.3.

Pharmacokinetics

Study #	Title	Module
Absorption		
DMPK-R0201773	Absorption, Metabolism, and Excretion Following an Intravenous or Oral dose of [^{14}C]PKC412 in the Rat	4.2.2.2.
DMPK-R0900139	Absorption, Metabolism and Excretion of [^{14}C]PKC412 following Oral and Intravenous Dosing in Rabbits	4.2.2.2.
DMPK-R0900185	Absorption, Metabolism and Excretion of [^{14}C]PKC412 Following Oral (3 mg/kg) and Intravenous (0.5 mg/kg) Dosing in Dogs	4.2.2.2.
DMPK-R0900185A	Absorption, Metabolism, and Excretion of ^{14}C-PKC412A Following Oral or Intravenous Administration to Dogs	4.2.2.2.
DMPK-R71993	Absorption and Disposition of ^{14}C-labeled CGP41251 in Rats and Dogs	4.2.2.2.
Distribution		
DMPK-R0300432	In Vitro Plasma and/or Serum Protein Binding of [^{14}C]PKC412 in the rat, dog and human	4.2.2.3.
DMPK-R0300868	In Vitro Human Plasma Protein Binding of [^{14}C]PKC412 in the Presence of CGP52421	4.2.2.3.
DMPK-R0301334	In Vitro Human Plasma Protein Binding of [^{14}C]PKC412 in the Presence of High Concentrations of α1-Acid Glycoprotein (AGP) (reviewed by Clinical Pharmacology)	4.2.2.3.
DMPK-R0800059	In Vitro Plasma Protein Binding of [^{14}C]PKC412 by Equilibrium Gel Filtration for the Rat, Dog, and Human	4.2.2.3.
DMPK-R0900286	Tissue Distribution of Radioactivity Following an Oral Dose of [^{14}C]PKC412 in the Pregnant Rat	4.2.2.3.
DMPK-R1500080	In Vitro Plasma Protein Binding of [14C]CGP52421 and [^{3}H]CGP62221 (Metabolites of PKC412) in Rat, Rog, and Human Plasma using Equilibrium Gel Filtration	4.2.2.3.
DMPK-R301996	Transfer of Radioactive Substance(s) to the Embryo-Fetal Compartment of Rats after Peroral Administration of 30 mg/kg ^{14}C-Labelled CGP41251	4.2.2.3.
DMPK-R51996	Transfer of Radioactive Substance(s) to the Embryo-Fetal Compartment of Rabbits after Peroral Administration of 20 mg/kg ^{14}C-Labelled CGP41251	4.2.2.3.
Metabolism		
DMPK-DM101993	Characterization of Radioactive Substances Excreted with Bile and Feces of Rats and Dogs Dosed with [^{14}C]CGP41251	4.2.2.4.
DMPK-R0200452	In Vitro Metabolism of NVP-PKC412 by Human Cytochrome P450 Enzymes	4.2.2.4.
DMPK-R1997520	Semiquantitative Determination of CGP41251 and Three Metabolites in Plasma of Rats after 6 Months of Peroral Treatment with 30 mg/kg CGP41251	4.2.2.4.
DMPK-R1997521	Semiquantitative Determination of CGP41251 and Three Metabolites in Plasma of Dogs after 6 and 12 Months of Peroral Treatment with 10 mg/kg CGP41251	4.2.2.4.
Excretion		
199DMPK-R0900142	Excretion in Milk after a Single Oral Dose of [^{14}C]PKC412 in the Rat	4.2.2.5.

Single Dose Toxicology

Study#	Title	Module
PCS-926034	CGP41251: Acute Oral Toxicity Study in Dogs	4.2.3.1.

Reference ID: 4051584

PCS-936215	CGP41251: Acute Oral Toxicity Study in Mice	4.2.3.1.
PCS-946016	CGP41251: Acute Oral Toxicity Study in Mice	4.2.3.1.
PCS-926033	CGP41251: Acute Oral Toxicity Study in Rats	4.2.3.1.
PCS-936216	CGP41251: Acute Oral Toxicity Study in Rats	4.2.3.1.

Repeat Dose Toxicology

Study#	Title	Module
DMPK-R1996089	Plasma Concentrations of CGP41251 in a 6/12 Month Oral Toxicity Study in Dogs	4.2.3.2.
PCS-R946003	CGP41251: 6-12 Month Oral Toxicity Study in Dogs	4.2.3.2.
DMPK-R91993	Plasma Concentrations of CGP41251 on Days 1 and 92 of a 3-Month Oral Toxicity Study in Dogs	4.2.3.2.
PCS-926041	CGP41251: 3-Month Oral Toxicity Study in Dogs	4.2.3.2.
DMPK-R1995083	Plasma Concentrations of CGP41251 in a 6/12-Month Pharmacokinetic Study in Rats	4.2.3.2.
PCS-R936281	6/12 Month Oral Toxicity Study in Rats	4.2.3.2.
PCS-956016	CGP41251: 26-week Oral (Gavage) Toxicity Study in Rats (MIN 954096)	4.2.3.2.
PCS-R111993	Plasma Concentrations of CGP41251 on Days 1 and 90 of a 3-Month Oral Toxicity Study in Rats	4.2.3.2.
PCS-926037	CGP41251: 3-Month Oral Toxicity Study in Rats	4.2.3.2.

Genetic Toxicology

Study#	Title	Module
PCS-926113	CGP41251: Salmonella and Escherichia/liver-Microsome Test	4.2.3.3.1.
PCS-926298	CGP41251: Gene Mutation Test with Chinese Hamster Cells V79 In Vitro	4.2.3.3.1.
PCS-926300	CGP41251: Cytogenetic Test on Chinese Hamster Cells In Vitro	4.2.3.3.1.
PCS-RAFP59	Bacterial Mutagenicity test on CGP41251	4.2.3.3.1.
PCS-R926299	CGP41251: Micronucleus Test, Rat In Vivo Study	4.2.3.3.2.

Reproductive Toxicology

Study#	Title	Module
PCS-964123	CGP41251: An Oral Study for Effects on Fertility and Early Embryonic Development in Rats	4.2.3.5.1.
PCS-936241	CGP41251: Segment II (Teratology) Study in Rats by Oral Administration	4.2.3.5.2.
PCS-936243	CGP41251: Segment II (Teratology) Study in Rabbits by Oral administration	4.2.3.5.2.
PCS-0770270	PKC412: An Oral (Gavage) Pre and Postnatal Study in the Rat	4.2.3.5.3.
PCS-R0870600	PKC412: An Oral (Gavage) Toxicity Study in the Juvenile Albino Rat	4.2.3.5.4.

Other Toxicity Studies:

Study#	Title	Module
	Metabolites	
PCS-LR1519360	In Silico Prediction of Potential Mutagenic Properties	4.2.3.7.5.
	Impurities	
PCS-R0770319	(b) (4) (Related Substance of PKC412): Mutagenicity	4.2.3.7.6.

Reference ID: 4051584

	Test using Salmonella Typhimurium	
PCS-R0770320	(b) (4) (Related Substance of PKC412): Chromosome Aberration Test with Cultured Human Peripheral Blood Lymphocytes	4.2.3.7.6.
Other		
PCS-R0770470	PKC412: A 4-Week Oral (Gavage) Impurity ((b) (4) Qualification Study in the Rat	4.2.3.7.7.

3.2 Studies Not Reviewed

See Appendix

3.3 Previous Reviews Referenced

Many studies were reviewed initially by Dr. Hua Zheng as part of the 30-Day safety decision under IND 57120. The reviewed studies were modified to maintain formatting consistency for this NDA review.

4 Pharmacology

Midostaurin was primarily developed and characterized as an inhibitor of the oncogenic mutant and wild-type forms of FLT3 and KIT receptor tyrosine kinases, a primary pharmacology that is useful in treating cancers that are driven by constitutively activated receptors. At the same time, due to the conserved arrangement of secondary structure elements that make up kinase proteins in general, midostaurin inhibits numerous other kinases which have been implicated in drug-related toxicities. Screens for secondary pharmacology revealed midostaurin also inhibits cycloooxygenase-1 (COX-1) and phosphodiesterase 4D (PDE4D). Safety pharmacology studies revealed high doses of midostaurin can result in reduced heart rates, mean arterial pressures, and respiration.

Of note, the canonical pathway of signal transduction mediated by FLT3 or KIT includes the receptor auto-phosphorylation after ligand stimulation followed by phosphorylation of secondary substrates like STAT proteins and MAPK (Mitogen-activated protein kinase) pathway activation. In neoplastic disease mediated by FLT3-ITD, additional downstream phosphorylation events have been described including PIM2, β-catenin and autaxin. In addition the PI3K/AKT (Phospho-inositol-3 kinase/ Protein kinase B) pathway is activated resulting in enhanced cell survival.

4.1 Primary Pharmacology

Kinase Selectivity and Anti-proliferative Activity of Midostaurin

Study title: Biological Profile of PKC412 and Two Metabolites

Study No.: RD-2008-01357
Report Date: July 10, 2015
Study report location: 4.2.1.1.
Conducting Laboratory: Novartis
GLP: No

Methods:

The inhibitory activity of compounds against a broad panel of kinases was determined using InVitrogen, SelectScreen™ (protocol available online). The Applicant noted that many of these kinases have been reported earlier to be susceptible to PKC412 inhibition.[7] Binding data is presented in the Appendix.

Antiproliferative activity was detected in the pro-B BaF3 cell line, expressing FLT3 wild-type or FLT3-ITD mutant or KIT mutants D816V or delVV599/560, following 48 or 72 hours (hr) incubation with PKC412, CGP52421, CGP62221, or vehicle (0.1 to 0.2% DMSO) using the The Alamar Blue™ Assay (Biosource International).

Results:
Midostaurin and its metabolites (1 μM) inhibited multiple kinases ≥ 80%. Some of these kinases (and others) had IC50 values within 30-fold of the most potent target, FLT3 D835Y, a TKD mutant.

Table 2 % Inhibition of Kinases (≥ 80%)

(Excerpted from the study report)

PKC412		CGP62221		CGP52421	
Kinase	% Inh	Kinase	% Inh	Kinase	% Inh
AURKA (Aurora A)	98	AKT1 (PKB alpha)	91	FLT3	91
EPHA2	89	AURKA (Aurora A)	93	FLT3 D835Y	96
FLT3 D835Y	89	EPHA2	93	NTRK1 (TRKA)	85
FYN	99	FLT3 D835Y	87	NTRK3 (TRKC)	83
NTRK2 (TRKB)	84	FYN	99	PDGFRA V561D	85
PTK2B (FAK2)	90	MAP4K2 (GCK)	101	RPS6KA1 (RSK1)	80
RPS6KA2 (RSK3)	96	PDGFRA D842V	84	BRSK1 (SAD1)	82
RPS6KA3 (RSK2)	90	PDGFRB (PDGFR β)	91	PHKG1	83
SGK (SGK1)	82	PRKCA (PKCα)	80	PKN1 (PRK1)	81
SRMS (Srm)	88	PRKCH (PKC eta)	80	STK4 (MST1)	81
TEK (Tie2)	90	RPS6KA1 (RSK1)	90	SYK	94
TYRO3 (RSE)	96	RPS6KA2 (RSK3)	82	TBK	87
		SGK (SGK1)	82	FLT3	91
		TYRO3 (RSE)	97		

[7] Fabbro D et al, 2000, PKC412- A Protein Kinase Inhibitor with a Broad Therapeutic Potential, Anti-Cancer Drug Design, 15: 17-28.

Table 3 IC50 Values for Inhibition

(Excerpted from the study report)

PKC412		CGP62221		CGP52421	
Kinase	IC50 [nM]	Kinase	IC50 [nM]	Kinase	IC50 [nM]
AURKA (Aurora A)	18.2	AURKA (Aurora A)	15.0	AURKA (Aurora A)	844
BRSK1 (SAD1)	31.8	BRSK1 (SAD1)	-	BRSK1 (SAD1)	-
CSF1R (FMS)	25.9	CSF1R (FMS)	-	CSF1R (FMS)	27.9
FLT3	19.8	FLT3	11.3	FLT3	59.5
FLT3 D835Y	3.64	FLT3 D835Y	2.29	FLT3 D835Y	10.2
MAP3K9 (MLK1)	22.6	MAP3K9 (MLK1)	39.0	MAP3K9 (MLK1)	47.4
NTRK1 (TRKA)	11.4	NTRK1 (TRKA)	14.2	NTRK1 (TRKA)	-
NTRK2 (TRKB)	50.8	NTRK2 (TRKB)	49.1	NTRK2 (TRKB)	-
NTRK3 (TRKC)	15.2	NTRK3 (TRKC)	8.35	NTRK3 (TRKC)	-
PDGFRA (PDGFRα)	34.2	PDGFRA (PDGFRα)	-	PDGFRA (PDGFRα)	223
PDGFRB (PDGFRβ)	35.2	PDGFRB (PDGFRβ)	-	PDGFRB (PDGFRβ)	-
PHKG1	11.9	PHKG1	-	PHKG1	-
PKN1 (PRK1)	15.6	PKN1 (PRK1)	-	PKN1 (PRK1)	81.0
PRKCA (PKC α)	203	PRKCA (PKC α)	152	PRKCA (PKC α)	827
PRKCB1 (PKC βI)	263	PRKCB1 (PKC βI)	-	PRKCB1 (PKC βI)	631
PRKCB2 (PKC β II)	119	PRKCB2 (PKC β II)	-	PRKCB2 (PKC β II)	-
RPS6KA1 (RSK1)	33.6	RPS6KA1 (RSK1)	10.9	RPS6KA1 (RSK1)	-
RPS6KA2 (RSK3)	18.8	RPS6KA2 (RSK3)	24.0	RPS6KA2 (RSK3)	-
RPS6KA3 (RSK2)	43.8	RPS6KA3 (RSK2)	18.5	RPS6KA3 (RSK2)	-
STK4 (MST1)	50.9	STK4 (MST1)	-	STK4 (MST1)	-
SYK	8.38	SYK	3.11	SYK	110
TBK1	15.5	TBK1	-	TBK1	93.2
		FER	9.72		
		ROS1	22.9		

Table 4 Activity in FLT3 and KIT Expressing Cells

(Excerpted from the study report)

	Mean EC_{50} Value (nM) ± SEM (number of replicates)	
Compound	**BaF3 FLT3-ITD**	**BaF3 wt**
PKC412	39.0 ± 1.7 (3)	388 ± 11 (3)
CGP52421	656 ± 155 (3)	3660 ± 459 (3)
CGP62221	27.9 ± 5.6 (3)	327 ± 89 (3)
PKC412 (Weisberg et al. 2002)	*<10*	*>100*

	Mean EC_{50} Value (nM) ± SEM (number of replicates)	
Compound	**BaF3 KIT D816V**	**BaF3 KIT delVV559/560**
PKC412	47.4 ± 12.4 (4)	97.7 ± 32.0 (4)
CGP52421	232.7 ± 85.7 (4)	436.5 ± 129.4 (4)
CGP62221	71.4 ± 40.1 (4)	124.5 ± 48.4 (4)
NVP-AMN107	2537 ± 1012 (4)	24.8 ± 11.7 (4)
PKC412 (Growney et al 2005)	*44 (1)*	*146 (1)*

Similar results that PKC412 is more potent for FLT3-ITD than FLT-3 wild-type and that CGP52421 was roughly 10-fold less potent for FLT3 than PKC412 have been observed

in different assays.[8,9] Growney at al. also compiled data from various published references that the PKC412 IC50 for the KIT (D816V) mutant was lower (44 nM), compared to KIT wild-type (IC50=138 nM without IL-3 and 345 nM with IL-3) and the KIT (delVV559/560) mutant (IC50=146 nM).

Study title: In Vitro Enzymatic and Cellular Profile of CGP41251, a Derivative of Staurosporine (Biology Report IT 55/93. 1993)

Study No.:	PKF-98-02545
Report Date:	November 3, 2009
Study report location:	4.2.1.1.
Conducting Laboratory:	Novartis
GLP:	No

In vitro purification and assay methods for protein kinases:
The following kinases were purified and the inhibitory activity of staurosporine and its derivative, CGP41251, was measured using different methods, which generally used baculovirus or viral construct transfection of protein encoding constructs into cells, purification of recombinant kinases (or kinase domains), and measurement of the transfer of radiolabeled phosphate from ATP to a substrate in the presence of recombinant kinases: bovine PKC-α, human PKC-βI, human PKC-βII, human/bovine PKC-γ, rat PKC-δ, rat PKC-ε, rat PKC-ζ and mouse PKC-η; ERK-1 and JNK; P70-S6 (purified from rat livers); PKB; cdk1/cycB (purified from starfish oocytes), cdk4/cycD1, cdk2/cycE; Plk1; PKA and PPK; CK-1 and CK-2; EGF-R, c-src and v-abl; c-lyn, SYK (TPK-IIB) and c-fgr (TPKIII); KDR, Flt-1, Flk-1, Tie1, Tie2/Tek, c-Met and FGF-R-1.

In vitro cellular methods to measure inhibition by CGP41251 or staurosporine:

- Methods described in the legends of Tables 6-8.

Results:
In kinase assays of in vitro enzymatic activity, CGP41251 preferentially inhibited the conventional PKC subtypes (cPKCs: PKC-α, −βI, −βII and −γ: IC50=22-31 nM). CGP41251 was less potent for the non-conventional PKCs (nPKCs: PKC−δ, −ε, −η: IC50=0.16 to 1.25 μM) and had low potency for the atypical PKC-ζ (IC50=465 nM). CGP41251 also inhibited KDR (VEGFR2).

Note: In MBA-MB-231 cells, removal of CGP41251 or staurosporine followed by incubation with medium alone for 60 minutes resulted in the complete restoration of the intracellular PKC activity to control levels indicating reversible inhibition of intracellular PKC activity. This observation suggests that permanent intracellular availability of the compound is required to inhibit PKC mediated functions in vitro and presumably also in vivo.

[8] Weisberg E et al, 2002, Inhibition of Mutant Flt3 Receptors in Leukemia Cells by the Small Molecule Tyrosine Kinase Inhibitor PKC412, Cancer Cell, 1:433-43.
[9] Growney JD et al, 2005, Activation Mutations of Human c-KIT Resistant to Imatinib Mesylate are Sensitive to the Tyrosine Kinase Inhibitor PKC412, Blood, Jul 15,106(2):721-4.

Table 5 In Vitro Kinase Inhibition by CGP41251 and Staurosporine

(Excepted from the study report)

Enzyme	Presumed Activator or Function	IC50 (uM) PKC412	IC50 (uM) Staurosporine
cPKC-α	DAG/Ca2+/PS	0.022 ± 0.008	0.003 ± 0.0005
cPKC-β1	DAG/Ca2+/PS	0.030 ± 0.018	0.008 ± 0.0036
cPKC-β_2	DAG/Ca2+/PS	0.031 ± 0.016	0.003 ± 0.0008
cPKC-γ	DAG/Ca2+/PS	0.024 ± 0.006	0.004 ± 0.001
nPKC-δ	DAG/PS	0.33 ± 0.071	0.034 ± 0.014
nPKC-η	DAG/PS	0.16 ± 0.095	0.013 ± 0.006
nPKC-ε	DAG/PS	1.25 ± 1.06	0.043 ± 0.011
aPKC-ζ	unknown/PS	465 ± 49	3.11 ± 2.68
cdk1/cycB	Cell cycle (G2/M)	0.57 ± 0.126	0.005 ± 0.002
cdk4/cycD1	Cell cycle (G1/S)	>10.0	<10.0
Plk-1	Triggers cdc25 (G2/M)	>1.0	>1.0
PKB/Akt	"Activated" by PIP3	>300	0.835 ± 0.395
Erk-1	GF/ras/mitogenesis	> 200	1.5
JNK	Stress/cytokines	>10.0	Nt
P38 kinase	Stress/cytokines	>1.0	Nt
P70-S6 kinase	Protein synthesis	5.0 ± 2.0	0.005 ± 0.002
PPK	Glycogen metabolism	0.038 ± 0.006	0.007 ± 0.003
PKA	cAMP	0.57 ± 0.20	0.035 ± 0.018
MLCK	Mysoin light chain	1.90 ± 0.5	0.003 ± 0.001
CK-2	Proliferation marker	>200.0	17
CK-1	Proliferation marker	>200.0	144
c-Src	Signaling	0.80 ± 0.30	0.22 ± 0.04
c-Fgr	B-cell signaling	0.79 ± 0.21	0.02 ± 0.02
c-Lyn	B-cell signaling	4.30 ± 2.4	0.036 ± 0.04
c-Syk	B-cells/Eosinophil sign.	0.095 ± 0.05	0.016 ± 0.08
CSK	Y527-phos. of c-Src	8.0 ± 4.3	0.35 ± 0.12
v-abl	Signal transduction and CML	>100.0	0.14 ± 0.15
Flt-1	VEGF-R-1-TPK (Fms-like tyrosine kinase; angiogenesis)	0.912 ± 0.424	0.040 ± 0.03
KDR	VEGF-R-2-TPK (Kinase insert Domain-containing Receptor ; angiogenesis)	0.086 ± 0.04	0.057 ± 0.04
Flk-1 (mouse homolog of KDR)	VEGF-R-1-TPK (Fetal liver kinase- 1; tumor angiogenesis)	0.39	Nt
Tie1	Angiogenesis.	>1.00	Nt
Tie2/Tek	Receptor for Angiopoietin 1 and 2 (angiogenesis)	>1.00	Nt
FGF-1-R	Fibroblast growth factor receptor (angiogenesis)	>1.00	Nt
c-Met	Receptor for the hepatocyte GF (HGF or SF: scatter factor: angiogenesis motility)	>1.00	0.48
EGF-R	R-TPK for EGF (mitogenesis)	1.1 ± 0.5	0.025 ± 0.01

Purification and assays were performed as described in Methods. Nt: not tested. VEGF-R-1-TPK: VEGF receptor tyrosine protein kinase. Results are expressed as means ± SD (of at least 2 independent experiments).

Boxes represent kinases with IC50 values for CGP41251 inhibition < 100 nM.

In cellular assays, CGP41251 was most potent for inhibiting PDGFR phosphorylation induced by PDGF (IC50=80 nM) compared to inhibition of IGF-1-R, EGF-R, Ins-R, KIT

wild-type, VEGFR2, and PKC mitogen-stimulated phosphorylation. CGP41251 was also more potent for inhibition PDGF mitogen-induced c-fos mRNA expression (IC50= 100 nM) compared to stimulation by EGF, FGF, or TPA. The IC50s of CGP41251 for inhibiting mitogen-induced MAP-kinase and p70-S6 kinase activity were ≥500 nM.

Table 6 Effect of CGP41251 and Staurosporine on Tyrosine Phosphorylation Induced by Mitogenic Agents and Inhibition of PKC in Cells

(Excerpted from the study report)

			IC50 (uM)	
Cells	Growth Factor (GF)	GF-R	CGP 41251	Staurosporine
A31	Platelet derived growth factor (PDGF)	PDGF-R	0.08	≤ 0.010
Rat-1-InsR	Insulin (Ins)	Ins-R	>100	nt
NIH3T3 LisNc4	Insulin like growth factor I (IGF-I)	IGF-I-R	>100	nt
A431	Epidermal growth factor (EGF)	EGF-R	>100	1.0
Mo-7e	Stem cell factor (SCF)	c-Kit	0.3	Nt
CHO-KDR	Vascular endotehlial growth Factor (VEGF)	(KDR)	1.0 ± 0.1	Nt
MDA MB-231	none	PKC	0. 5 ± 0.43	0.05 ± 0.06

Cellular ELISAs as well as Western blotting were used to monitor the effects of compounds on the ligand induced tyrosine phosphorylation of the PDGF-R, the EGF-R, the c-kit, IGF-I-R, the Ins-R and the KDR measured by anti-phosphotyrosine antibodies after stimulation of of cells with the cognate growth factor as described in Methods. For the determination of the intracelllular PKC activity confluent human breast cancer MDA-MB-231 cells were incubated for 30 min at 37 C with increasing concentrations of CGP 41251 and staurosporine. Cell extracts were prepared and protein kinase activity was determined using protamine sulfate as exogenous substrate as described in Methods. Results are expressed as means ± SD (of at least 2 independent experiments).

Table 7 Inhibition by CGP41251 and Staurosporine of c-fos mRNA Expression Induced by Mitogenic Agents in Cells

(Excerpted from the study report)

	CGP 41251	staurosporine
Stimulus	IC50 (µM)	
PDGF-BB	0.1	0.05
EGF	>10.0	0.5
bFGF	1.0	nt
TPA	5.0	0.05

Quiescent BALB/c 3T3 (A31) cells were incubated for 90 min with increasing concentrations of CGP 41251. After stimulation with PDGF-BB (10 ng/ml), EGF (20 ng/ml), bFGF (10 ng/ml) or TPA (25 ng/ml) for 30 min, samples of total RNA were analyzed by Northern blotting using a v-fos probe as described in Methods. nt: not tested

Table 8 Inhibition by CGP41251 and Staurosporine of MAP kinase and p70s6 Kinase Activation Induced by Mitogenic Agents in Cells

(Excerpted from the study report)

	Inhibition of Map Kinase activation IC50 (μM)		Inhibition of p70S6 kinase activation IC50 (μM)	
	CGP 41251	Staurosporine	CGP 41251	Staurosporine
PDGF-BB	0.5 ± 0.4	0.3 ± 0.2	0.5 ± 0.8	0.05 ± 0.04
EGF	>10.0	1.0 ± 0.9	0.5 ± 0.6	0.01 ± 0.03
bFGF	2.0 ± 1.5	0.3 ± 0.2	0.5 ± 0.5	0.01 ± 0.02
TPA	5.0 ± 3.0	0.5 ± 0.4	0.5 ± 0.6	0.05 ± 0.03
VEGF	5.0	nt	nt	nt

Quiescent Swiss 3T3 cells in 24-well dishes were incubated for 30 min with CGP 41251 before stimulation with EGF (1 nM, 6 ng/ml), PDGF-BB (100 ng/ml), bFGF (50 ng/ml), TPA (100 nM) for exactly 5 min at 37° C. Map kinase activation by VEGF was performed in CHO-KDR as described in Methods. MAP-kinase and p70-S6 kinase activities were measured as described in Methods. Maximal stimulation of MAP-kinase and p70-S6 kinase activity (100%) was determinded in the absence of CGP 41251. Results are expressed as means ± SD (of at least 2 independent experiments).

Study title: Kinetic Properties and Pharmacological Profile of CGP41251 (Biology Report 90/89, Annex to BBB/IT 10/89)

Study No.: PKF-98-02546
Report Date: November 3, 2009
Study report location: 4.2.1.1.
Conducting Laboratory: Novartis
GLP: No

Methods:

- Inhibition of PKC (purified from porcine brain) activity by CGP41251 was measured, with transfer of radiolabeled phosphate from ATP to a substrate as the readout.
- Binding of the PKC activator [^{3}H]phorbol dibutyrate (PDBu) to PKC in the presence of CGP41251 or synthetic diacylglycerol DiC8 (inhibitor of PDBu to the regulatory domain of PKC) was measured using scintillation counting.
- Inhibition of PKC-α activity/phosphorylation of a protamine sulfate substrate was measured in ER-negative MDA-MB231 cell line lysates.
- In vitro antiproliferative effects of CGP41251 were measured in various cell lines.

Results:

- CGP41251 inhibited PKC activity with an IC50 of 50 nM and Ki of 45 nM. The inhibition caused by CGP41251 was reversed by increasing concentrations of ATP, indicating competitive binding with ATP. IC50 values for isozymes: PKC-α=16 nM, PKC-β=29 nM and PKC-γ=11 nM.
- Binding of the PKC activator [^{3}H]phorbol dibutyrate to the enzyme was not affected by CGP41251. Together, this suggests binding of CGP41251 to the catalytic domain.
- In MDA-MB-231 cells, staurosporine was one order of magnitude more potent than CGP41251. In repeat experiments the IC50 for the inhibition of intracellular PKC activity by CGP41251 was found to vary between 0.5 to 5.0 μM depending on the time of exposure to CGP41251.

- CPG41251 was most potent for antiproliferative activity of human promyelocytic leukemia cells (IC50=130 nM) of 6 tested cell lines.

Table 9 Anti-Proliferative Effects In Vitro (IC50 in µM)

(Excerpted from the study report)

	Cell lines					
(IC50: µM)	B 16	CT 26	2661	T 24	HL-60	CEC
CGP 41 251	0.77	0.32	0.37	0.23	0.13	0.20

B 16: mouse B16/BL6 melanoma cells
CT 26: mouse colon carcinoma cells
2661: mouse mamma adenocarcinoma 2661/61 cells
T24: human bladder carcinoma T 24 cells
HL-60: human promyelocytic leukemia cells
CEC: bovine corneal endothelial cells

- In preliminary toxicity studies in which BL/6 mice bearing B16/BL6 melanoma were administered CGP41251, the maximally tolerated dose (MTD) was estimated to be 250 mg/kg intraperitoneally (IP) and 750 mg/kg orally, with dose limiting toxicities of anemia (IP only) and leukopenia.

Study title: Antiproliferative Activity by PKC412 in Various Human AML Cell Lines

Study No.: RD-2004-01878
Report Date: September 21, 2015
Study report location: 4.2.1.1.
Conducting Laboratory: Novartis
GLP: No

Methods:
Leukemia cells in 96 well plates were assessed for viability following 48 hr incubation with PKC412 (alone or combination with daunorubicin, Ara-C, the farnesyl-transferase inhibitor tipifarnib, the differentiation inducing agent all-trans retinoic acid, and the HDAC inhibitor LAQ824), or vehicle (0.1% DMSO).

Results:

Table 10 Inhibition of Cell Proliferation by PKC412 in Various Leukemia Lines

(Excerpted from the Pharmacology Written Summary)

	Mean IC50 [nM] ± SEM (number of determinations)		
Cell line	Mutation	Growth Factor	Proliferation
MV4-11	FLT3-ITD homozygous K-ras-G12D/A18D	none	26.3 ± 7.1 (6)
MOLM13	FLT3-ITD heterozygous	none	48.4 ± 6.9 (3)
PL-21	FLT3-ITD heterozygous	none	>1000 (3)
OCI-AML5	wtFLT3	none	821 (1)
	wt-ras	GM-CSF	639 ± 70 (3)
MUTZ-2	wtFLT3 wt-ras	SCF	902 (1)
SEM	wtFLT3 wt-ras	none	91 ± 1 (2)
SEM-K2	wtFLT3 wt-ras	none	157 ± 76 (3)
EOL-1	FIP1L-PDGFR	none	8.1 ± 1.4 (3)
M07e *	wtFLT3	GM-CSF	183 ± 16 (3)
	wt-ras	SCF	186 ± 24 (3)
		IL-3	158 (1)
HL-60	wtFLT3 N-ras-Q61L	none	1334 ± 536 (3)

The effect of midostaurin on cell proliferation was determined with AlamarBlue (* or ATPLite, respectively) and calculated as percent inhibition and dose-response curves were used to calculate IC50 values, expressed as mean ± SEM, ( ) = number of experiments.

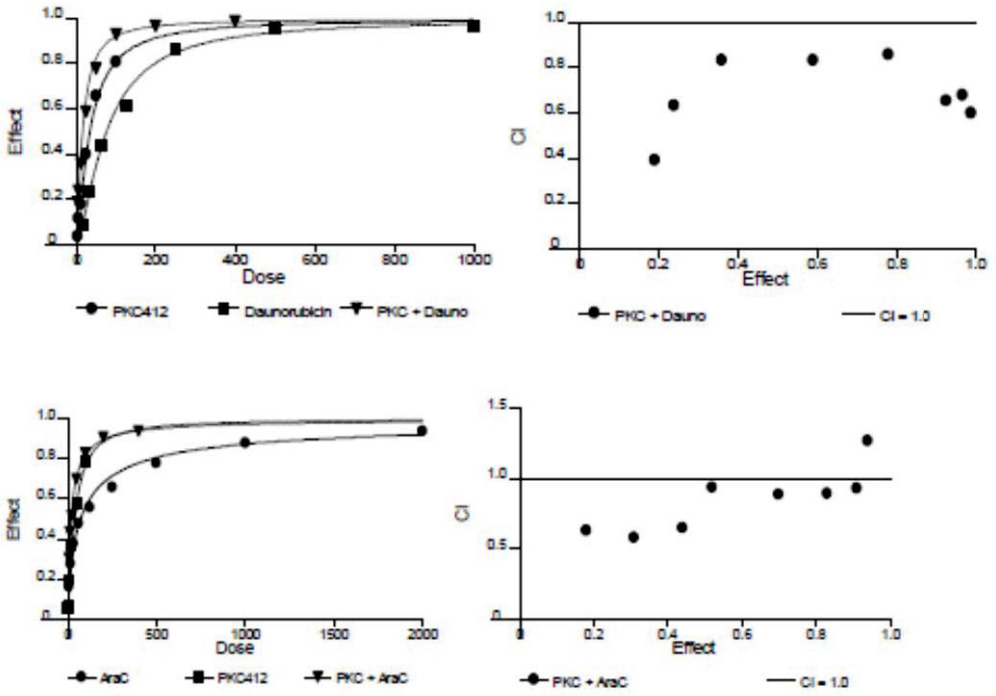
The antiproliferative activity of PKC412 in MOLM13 and MV4-11 cells is consistent with what has been observed in other laboratories using different assays.[10,11,12]

Appears this way on original

[10] Furukawa Y et al, 2007, Divergent Cytotoxic Effects of PKC412 in Combination with Conventional Antileukemic Agents in FLT3 Mutation-Positive Versus -Negative Leukemia Cell Lines, Leukemia, 21(5):1005-14.
[11] Odgerel T et al, 2008, The FLT3 inhibitor PKC412 exerts differential cell cycle effects on leukemic cells depending on the presence of FLT3 mutations, Oncogene, 27:3102-3110.
[12] Zarrinkar P et al, 2009, AC220 is a Uniquely Potent and Selective Inhibitor of FLT3 for the Treatment of Acute Myeloid Leukemia (AML), Blood,114(14): 2984-92.

Figure 1 Dose-Effect Curve and Combination Index Plot for PKC412 and Daunorubicin or Ara-C in MOLM-13 cells

(Excerpted from the Pharmacology Written Summary)



MOLM-13 cells were treated for 48 hrs with decreasing concentrations of either midostaurin, Daunorubicin (or Ara-C) or a combination of fixed-ratio (1:1) of midostaurin and Daunorubicin (or Ara-C). Then, 10 µl of AlamarBlue was added per well and the plates incubated for 6 hrs at 37°C and 5% CO_2. Fluorescence was measured using a Cytofluor II 96-well plate reader. Data were analyzed using the CalcuSyn software program. Shown are mean values of triplicate determinations for one representative experiment.

Combinations of midostaurin with daunorubicin or Ara-C repeatedly yielded combination indices <1, which are indicative of synergism according to the combination index method of Chou and Talaly.[13] Combinations with tipifarnib and all-trans retinoic acid also had a combination index <1 (not shown).

Synergistic interactions of midostaurin with other cytotoxic agents (cytarabine, doxorubicin, idarubicin, mitoxantrone, etoposide, 4-Hydroperoxycyclophosphamide, and vincristine) were also observed in three FLT3-ITD expressing AML cell lines (MOLM13, MOLM14, MV4-11), whereas mainly additive or antagonistic interactions were observed in the B-cell ALL line BALL-1 and KG-1 or U937 (not shown).[10]

In study PKF-98-02547, previously reviewed as BIO-4 by Dr. Hua Zheng, midostaurin inhibited proliferation of a broad spectrum of tumor cell lines in vitro.

[13] Chou TC et al, 1991, The Median-Effect Principle and the Combination Index for Quantitation of Synergism and Antagonism, In Synergism and Antagonism in Chemotherapy (Chou TC and Rideout DC eds.), pp.61-102, Academic Press, San Diego.

Table 11 Midostaurin In Vitro Inhibition of a Panel of Tumor Cell Lines

(Excerpted for the 30-Day Review by Dr. Hua Zheng)

Cell lines	$IC_{50} \leq 0.10$ µM	1.0 µM ≤ IC_{50} ≥ 0.10 µM	$IC_{50} \geq 1.0$ µM
Number of cell lines	17	69	12
Types of cell lines tested	Leukemia, NSCLC, Colon, CNS, and renal Cancer	Leukemia, lymphoma, NSCLC, small cell lung cancer, large cell lung cancer, Colon, CNS, breast, ovarian, prostate, and renal cancer, and melanoma	Melanoma, leukemia, lymphoma, colon, breast, and bladder cancer Mouse mammary and colon carcinoma, mouse B16/BL melanoma

Glioblastoma, bladder, epithelial, and lymphoblastoid cell lines also had IC50s between 0.1 and 1 µM; one melanoma cell line (MALME-3M); and, 2 prostate cell lines (FC and RB) also had IC50s <0.1 µM.

Activity of Midostaurin in Mast Cells

The Applicant did not submit their own studies, but rather referenced data from Gleixner et al (2006).[14] In summary, IC50 values for antiproliferative effects by midostaurin ranged from 50 to 250 nM in HMC1.2 (D816V/V560G KIT mutant expressing) and HMC-1.1 (V560G KIT mutant only expressing) mast cell lines; and, with an IC50 of 50 nM in primary mast cells (with KIT D816V) from a single patient with smoldering systemic mastocytosis (SM). Proapoptotic effects were observed with 24 hr midostaurin treatment in HMC1.2 cell lines. Midostaurin inhibited the phosphorylation of the KIT receptor, and its downstream targets STAT3 and STAT5 in a dose-dependent manner in murine BaF3 cells expressing several constitutively active oncogenic KIT mutants which had been identified in SM patients. Reduction of the phospho-signal was observed at midostaurin concentrations of 10 to 50 nM for the KIT-D816V mutants.

Summary of MOA – Apoptosis Induction and Signaling Inhibition

Published references showed that midostaurin induced apoptosis measured by Annexin V and/or Tunnel staining in FLT3-ITD expressing (MV4-11) or wild-type FLT3 overexpressing (SEMK2-M1) AML cell lines.[11,15] In AML patients with FLT3-ITD mutations, upregulation of apoptosis regulators Bim and Puma mRNA expression was observed in some samples.[16]

Reduction of the phospho-FLT3 signal and downstream effectors STAT5 and ERK was observed at midostaurin concentrations of 30 nM for the FLT3-ITD mutant and 100 nM for the FLT3-TKD mutant BaF3 cells.[17]

[14] Gleixner KV et al, 2006, PKC412 Inh bits In Vitro Growth of Neoplastic Human Mast Cells Expressing the D816V-Mutated Variant of KIT: Comparison with AMN107, Imatinib, and Cladribine (2CdA) and Evaluation of Cooperative Drug Effects, Blood, 107(2):752-9.
[15] Armstrong SA et al, 2003, Inh bition of FLT3 in MLL: Validation of a Therapeutic Target Identified by Gene Expression Based Classification, Cancer Cell, 3(2):173-83.
[16] Nordigarden M et al, 2009, BH3-Only Protein Bim More Critical than Puma in Tyrosine Kinase Inhibitor–Induced Apoptosis of Human Leukemic Cells and Transduced Hematopoietic Progenitors Carrying Oncogenic FLT3, Blood, 113: 2302-2311.
[17] Barry EV et al, 2007, Uniform Sensitivity of FLT3 Activation Loop Mutants to the Tyrosine Kinase Inhibitor Midostaurin, Blood, 2007,110(13):4476-9.

In vivo anti-tumor activity

Study title: Dose Dependent Anti-Tumor Efficacy of Midostaurin in an AML Xenograft Model

Study No.: RD-2014-00163
Report Date: November 11, 2015
Study report location: 4.2.1.1.
Conducting Laboratory: Novartis
GLP: No

Methods:
See legend in Table 12. Animals were treated with NVP-PKC412 for 12 days.

Results:
NVP-PKC412 exhibited dose dependent antitumor activity when administered orally daily at doses from 5 to 150 mg/kg. Statistically significant smaller tumors were obtained in mice treated at dose levels of 50 and 150 mg/kg compared to vehicle treated (tumor regression 58 to 100%, respectively).

Table 12 Efficacy and Tolerability of NVP-PKC412 against MV4-11 Xenografts

(Excerpted from the study report)

		Tumor response			Host response		
Compound	Dose, route, schedule	T/C (%)	Regres-sion (%)	ΔTumor volume (mm^3)	Δbody weight (g)	Δbody weight (%)	Survival (alive/total)
Vehicle	10 ml/kg, qd, p.o.	100	-	1406 ± 317	3.2 ± 0.8	13.4 ± 3.3	6/6
PKC412	5 mg/kg qd, p.o.	53	-	748 ± 104	2.5 ± 0.4	9.4 ± 1.5	6/6
PKC412	20 mg/kg, qd, p.o.	42	-	595 ± 251	2.7 ± 0.5	10.8 ± 2.1	6/6
PKC412	50 mg/kg qd, p.o.	-	58	-51 ± 13*	2.4 ± 1.1	9.7 ± 4.3	6/6
PKC412	150 mg/kg qd, p.o.	-	100	-75 ± 20*	0.4 ± 1.1	2.0 ± 4.6	5/6

* p<0.05 versus vehicle (one-way ANOVA *post hoc* Dunns)

Tumors were established in female athymic nude mice by subcutaneous inoculation of human acute myeloid leukemia MV4-11 cells ($5x10^5$ cells/200µl). Ten days after inoculation, treatment was started with NVP-PKC412 at dose levels of 5, 20, 50 and 150 mg/kg; q.d., p.o. and compared with control vehicle (b) (4)) administered at 10ml/kg; q.d. p.o. Tumor volumes were measured by multiplying the three largest diameters (LxWxH) with π/6. Where applicable, data are presented as mean ± SEM. Δ tumor volumes represent the tumor volume on the last recording day minus the tumor volume on the first treatment day. Antitumor activity is expressed as %T/C (Δtumor volume of treated animals divided by the Δtumor volume of control animals expressed in percent). When %T/C is negative a regression value is calculated by dividing the absolute Δtumor volume with the tumor volume on the first treatment day expressed in percent.

Pharmacokinetic (PK) data from non-tumor bearing mice is presented below, but may not be fully correlative with actual exposures in the mouse xenograft study since a different formulation was used ((b) (4)).

Table 13 Summary of Single Oral Dose PK Parameters from Non-Tumor-Bearing Mice

(Adapted from Tables 2-8 and 2-9 from the Pharmacology Written Summary)

	Cmax (µM)		Cmaxlast (µM)		Tmax (h)		Tmaxlast (h)		AUC last (h.µmol/L)	
Dose PKC412 (mg/kg)	25	100	25	100	25	100	25	100	25	100
PKC412	1.04	1.73	0.14	0.43	0.5	1	8	24	3	17
CGP52421	2.56	4.44	1.26	0.3	1	6	8	24	16	53
CGP62221	0.06	0.2	0.02	0.09	1	6	6	24	0.1	4

Female OF1 mice received an oral dose of 25 or 100 mg/kg of PKC412 formulated as a [redacted] (b) (4) The pharmacokinetic parameters Cmax, Clast, Tmax and Tlast were determined by inspection of the data. Data are expressed as mean values, n = 4. Area under the plasma concentration versus time curve (AUC) was calculated from the mean plasma concentrations by linear trapezoidal rule using noncompartmental analysis (WinNonlin v. 4, Pharsight). Source: Study No. RD-2009-00618.

Of note, a published report showed that oral administration of midostaurin at 100 mg/kg from Days (D) 30–88 in Trial 1 and D25–68 in Trial 2 (post bone marrow transplantation of cells expressing FLT3-ITD in mice) resulted in a survival benefit compared to placebo control.[8] In another published report, noninvasive imaging of Taconic Line NCr nude mice inoculated with FLT3-ITD BaF3 cells engineered to stably express firefly luciferase also showed that 6 days of 100 mg/kg midostaurin treatment had antitumor activity.[18] The Applicant noted that midostaurin was not tested in ASM models since positive clinical data was available from a compassionate use study.[19]

4.2 Secondary Pharmacology

General Methods for Study Nos. RD-2016-00522-v2, RD-2016-00523-v2, and RD2016-00531:

Off-target activity was assessed for G-protein coupled receptors (GPCRs), transporters, ion channels, nuclear receptors, and enzymes, which included nine targets identified by the Applicant as being potentially involved in suicidality.[20] For GPCR and transporter radioligand binding assays, membranes were prepared from the rat brain cortex or cell lines expressing recombinant or native receptors. Radioactivity was detected using filtration or scintillation proximity assay (SPA). For some GPCRs, functional cAMP quantification and calcium flux cellular assays were also conducted in agonist and antagonist mode. Binding to PXR was detected using LanthaScreen TR-FRET technology. Activity against cyclooxygenase-1, monoamine oxidase A, or phosphodiesterases 3 and 4D were measured using an amplex red method, luminogenic substrate, or radiolabeled cAMP, respectively. Binding to the hERG potassium channel was also measured using a radioligand binding assay.

Metabolite CGP52421

CGP052421-NX-1 was assessed for its off-target activity on 40 targets (25 GPCRs, 3 transporters, 7 ion channels, 1 nuclear receptor (PXR) and 4 enzymes). The panel for

[18] Weisberg E et al, 2008, Antileukemic Effects of the Novel, Mutant FLT3 Inhibitor NVP-AST487: Effects on PKC412-Sensitive and -Resistant FLT3-Expressing Cells, Blood, 112: 5161-5170.
[19] Gotl b J et al, 2005, Activity of the Tyrosine Kinase Inhibitor PKC412 in a Patient with Mast Cell Leukemia with the D816V KIT Mutation, Blood, 106(8):2865-70.
[20] Muller PY et al, 2015, Integrated Risk Assessment of Suicidal Ideation and Behavior in Drug Development, Drug Discov Today, 20(9):1135-42.

CGP52421 was the broadest and included additional adrenoceptors, dopamine and opiate receptors, cannabinoid 1 receptor, 5-HT2B, GABA receptors, and the NMDA channel, which were not evaluated for CGP62221 or PKC412. Activities of >50% inhibition at 10 µM were found only for phosphodiesterase PDE4D (62%; IC50=4.7 µM). A weaker activity was also found in the 5-HT2B antagonist assay (IC50=18 µM). All other profiled targets had IC50s >30 µM.

Metabolite CGP62221
CGP062221-NX-1 was tested in secondary pharmacology profiling assays comprising 23 targets (13 GPCRs, 3 transporters, 2 ion channels, 1 nuclear receptor and 4 enzymes) (Note: Did not evaluate late calcium channel). Activities of >50% inhibition at 10 µM were found only for phosphodiesterase PDE4D (62%, IC50=6.9 µM) and adenosine transporter ENT1 (AdT) (55%, IC50=8.3 µM). A weaker activity was also found in the COX-1 assay (IC50=13 µM). All other profiled targets had IC50s >30 µM.

PKC412
PKC412 was tested against 25 targets (13 GPCRs, 3 transporters, 4 ion channels, 1 nuclear receptor and 4 enzymes)(Note: also evaluated inhibition of the human voltage-gated sodium channel (Nav1.5)). Activities of >50% inhibition at 10 µM were found only in the COX-1 assay (98%, IC50 = 2.3 µM) and phosphodiesterase PDE4D assay (75%, IC50=3.2 µM). All other profiled targets had IC50s >30 µM.

4.3 Safety Pharmacology

Study title: Electrophysiological Safety Measurements of hERG Currents in Stably Transfected HEK293 Cells (not GLP)

Study no:	PCS-R0250111
Study report location:	4.2.1.3.
Conducting laboratory and location:	Novartis, Basel, SWISS
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	PKC412-NXA.001; Batch No. 840296; Purity: 98.9%

Key study findings:

- PKC412 did not inhibit the hERG channels in stably expressed in HEK293 cells at its highest soluble concentration of 12 µM (6.8 µg/mL).

Methods:

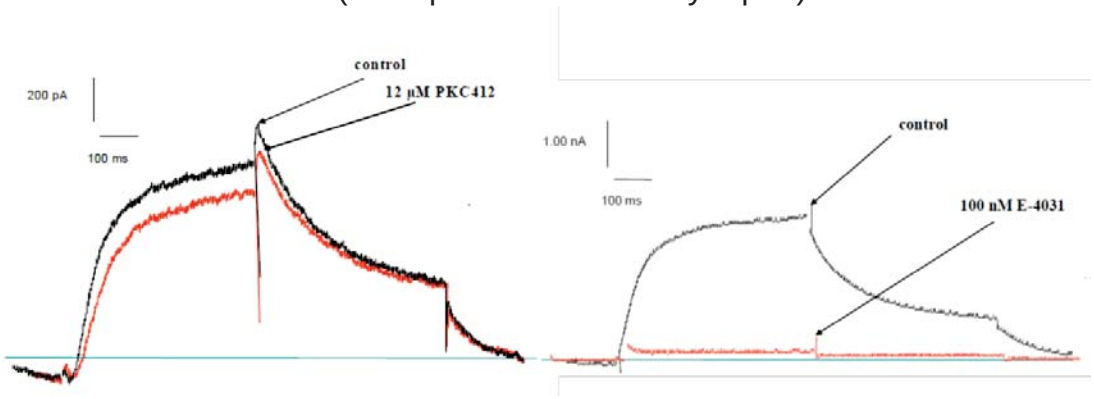
Strains/species/cell line:	HEK293 cells stably transfected with hERG cDNA
Controls:	
Negative Control	DMSO (0.1%) (mean 94.6% of control, n=5)
Positive control	E-4031 (100 nM) (mean 5.21% of control, n=2)
Concentrations:	12 µM PKC412 (mean 93.41% of control, n=4)
Test system:	Whole-cell patch-clamp

Reference ID: 4051584

Results:

Figure 2 Effect of PKC412 and Positive Control on hERG Current

(Excerpted from the study report)



Study title: Effects of 512-03 (Hydroxy-PKC412) on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

Study no:	PCS-R0770504
Study report location:	4.2.1.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 30, 2007 (report date)
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	512-03 (Hydroxy-PKC412); Batch No. 07/2; Purity: 97.9%

Key study findings:

- The IC50 for the inhibitory effect of 512-03 (Hydroxy-PKC412, CGP52421) on hERG potassium current was not determined but estimated to be higher than 1.5 µM.

Methods:

Strains/species/cell line:	HEK293 cells stably transfected with hERG cDNA
Controls:	
Negative Control	HB-PS + 0.3% v/v DMSO (n=3)
Positive control	Terfenadine (60 nM inhibits 78.9%), E-4031 (500 nM) (n=2)
Concentrations:	0.34, 1.5, 4.74 µM (actual) (1, 3, and 10 µM, intended)(i.e., mid and high concentration formulations were not stable)
Test system:	Whole-cell patch-clamp

Results:

- Precipitate was observed at ≥1.5 µM, which may have contributed to the instability of 512-03 (losing up to 21% of the target concentration between the beginning and end of the day).

Table 14 hERG Current Block at Each Concentration of 512-03 (Hydroxy-PKC412)

(Excerpted from the study report)

Concentration (µM)	Mean	SD	SEM	N
0	0.3%	0.3%	0.1%	3
0.34*	16.5%	1.2%	0.7%	3
1.50*	38.5%	1.5%	0.9%	3
4.74*	26.4%	2.5%	1.5%	3

* Value is statistically different than vehicle alone.

(Concentrations were taken from the perfusion outflow apparatus)

Study title: Effects of Desmethyl-PKC412 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

Study no:	PCS-R0770505
Study report location:	4.2.1.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 30, 2007 (report date)
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	545-06 (Desmethyl-PKC412); Batch No. 06/1; Purity: 93.3%

Key study findings:

- Desmethyl-PKC412 (CGP62221) in patch voltage clamp recordings of HEK293 cells stably expressing hERG current did not inhibit more than 20% of hERG currents when tested at its nominal limit of solubility.

Methods:

Strains/species/cell line:	HEK293 cells stably transfected with hERG cDNA
Controls:	
Negative Control	HB-PS + 0.3% v/v DMSO (n=3)
Positive control	Terfenadine (60 nM inhibits 83%), E-4031 (500 nM) (n=2)
Concentrations:	1.21 (actual) (3 µM, intended)(i.e., formulation was stable)(10 µM was insoluble)
Test system:	Whole-cell patch-clamp

Results:

Table 15 hERG Current Block for Desmethyl-PKC412

(Excerpted from the study report)

Desmethyl-PKC412 Concentration (μM)	Mean % hERG Inhibition	SD	SEM	n
0 (Vehicle)	0.2%	1.4%	0.8%	3
1.21	11.3%	1.4%	0.8%	3

(Concentrations were taken from the perfusion outflow apparatus)

Study title: Effects of CGP41251 (Protein Kinase C Inhibitor) on Neurotransmitter Release and Uptake and on Phosphatidylinositol Turnover (Biology Report 18/90)

Study no:	PKF-90-02620
Study report location:	4.2.1.3.
Conducting laboratory and location:	Novartis
Date of study initiation:	November 20, 2009 (report date)
GLP compliance:	No
QA statement:	NA
Drug, lot #, and % purity:	CGP41251 (lot # and purity were not provided)

Key study findings:

- At 10 μM, CGP41251 increased stimulation induced and/or basal [^{3}H]NA and [^{3}H]5-HT release and reduced [^{3}H]GABA release, but did not significantly affect [^{3}H]Ach release. Most markedly it induced basal release of [^{3}H]DA by 81% compared to controls, which could be therapeutically relevant since the EC50 is likely less than 10 μM.
- CGP41251 weakly inhibited uptake of neurotransmitters with IC50s >100 μM.

Methods:

Strains/species/cell line:	Tif:RAI (SPF) rats (brain slices, mesencephali and diencephalic homogenates, or whole brain homogenates)
Controls:	
Negative Control	Assumed to be bicarbonate buffer with DMSO
Positive control	Imipramine ([^{3}H]NA and [^{3}H]5-HT uptake), nipecotic acid ([^{3}H]GABA uptake)
Concentrations:	10 μmol/L
Test system:	Release of [^{3}H] noradrenaline (NA), [^{3}H] serotonin (5-HT), [^{3}H] dopamine (DA), [^{3}H]GABA and [^{3}H]choline (Ach) from relabeled brain slices (test article was added prior to second electrical stimulation); uptake of [^{3}H]NA, [^{3}H]5-HT and [^{3}H]GABA into rat midbrain synaptosomes; presence of ^{3}H-inositol 1-phosphate after incubation with test article (measure of inositol monophosphate phosphatase activity)

Table 16 Effects of CGP41251 on Neurotransmitter Release

(Excerpted from the study report)

Table 1: Effects of 10 µmol/l CGP 41251 on the [^{3}H]overflow from [^{3}H]NA, [^{3}H]5-HT, [^{3}H]DA, [^{3}H]GABA, [^{3}H]choline and [^{3}H]DA prelabelled brain slices

Transmitter	[^{3}H]NA	[^{3}H]5-HT	[^{3}H]GABA	[^{3}H]DA	[^{3}H]ACh
Brain area	cortex	cortex	cortex	c. striatum	hippocampus
Uptake inhibition	cocain	citalopram	SK&F89976	nomifensine	-
S_1 controls (% fractional release)	4.88	2.08	1.09	4.67	2.84
S_2/S_1 controls	0.94	0.99	1.10	0.84	0.99
CGP 41251 (S_2/S_1 as % of S_2/S_1 of controls)	109 [a]	115	99	110	98
5%-confidence limits	107,111	103,128	96,103	102,119	91,106
Basal release (% of controls)	120	126 [a]	81 [b]	181 [a]	88
5%-confidence limits	103,140	115,139	71,92	136,253	74,103

a $2p < 0.01$
b $2p < 0.05$

Legend: For release experiments with [^{3}H]NA, [^{3}H]5-HT and [^{3}H]GABA, cross-chopped (0.35 mm) slices of a superficial layer of the cerebral cortex and of the hippocampus for release experiments with [^{3}H]ACh, respectively, were used. For release experiments with [^{3}H]DA, the slices were cut transversely from the c. striatum (thickness 0.3 mm). The slices were electrically stimulated twice and the drug added to the superfusion medium between the two stimulation periods. The stimulated [^{3}H]overflow (S_1 and S_2) was calculated by subtracting the interpolated basal overflow. For further details see methods.

- The IC50s for CGP41251 and imipramine on the [^{3}H]NA-uptake into rat midbrain synaptosomes were approximately 100 and 1 µM, respectively.
- The IC50s for CGP41251 and imipramine on the [^{3}H]5-HT-uptake into rat midbrain synaptosomes were >1 mM and approximately 1 µM, respectively.
- The IC50s for CGP41251 and nipecotic acid on the [^{3}H]GABA-uptake into rat midbrain synaptosomes were >1 mM and approximately 10 µM, respectively.
- At a concentration of 1 mM, CGP41251 did not inhibit inositol 1,4,5-trisphosphate 5-phosphatase (raw data not provided in study report).

Study title: CNS Evaluation of CGP41251 (Protein Kinase C Inhibitor) (Biology Report 36/90)

Study no:	PKF-90-02621
Study report location:	4.2.1.3.
Conducting laboratory and location:	Novartis
Date of study initiation:	November 20, 2009 (report date)
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CGP41251 (lot # and purity were not provided)

This study was initially reviewed by Dr. Hua Zheng, but has been formatted to fit this review and the slight rotarod effect was added by this reviewer.

Key study findings:

- The only notable changes observed were slight hyperthermia, slight reduction in time to stay on rotarod, and marginal, not dose-related ataxia.

Methods:

Doses:	10, 30, 100, 300, or 1000 mg/kg (general behavior); 10, 30, 100 or 300 mg/kg (rotarod, body temp, EtOH-induced narcosis, and motility)
Frequency of dosing:	Single
Route of administration:	Oral
Dose volume:	Unknown
Formulation/Vehicle:	(b) (4) the final DMSO concentration being 4%
Species/Strain:	Tif:MAGf (SPF) male mice
Number/Sex/Group:	4/group (general behavior);10/group (rotarod, body temp, EtOH-induced narcosis, motility)
Age:	Unknown
Weight:	22 to 28 g
Satellite groups:	None
Test system:	General behavior, rotarod (300 sec), body temperature, EtOH-induced narcosis, motility

Results:

- Slight, not dose-related ataxia, which appeared at all doses tested up to 4 hr after drug administration.
- Slight reduction in length of time able to stay on rotarod for 300 mg/kg group, which normalized to control lengths by 4 hrs postdose.
- When compared to vehicle treated controls (body temperature between 35.5 and 36.2°C), body temperature was significantly increased 1 hr (300 mg/kg, maximal effect of +0.7°C) and 2 hr (10, 30, 100 and 300 mg/kg), but not 4 hr after treatment with CGP41251.

Reference ID: 4051584

Study title: Evaluation of Cardiovascular and Renal Effects of CGP41251 (PKC412) (Protein Kinase C Inhibitor; Antitumour Agent) (Biology Report 43/90)

Study no:	PKF-90-02622
Study report location:	4.2.1.3.
Conducting laboratory and location:	Novartis
Date of study initiation:	February 16, 2011 (report date)
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	CGP41251 (lot # and purity were not provided)

This study was initially reviewed by Dr. Hua Zheng, but has been formatted to fit this review and the slight urine and electrolyte effects were added by this reviewer.

Key study findings:

- CGP41251 administered intravenously (IV) at doses ≥ 8 mg/kg in male rats caused ↓MAP and HR; no effects were seen orally up to 300 mg/kg.
- CGP41251 inhibits NA- and KCl-induced vasoconstriction with a similar efficacy and potency and are indicative of a vasodilatory property of the compound.
- CGP41251 administered orally at ≥ 100 mg/kg in female rats slightly increased sodium and chloride excretion.

Methods:

Doses:	3, 8, 25 mg/kg CGP41251 IV (rate 10 µL/min); 300 mg/kg (oral)
Frequency of dosing:	Single
Route of administration:	IV (anaesthetized rats); oral (conscious rats)
Dose volume:	2 mL/kg (oral)
Formulation/Vehicle:	DMSO (IV); solution of DMSO, (b) (4) (oral)
Species/Strain:	Tif:RAIf, SPF male rats
Number/Sex/Group:	4/group
Age:	Unknown
Weight:	350 to 450 g
Satellite groups:	None
Test system:	Catheterization to measure heart rate, femoral artery, and jugular vein (IV route only) pressure

Results:

- Intravenous infusion (30 min) of PKC412 at doses 8 and 25 mg/kg caused a dose-related decrease in mean arterial pressure (MAP) and heart rate (HR) in anesthetized rats (up to 47 and 21%↓, respectively). Both effects were of very slow onset.
- The highest dose of 2.5 mg/kg/min administered intravenously induced severe hypotension and respiratory arrest in three rats and was lethal after

Reference ID: 4051584

14 to 21 minutes of infusion that corresponded to total doses of 35 to 53 mg/kg.

- By contrast, oral administration of PKC412 in a single dose of 300 mg/kg had no effect on MAP and HR over six hours in conscious rats.
- Oral administration of PKC412 (30 to 300 mg/kg) in the same species, but females, with the same vehicle (n=8/dose) increased sodium and chloride excretion by about 100% and 50% at both 100 and 300 mg/kg, respectively, but the study report noted that this is weak compared to diuretic agents.

Methods:

Concentrations:	0.18, 1.8 and 18 µmol/L
Formulation/Vehicle:	DMSO (0.5%)
Species/Strain:	Tif:DHP(SPF) white Pirbright guinea-pigs
Number/Sex/Group:	n=3/concentration (n=4 for controls)
Age:	Unknown
Weight:	365 to 420 g
Satellite groups:	None
Test system:	Stimulated isolated atria were treated with test article for 60 min

Results:

- In isolated guinea pig atria, PKC412 applied in single concentrations of 0.18, 1.8 and 18 µM had no consistent effects on either the force or the rate of contraction.

Methods:

Concentrations:	0.18, 1.8 and 18 µmol/L
Formulation/Vehicle:	DMSO (0.5%)/EtOH (0.36%)
Species/Strain:	Tif:RAIf, SPF male rats
Number/Sex/Group:	n=3/concentration (n=4 for controls)
Age:	Unknown
Weight:	340 to 370 g
Satellite groups:	None
Test system:	Mesenteric artery was dissected and perfused at a constant rate of 5 mL/min with physiological salt solution (PSS), noradrenaline (2 µg, NA) and potassium chloride (200 µmol, KCl) were added to the perfusion system, in the presence or absence of test article.

Results:

- PKC412 caused concentration-dependent inhibition of both noradrenaline- and potassium chloride-induced vasoconstriction at all concentrations.

- The calculated IC50 for NA- and KCl-induced contractions were 0.67 and 0.41 µmol/L, respectively.

Methods:

Concentrations:	1, 10, 30 µmol/L
Formulation/Vehicle:	DMSO (0.5%)
Species/Strain:	Male Chinchilla rabbits (KA 47)
Number/Sex/Group:	n=3/concentration (n=4 for controls)
Age:	Unknown
Weight:	2 to 2.5 kg
Satellite groups:	None
Test system:	Descending thoracic aorta removed and immersed in a tissue bath; at 20-minute intervals, 10 nmol/L angiotensin II and 50 nmol/L noradrenaline hydrochloride were added

Results:

- PKC412 caused no effect on contractions induced by either angiotensin-II or noradrenaline.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Pharmacokinetic parameters for midostaurin and its predominant metabolites hydroxy-PKC412 (CGP52421, 512-03) and desmethyl-PKC412 (CGP62221) were evaluated in rats, dogs, rabbits and humans using an earlier (b) (4) formulation and a more clinically relevant (b) (4) In the species tested, midostaurin was absorbed between 1.7 and 8 hrs, with the dog being relevant to humans in terms of bioavailability and metabolism (CGP62221 was absent in rats). Plasma protein binding was high (>98%) for midostaurin and the predominant metabolites. Midostaurin was well distributed in the majority of tissues in rats, was excreted primarily via the hepatobiliary route (rat) and feces (all species), and, in rats, could pass to the fetus in be excreted in milk.

Absorption

- Midostaurin ((b) (4) formulation) is absorbed slower in the rat, dog, and rabbit compared to humans (4 to 8 hrs versus 1.7).
- The extent of absorption was estimated to be high in rats and humans (>90%) and moderate in dogs and rabbits (40 to 64%).
- Bioavailability with the (b) (4) formulation was low in rats (9.3%) and rabbits (1.8%), and moderate in dogs (48.5%), indicating high to moderate first pass metabolism (note: bioavailability >90% in humans based on Clinical Pharmacology discipline assessment).
- Clearance was moderate, less than 35% of hepatic blood flow.
- Vss suggests midostaurin was well distributed to tissues/organs.

Reference ID: 4051584

- Concentration versus time profiles were biphasic with a long terminal half-life.

Table 17 PK Parameters of Radioactivity in Plasma after a Single Oral Dose Across Species

(Excerpted from the PK Written Summary)

PK parameters	Rat	Rabbit	Dog	Human
p.o. dose (mg/kg) [a]	10	10	3	0.69 (50mg)
Tmax (h)	Range: 4-8	6.7	4.0	1.7 (median 1)
Cmax (ng/mL) [nM]	46.6 [81.8]	30.5 [53.5]	144 [252]	1210 [2120]
AUClast (ng·h/mL) [nM·h]	NR	731 [1280]	1640 [2870]	15200 [26700]
AUCinf (ng·h/mL) [nM·h]	946 [1660]	742 [1300]	1660 [2910]	15700 [27500]
Apparent terminal T1/2 (h)	10	15	9.6	20
Absorption (%) [b]	High (>90%)	Moderate (64%)	Moderate (40-47%)	High (>90%)
Bioavailability (%)	9.3	1.8	48.5	NR

NR = Not reported. Data represent mean values (except where noted) and were rounded to 2 or 3 significant figures.

[a] Doses were (b) (4)

[b] Absorption was estimated based on % metabolites of the dose detected in excreta together with the stability data of midostaurin in feces after an oral dose or based on the AUC ratio of radioactivity in blood or plasma.

Sources: [Table 2.6.5.3A ADME(US) R0201773], [Table 2.6.5.3A DMPK R0900185a and DMPK R0900185], [Table 2.6.5.3A DMPK R0900139], [Summary of Clinical Pharmacology studies 2.7.2 - Study CPKC412A2107], [Table 2.6.5.3A CPKC412A2107, CPKC412A2107 Amendment 1, and CPKC412A2107 Amendment 2].

Rat, rabbit and dog formulations contained (b) (4) (Rat batches: E-5625-106-35 and WSJ-88.1.01 (unlabeled); Rabbit batch: E-42109-27-47, Purity: 98.1%; Dog Lot: RSE 044-23, Purity 98.6%, Batch: E-42109-31-38). All above studies were conducted at Novartis, East Hanover, NJ, USA or (b) (4) (dog) with report dates between May 2005-November 2009.

Table 18 Midostaurin PK Parameters after a Single IV Dose in Animals

(Excerpted from the PK Written Summary)

PK parameters	Rat	Rabbit	Dog
i.v. dose (mg/kg)	1	2	0.5
Cmax (ng/mL) [nM]	1850 [3250]	4630 [8110]	175 [307]
AUClast (ng·h/mL) [nM·h]	NR	8280 [14500]	562 [985]
AUCinf (ng·h/mL) [nM·h]	1020 [1790]	8280 [14500]	570 [999]
CL (L/h/kg)	0.98	0.24	0.90
Vss (L/kg)	1.20	1.36	3.77
T1/2 (h)	λ1 0.5, λ2 3.2	10.4 (λ1 0.2, λ2 7.3)	4.54 (λ1 0.9, λ2 4.0)

Sources: [Table 2.6.5.3C ADME(US) R0201773], [Table 2.6.5.3C DMPK R0900185a and DMPK R0900185], [Table 2.6.5.3C DMPK R0900139],

Rat and rabbit formulations contained (b) (4) :sterile water ((b) (4))(Rat batch: E-5625-106-35)(Rabbit batch: E-42109-27-04, Purity: 98.1%). The dog formulation contained (b) (4) in sterile saline (Lot: RSE 044-22, Purity: 98.6%, Batch: E-42109-31-19). All above studies were conducted at Novartis, East Hanover, NJ, USA or (b) (4) (dog) with report dates between May 2005-November 2009.

Distribution

Plasma Protein Binding:

- In vitro protein binding of midostaurin, (>98%). Binding was concentration independent in Study No. DMPK-R0300432, but was temperature dependent in rats, with a lower average binding of 0.932± 0.011 at 25°C. High concentrations of CGP52421 (up to 20,000 ng/mL) did not affect protein binding of PKC412 (Study No. DMPK-R0300868).

Table 19 Summary of Plasma Protein Binding of PKC412 Across Species

(Excerpted from Study No. DMPK-R0300432)

[^{14}C]PKC412 concentration (ng/mL)	Fraction (mean ± SD)					
	Rat[a]	Dog[a]	Human Subject 1[b]	Human Subject 2[b]	Human Subject 3[b]	Human Average[c]
100	0.988 ± 0.003	0.987 ± 0.002	0.993	BLD[d]	0.946	0.969
500	0.984 ± 0.013	0.987 ± 0.001	0.988	0.996	0.988	0.991 ± 0.004
1000	0.988 ± 0.007	0.986 ± 0.003	0.990	0.997	0.990	0.992 ± 0.004
5000	0.991 ± 0.001	0.987 ± 0.002	0.988	0.997	0.991	0.992 ± 0.005
Overall average	0.988 ± 0.007	0.987 ± 0.002	0.990 ± 0.002	0.996 ± 0.001	0.979 ± 0.022	0.988 ± 0.014

[a] pooled blood (n ≥ 3) and data obtained from triplicate analyses

[b] data obtained from single analysis

[c] mean data obtained from the three individual human subjects

[d] below the limit of detection

Fresh blood was collected with a heparinized tube from male Wistar-Hanover rats (HsdBrl:WH; 250 to 300 g; n >3, Beagle dogs (8 to 12 kg, n = 3) and male human volunteers (n = 3) who gave written informed consent. [^{14}C]PKC412 (Batch No. E-5625-106-35) was used in the study. Heparin did not appear to affect plasma protein binding.

- In a different plasma protein binding study using gel filtration (Study No. DMPK-R08000059), binding of PKC412 to human plasma proteins was concentration dependent. At higher concentrations, free drug content increased by about 7-fold (from 0.01 to 0.07%), most probably due to saturation of some plasma proteins e.g. α-acid glycoprotein.

Table 20 Mean Plasma Protein Binding of PKC412 in Rats, Dogs, and Humans Measured Using Equilibrium Gel Filtration

(Excerpted from Study No. DMPK-R08000059)

Species	Bound fraction (mean) (%)	Unbound fraction (%)	
		Mean	SD
Rat	99.90	0.10	0.02
Dog	99.92	0.08	0.01
Human (range)	99.93 – 99.99	0.01 – 0.07	

Frozen animal plasma was supplied by [redacted](b) (4). Human plasma was from healthy male volunteers and it was delivered frozen from the [redacted](b) (4) Plasma was defrosted from storage at –20°C. Pools with n ≥ 3 for animal and human plasma were used. [C]PKC412 (RSE 044-16, Preparation Batch No. E-30585- 97) was used in the study.

- Plasma protein binding for CGP52421 and CGP62221 was high in all species tested (>99%).

Table 21 Mean Plasma Protein Binding of CGP52421 and CGP62221 in Rats, Dogs, and Humans Measured Using Equilibrium Gel Filtration

(Excerpted from Study No. DMPK-R1500080)

Species	[^{14}C]CGP52421 Total concentration range (ng/mL)	Fraction unbound (%)	Fraction bound (%)	[^{3}H]CGP62221 Total concentration range (ng/mL)	Fraction unbound (%)	Fraction bound (%)
rat	141 - 39700	0.239 ± 0.0351	99.8	860 – 27200	0.227 ± 0.0907 (0.188, 0.164, 0.330)	99.8
dog	319 - 45200	0.204 ± 0.0454	99.8	925 – 47300	0.193 ± 0.0140	99.8
human	1970 - 34600	0.126 ± 0.0855 (0.0214, 0.155, 0.202)	99.9	5470 – 43500	0.111 ± 0.0706 (0.0375, 0.0976, 0.198)	99.9

Plasma from all species was prepared at Novartis and stored at -20°C or purchased from external vendors. Plasma was defrosted from storage at –20°C before usage. Plasma pools of n ≥ 3 animals/subjects were used for all performed studies. K3-EDTA was used as anticoagulant in all cases. [^{14}C]CGP52421 (Batch No. RSU6221-2013-10504-01) and [^{3}H]CGP62221 (Batch No. RSU6222-2015-13543-01) were used in the study.

- The Applicant noted that overall, the fraction unbound (%) of midostaurin and its metabolites at a clinically relevant steady state concentration range did not appear to be concentration dependent (Cmax 1160 to 5560 ng/mL [2 to 9.74 µM] at the dose of 50 or 100 mg BID in AML and ASM patients.

Tissue Distribution Male Rats:
Methods (Study No. DMPK-R71993):

- Tif:RAIf (albino) rats were treated with 1 mg/kg single IV administration of [^{14}C]PKC412 (various batches, vehicle of PEG400/0.9% saline). The concentration of ^{14}C was determined in homogenized tissues (n=3 rats/timepoint at 0.083 and 168 hrs).
- Long Evans (LE) rats were also treated with 1 mg/kg single IV administration [^{14}C]PKC412 and the concentration of ^{14}C was determined using whole body audioradiography (WBA) and lyophilized samples from freeze-dried whole body sections (n=1 rat/timepoint at 0.083, 2, 8, 24, 168 hrs) by liquid scintillation counting.
- Tif:RAIf rats were treated with 3, 10, or 22 mg/kg single oral administration of [^{14}C]PKC412 (various batches, vehicle of (b) (4)). Radioactivity in homogenized tissues was determined (at 1, 24, and 168 hr postdose for the 3 mg/kg dose and only 168 hr postdose for the 10 and 22 mg/kg doses) by liquid scintillation counting (n=3 rats/timepoint).
- Tif:RAIf rats were treated with multiple oral doses of 3 mg/kg [^{14}C]PKC412 (various batches, vehicle of vehicle of (b) (4)) daily for 10 to 18 days. Radioactivity was determined 24 hr postdose on D10 and D18 in homogenized tissues by liquid scintillation counting (n=3 rats/timepoint).

Results:

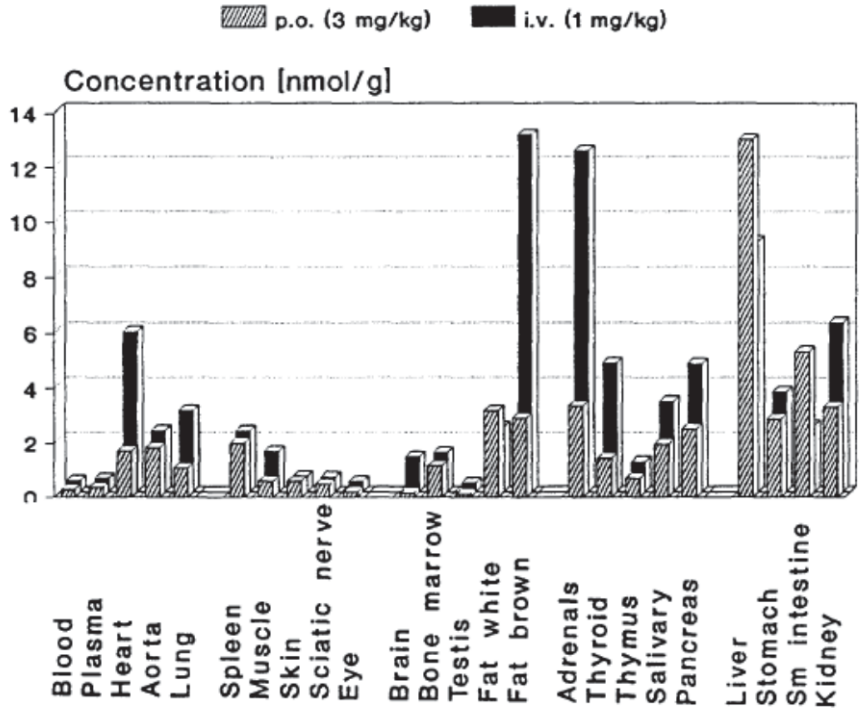
- Highest concentrations were detected 0.083 hr and 1 hr postdose following IV and oral administration, respectively.
- Whole body autoradiography showed that [^{14}C]PKC412 was taken up by the pituitary gland and crossed the blood brain barrier, with the highest [^{14}C]

concentrations in the frontal cortex following IV administration. No melanin binding was observed.

- Relative distribution in various organs is noted in the following Figures.

Figure 3 Blood and Tissue Distribution in Male Rats 5min and 1hr after IV and Oral Administration of [^{14}C]PKC412 (Means of 3 Animals)

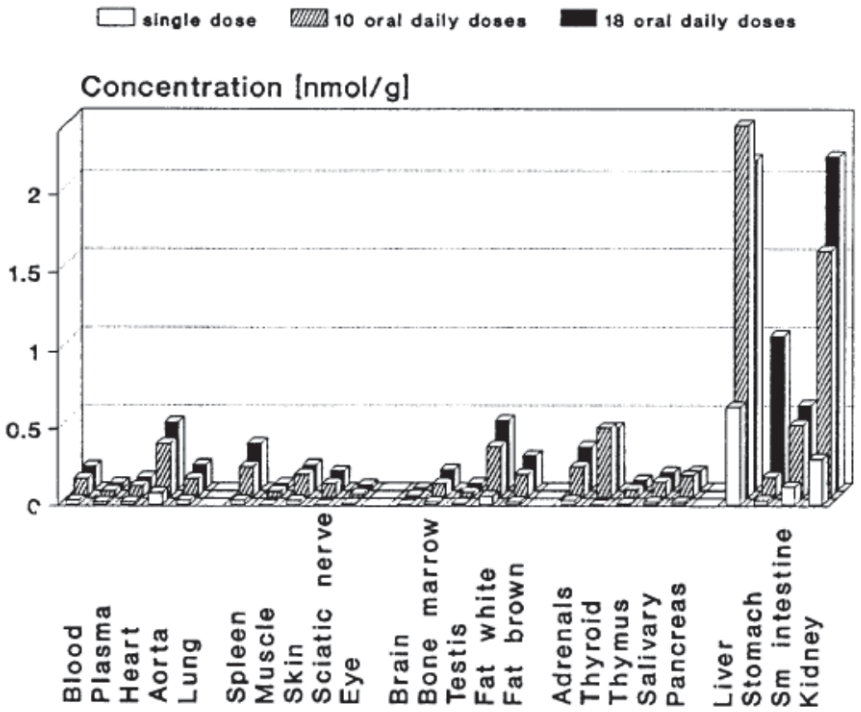
(Excerpted from Study No. DMPK-R71993)



Appears this way on original

Figure 4 Blood and Tissue Distribution in Male Rats 24hr Single and Repeated Oral Administration of [^{14}C]PKC412 (Mean of 3 Animals)

(Excerpted from Study No. DMPK-R302996)



Tissue Distribution Pregnant Rats and Rabbits:
Methods (DMPK-R301996):

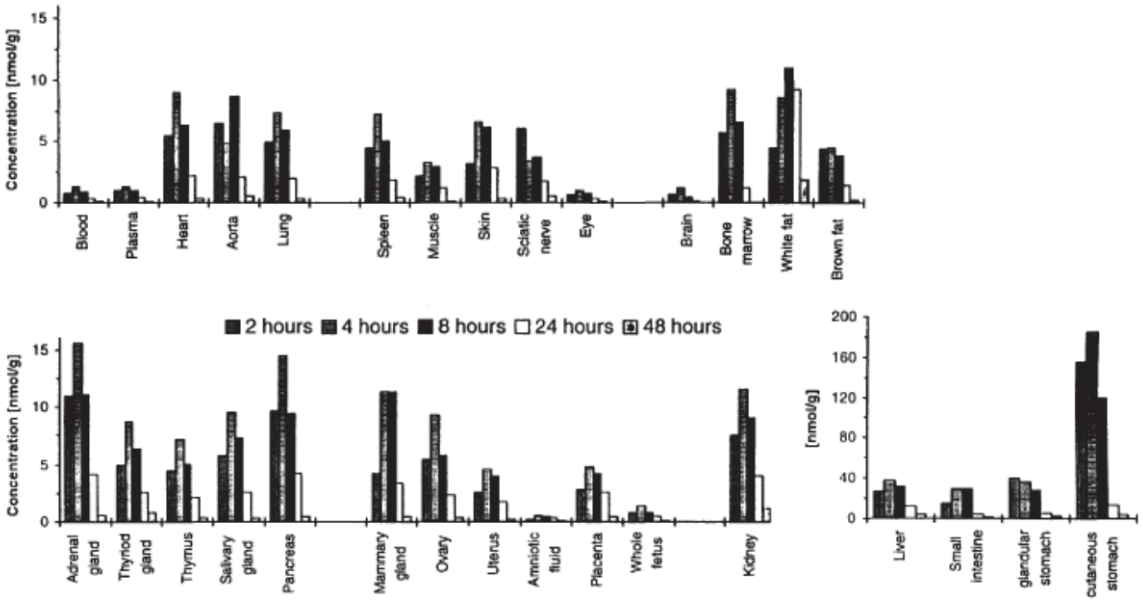
- A single oral dose of 30 mg/kg [^{14}C]CGP41251 (Batch: Ot-7.1, Purity: 99%, (b) (4) diluted in water) was administered to pregnant Hsd/Ola Sprague Dawley rats on gestational day (GD) 13. Radioactivity was determined in homogenized tissues by liquid scintillation counting, n=3 to 6 dams/timepoint.

Results:

- The highest concentrations were in the stomach, small intestine, and liver, with very little in the whole fetus and amniotic fluid (similar levels to blood).

Figure 5 [^{14}C]CGP41251 Concentrations in the Dam and Fetus of Rats [redacted] (b) (4) Formulation)

(Excerpted from Study No. DMPK-R301996)



Concentrations of radioactive substance(s) in blood, plasma, selected organs and tissues of the dam, and in the amniotic fluid, placenta, and whole fetus 2, 4, 8, 24, and 48 hrs after single peroral administration of 30 mg/kg [^{14}]CGP41251 on Day 13 of gestation (mean values).

Methods (DMPK-R0900286):

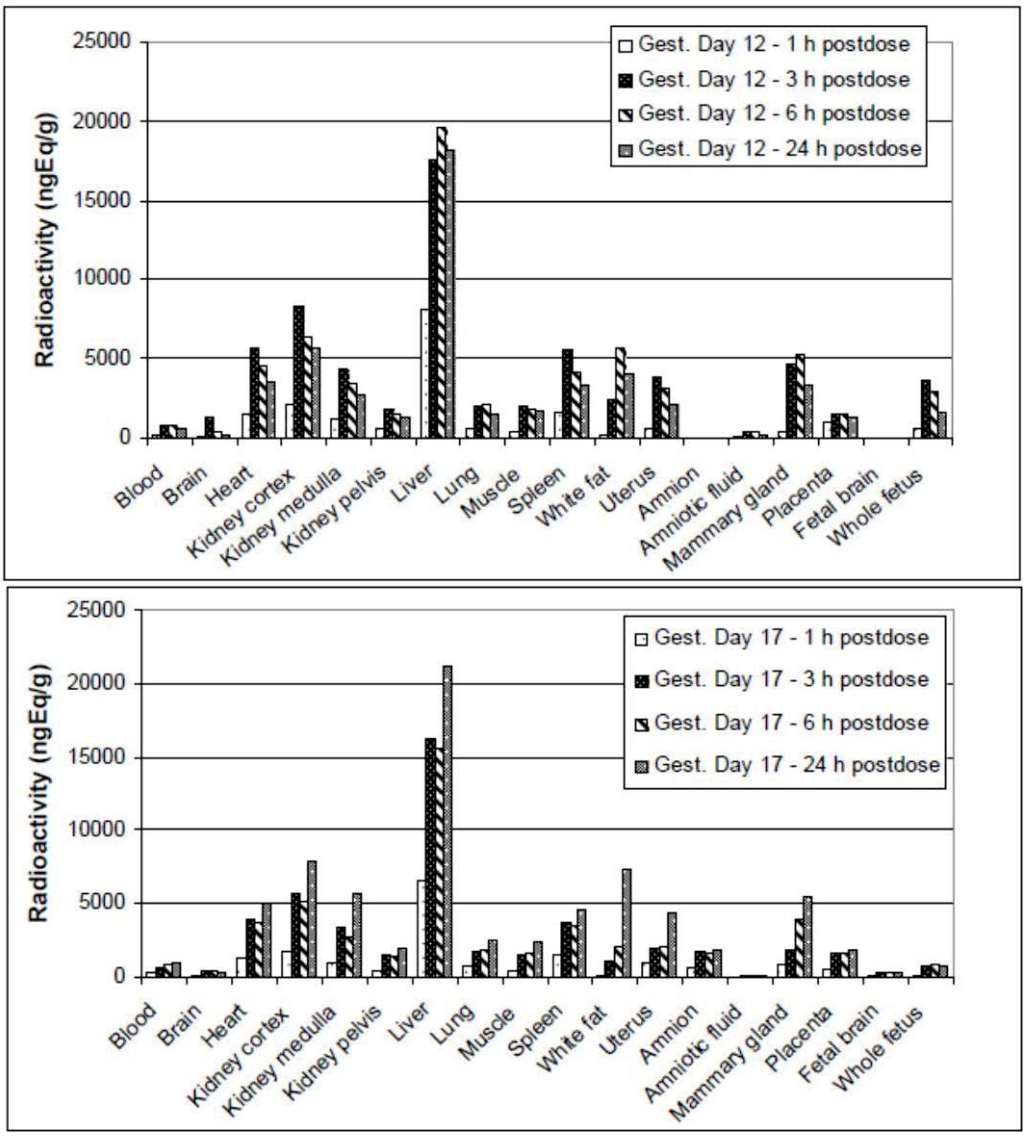
- A single oral dose of 30 mg/kg [^{14}C]PKC412 (Batch: RSE-044-20, Purity:>98%, in the more clinically relevant [redacted] (b) (4)) was administered to pregnant Wistar/Han (albino) rats on GD12 or GD17. Radioactivity was measured using WBA (n=1 dam/timepoint at 1, 3, 6 and 24 hr postdose).

Results:

The highest concentrations were in the liver, kidney, spleen, and heart, displaying the highest radioactive concentration at 3 or 6 hr postdose (24 hr on GD17, suggesting delayed absorption). By 24 hr postdose on GD12, the radioactivity concentrations in most tissues were >70% of their peak concentration. On GD12, the fetus/maternal blood ratio=2.4 to 4.9, brain/blood ratios ranged from 0.27 to 1.7, and tissue/blood ratios were 0.4 to 0.6 in amniotic fluid and 1.7 to 4.4 in placenta. On GD17, fetus/maternal blood and brain/blood ratios were lower than GD12 and distribution to amniotic fluid was very low (<0.1 to 0.18-fold of maternal blood). It is noted that the fetus on GD12 showed >2-fold radioactivity than the fetus on GD17. Very little drug appeared to enter the fetal brain.

Figure 6 [^{14}C]PKC412 Concentrations in the Dam and Fetus of Rats ([redacted] (b) (4) Formulation)

(Excerpted from Study No. DMPK-R0900286)



Methods (DMPK-R51996):

- An oral dose of 20 mg/kg [^{14}C]CGP41251 (Batch: Ko-80, 1A-2, Purity: >98%, [redacted] (b) (4) diluted in water) was administered to New Zealand white rabbits on GD17. Radioactivity was determined in homogenized tissues by liquid scintillation counting 24 hrs postdose only (n=2).

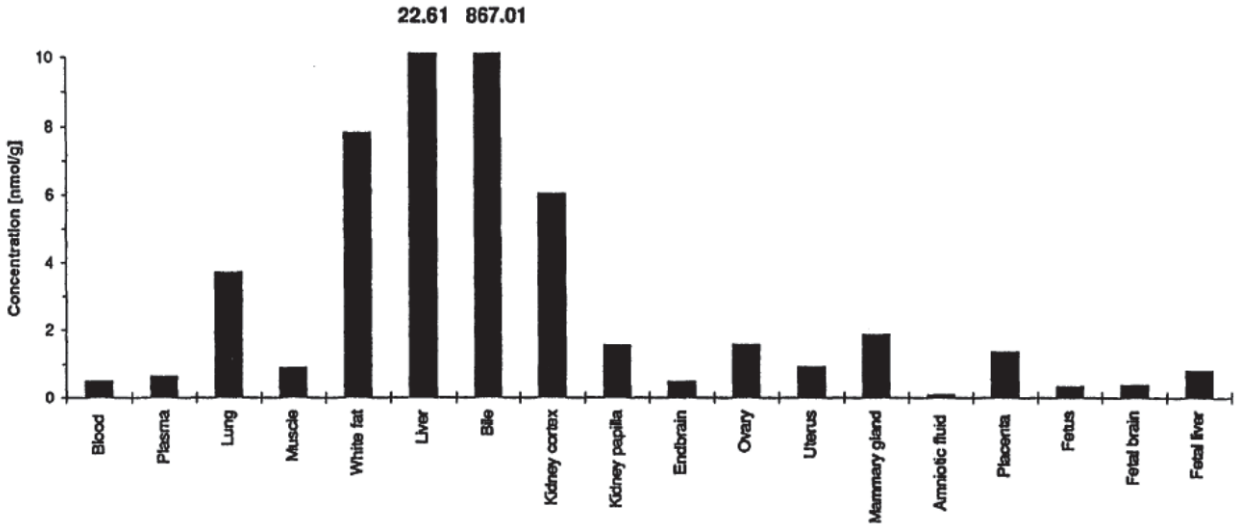
Results:

- The highest concentrations were found in the bile, liver, white fat, kidney, and lung. Uptake of radioactivity into the mammary glands suggests that the compound may be excreted with the milk in lactating animals. Transfer to the

fetus was not extensive (^{14}C levels in the fetus were lower than that in maternal blood).

Figure 7 [^{14}C]CGP41251 Concentrations in the Dam and Fetus of Rabbits (b) (4) Formulation)

(Excerpted from Study No. DMPK-R51996)

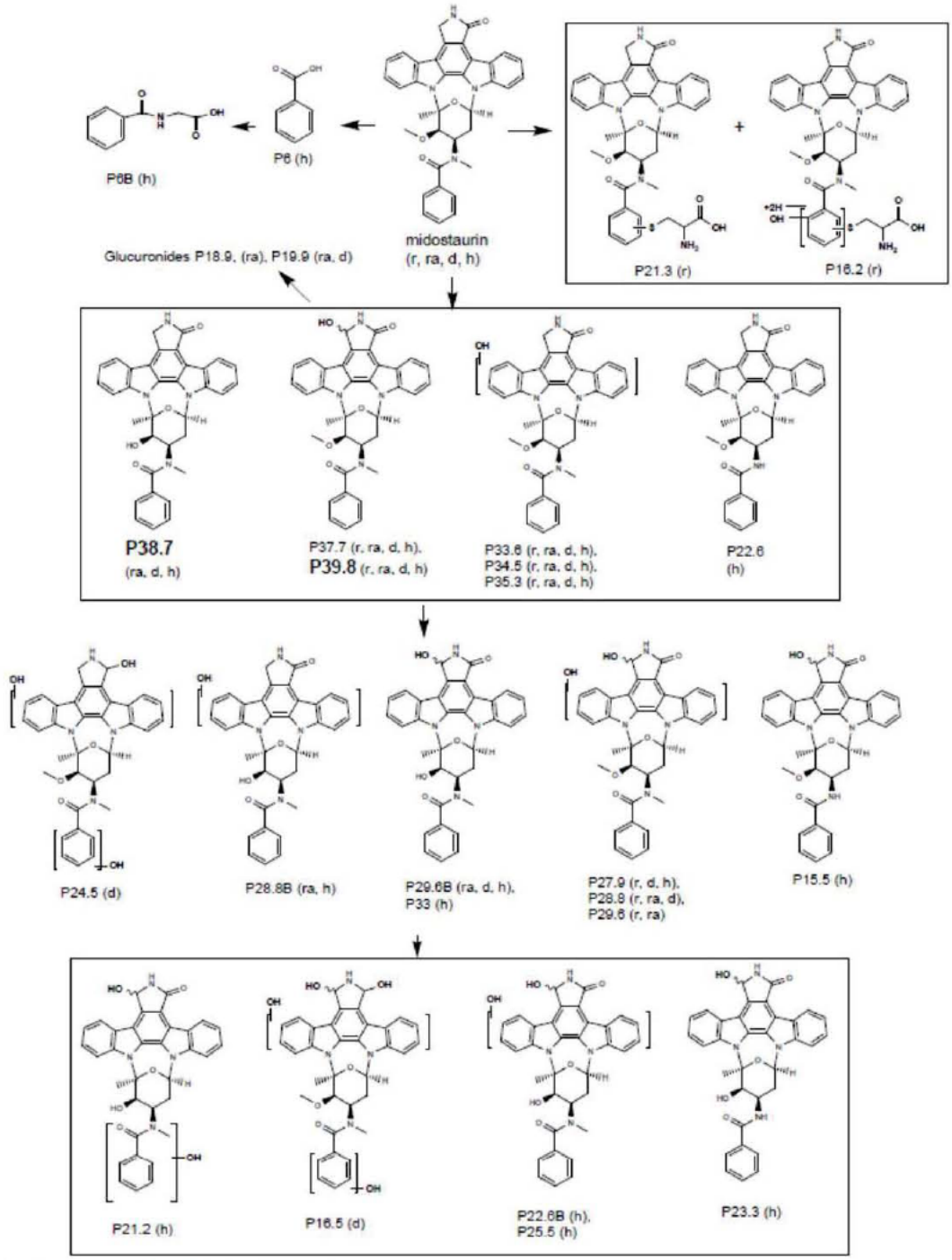


Metabolism

- Midostaurin is highly metabolized representing 22% to 57% of the total drug-related AUC. The major circulating components (>10% of the total drug-related AUC) were midostaurin, CGP52421 (epimers 1 and 2, mono-hydroxylation metabolites) and CGP62221 (O-demethylation metabolite) in animals and humans. CGP52421 (both epimers) is present in the rat, rabbit, dog and human. CGP62221 was not detected at all in the rat and only as 1.9 and 4.6% of total AUC in rabbits and dogs, respectively (See Table 22).
- In human liver slices, biotransformation occurred mainly via oxidative pathways, whereas, in rat, dog and monkey liver slices it occurred via both oxidative and glucuronidation of the oxidative metabolites. Rates were similar for rat, monkey and human (Study No. DMPK-R0201774).
- CYP3A4 was mainly involved in both hydroxylation and demethylation pathways of midostaurin, with a very minor contribution of CYP1A1. The hydroxylation and demethylation reactions appeared to have comparable catalytic efficiencies in both recombinant CYP3A4 and human liver microsomal preparations.

Figure 8 Metabolic Pathways for Midostaurin in All Species

(Excerpted from Figure 5.1. of the Pharmacology Written Summary)



*r: rat; ra: rabbit; d: dog; h: human

In bold are the major human metabolites

Note: Major human metabolites in bold are P38.7= CGP62221; P39.8=epimer 2 of CGP52421

Table 22 Plasma AUC% of Midostaurin and Circulating Human Metabolites after an Oral Radiolabeled Dose Across Species

(Excerpted from the PK Written Summary)

	AUC % of midostaurin and circulating metabolites[a]			
Metabolite[b]	Rat (10 mg/kg)	Rabbit (10 mg/kg)	Dog (3 mg/kg)	Human (0.71 mg/kg)[c]
Midostaurin	25	41	57	22
P15.5	-[d]	-	-	0.77
P19.9	-	-	4.0	-
P22.6	-	-	-	0.95
P23.3	-	-	-	1.1
P29.6B	-	4.1	-	2.5
P33	-	-	-	7.1
P33.6	-	-	5.0	-
P34.5	1.7	-	1.9	-
P35.3	2.5	-	0.68	-
P37.7 (epimer 1 of CGP52421)	20	22	22	5.3
P38.7 (CGP62221)	-	1.9	4.6	28
P39.8 (epimer 2 of CGP52421)	37	19	3.8	33

[a] The AUC % based on total radioactivity were obtained from the ADME studies.

[b] See Figure 5-1 for structures.

[c] Based on a dose of 50 mg and a mean body weight of 70 kg.

[d] Not detected or not quantifiable.

Sources: [Table 2.6.5.9A ADME(US) R0201773], [Table 2.6.5.9F DMPK R0900139], [Table 2.6.5.9D DMPK R0900185a and DMPK R0900185], [Summary of Clinical Pharmacology studies 2.7.2 - Study CPKC412A2107], [Table 2.6.5.9H CPKC412A2107, CPKC412A2107 Amendment 1, and CPKC412A2107 Amendment 2]

Excretion

- In an extension study of Study No. DMPK-R71993, rats excreted predominantly conjugated metabolites and the dogs excreted primarily unconjugated metabolites (Study No. DMPK-DM101993).
- Excretion of the CGP52421 and CGP62221 metabolites was primarily fecal, like the parent (0.5 to 4% excreted in urine). Total recovery of radioactivity in all species was high (81.5 to 99.4%). Unchanged drug accounted for 0.91-18.7% in feces across species (highest in the rabbit).
- Biliary excretion is a major pathway of excretion as evidenced in bile cannulated rats.

Excretion in Lactating Rats:
Methods (Study No. DMPK-R0900142):

- Lactating Wistar/Han rats were treated with a single 30 mg/kg oral dose on postnatal day (PND) 8 or 9 (n=3 rats/timepoint at 1, 2, 4, 8 and 24 hr postdose).

Results:

- Unchanged midostaurin was present at higher relative concentrations in rat milk (51%) compared to that observed in plasma (38%). Epimers of CGP52421 represented 28 to 32% of the total AUC in rat plasma and 12 to 22% of rat milk (CGP62221 is not present in the rat). Transfer to milk occurred within 1 hr

postdose, with Tmax at 4 hr and a half-life of 24 hr. Minor metabolites included P27.9 (dihydroxy PKC412), P28.8 (dihydroxy PKC412), P29.6 (dihydroxy PKC412), P35.3 (monohydroxy PKC412) and P43.5, each accounting for no more than 4.1% of total AUC(0-24hr) .

Table 23 Estimated Percent Transfer of Midostaurin and Metabolites

Applicant's Estimate:

	[Plasma] at 50 mg BID*	Mean Milk:plasma ratio	Estimated [Milk]	Estimated mg/L in Human Milk	% of Dose in Human Milk
PKC412 and metabolites	13750 ngEq/mL	5.3	72875 ngEq/mL	73	73
PKC412	1350 ngEq/mL	7.1	9590 ngEq/mL	9.6	9.6

*Human plasma concentration data is from Study No. CPKC412A2107.

Animal:Human Exposure Comparisons

- Human AUCs compared to AUCs in the rat and dog, the general toxicology species, were higher for midostaurin, epimer 1 and epimer 2 of CGP52421, and CGP62221. Of note, in humans, midostaurin has time-dependent PK and based on trough concentration data, 50 mg and 100 mg BID doses in patients seem to have similar exposure at steady state (based in communication with the Clinical Pharmacology team). The highest concentration observed was on Day 3 with an AUC(0-24hr) of 95009 ng.hr/mL (or 166.496 µmol.hr/mL, factoring in the molecular weight of midostaurin), which is about double the human exposure noted by the Applicant in the table below. Therefore, the most conservative animal exposure multiples relative to humans in Table 24 below can be assumed to be about half of what is listed.

Table 24 Comparative Systemic Exposure Ratios of PKC412 and the Metabolites CGP52421 and CGP62221

(Excerpted from the Nonclinical Overview)

Species Study	Dose (mg/kg/day)	Animal[a] and Human[b] Exposure AUC0-24h (nmol*h/L)			Exposure Multiple relative to Human exposures at steady state 50/100 mg bid		
		PKC412	CGP52421	CGP62221	PKC412	CGP52421	CGP62221
Rat 26/52-week[c] [936281]	30[d]	4370 **4.37** (fu)	13000 31.1 (fu)	-[e]	0.06/0.05 **0.3/0.25**	0.06/0.11 **0.68/1.19**	-
Rat MTD 26-week [956016]	60	10083 **10.1 (fu)**	NM	NM	0.14/0.12 **0.7/0.6**	-	-
Dog 26/52-week[c] [946003]	10[d]	6560 **5.2 (fu)**	6880 **14 (fu)**	1460 **2.8 (fu)**	0.09/0.08 **0.35/0.3**	0.03/0.06 **0.31/0.5**	0.02/0.01 **0.11/0.07**
Dog MTD 13-week [926041]	30	11164 **8.9 (fu)**	NM	NM	0.15/0.13 **0.6/0.5**	-	-
Monkey 13-week[c] [956014]	6	9560	4830	2150	0.13/0.11	0.02/0.04	0.03/0.02
Juvenile Rat [0870600]	15[d]	4106	NM	NM	Range 0.26-0.13[i]		
Rat Fertility LOAEL[f] [964123]	60	10083[g]	NM	NM	0.13/0.11		
Rat EFD LOAEL[f] [936241]	3	334[g]	NM	NM	0.004/0.004		
Rabbit EFD LOAEL[f] [936243]	10	1581[h]	NM	NM	0.02/0.02		

Species Study	Dose (mg/kg/day)	Animal[a] and Human[b] Exposure AUC0-24h (nmol*h/L)			Exposure Multiple relative to Human exposures at steady state 50/100 mg bid		
		PKC412	CGP52421	CGP62221	PKC412	CGP52421	CGP62221
Human	50 mg bid AML	75550 **15.1 (fu)**	224960 **48 (fu)**	65200 **24.5 (fu)**			
	100 mg bid ASM	88220 **17.6 (fu)**	113280 **24 (fu)**	103630 **38.9 (fu)**			

fu= fraction unbound or free fraction, NM = Not measured; MTD = Maximum Tolerated Dose, EFD = embryo fetal development, LOAEL= lowest observed adverse effect level; bid= twice a day; Bold numbers indicate the free fractions and associated exposure multiples

[a]-Average of the mean values from male and female animals after the last dose or at steady state.

[b]-Predicted exposures of midostaurin and its two major metabolites, CGP52421 and CGP62221 at steady state based on the final PopPK models in AML developed with pooled data from studies A2104, A2104E1, A2104E2 and A2106 popPK report AML and for ASM, developed with pooled data from A2213 and D2201 [popPK report ASM].

[c]-Semi-quantification of metabolites / parent were performed using non-validated method in the preclinical species, see [Table 3-2; DMPK R1500239].

[d] – highest dose tested in the study

[e] - Trace quantities below the calibration standards detected

[f] - LOAEL doses listed are from the respective studies as indicated in the table. Exposure data are from other studies (see footnote [g] and [h]).

[g] - Exposures reported for rat fertility and rat EFD are based on exposures measured at the respective doses at steady state in females in [study 956016] [BPK (CH) 1995/083]

[h] – Measured from rabbit pilot EFD [study 936242] [BPKCH 1995/069]

[i] - Exposures used in deriving these ratios are based on prediction of AUCs in pediatric patients given midostaurin at 60 mg/m^2. The patient ranged in weight from 7- 75 kg with AUCs of 30475 -15561 respectively [Source: /vob/CPKC412A/pool/pkpd_001/nonmem/PK_Ped/Simulations3.R -> .Rout]

5.2 Toxicokinetics

See general toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Single dose studies were reviewed by Dr. Hua Zheng during the 30-Day safety review period and are summarized in the Integrated Summary.

6.2 Repeat-Dose Toxicity

Studies 926037, 954096, 936281, 926041, and 946003 were previously reviewed under IND 57120 by Dr. Hua Zheng. The format and content of the reviews were modified to fit this NDA review.

In a GLP-compliant 3-month oral toxicity study in Tif:Ralf rats (SPF) (Study No. 926037), the PKC412 (b) (4) formulation was administered at daily doses of 0 ((b) (4); Batch 1058/4), 10, 20 and 30 mg/kg/day (low dose (LD), mid dose (MD), high dose (HD), respectively) to 10 rats/sex/main study group and 5 rats/sex/recovery group. The following findings are noted:

- Clinical signs: reversible mild to moderate salivation ≥ 20 mg/kg/day.
- Irreversible ↓RBC parameters (up to 15%↓), observed as early as week (wk) 5 at 30 mg/kg/day.
- Reversible ↑ALT and AST (roughly 2-fold↑ at wk 13), correlating with ↑liver weights (14 to 19%↑) mainly at 30 mg/kg/day
- ↓ mean relative thymus weights (11 to 23%) in HD and MD female (♀) groups; not reversible
- ↓ mean thyroid weights (21 to 29%) in recovery HD♀ groups
- Microscopic findings (No histopathological data was available for recovery animals):
    - Mineralized lymphoid follicles of the ileum in 1 HD male (♂) (correlated with macroscopic dark mesenteric lymph nodes (LN))
    - Moderate eosinophilic debris of the epididymis 2 HD♂
    - Aspermia of the epididymis in 1 HD♂
    - Marked tubular atrophy of the testis in 1 HD♂
    - Congestion of the mesenteric LN in 2 HD♂ (correlated with macroscopic dark mesenteric LN macroscopically)
    - Trace to minimal focal heart necrosis in 5/10 HD♂ and 1/10 HD♀
    - Marked to moderate goblet cell hyperplasia of the ileum and jejunum in 1 HD♂
    - Minimal perivascular edema in 1 HD♂ and 2 HD♀ (correlated with ↑ lung weights)
    - Moderate pleural thickening was observed in one MD♀
- Toxicokinetics (TK) (Study No. PCS-R111993): The systemic exposure (AUC) and Cmax were dose proportional both on Day 1 and at steady-state. Both Cmax

and AUC values were higher at the steady-state than that at Day 1. Tmax = 1 to 2 hours.

Study title: CGP41251: 26-Week Oral (Gavage) Toxicity Study in Rats

Study no.:	956016 (originally 954096)
Study report location:	4.2.3.2.
Conducting laboratory and location:	Novartis, Summit, NJ USA
Date of study initiation:	July 25, 1995
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	PKC412 ([redacted (b) (4)] (Lot 800192) [redacted (b) (4)] Batch 1058/11; Purity: >99%)

Key Study Findings

- All males at 100 mg/kg/day were moribund by wk 3. Changes secondary to moribundity/mortality consisted of toxicity to the glands (adrenal, pancreas, lacrimal and salivary glands) and changes in the stomach mucosa (e.g., resident bacterial rods).
- Toxicity to the heart (females only), bone marrow and spleen (males only), small and large intestines, and seminiferous tubules (males only) occurred at 100 mg/kg/day.
- Toxicity to the thymus, mesenteric lymph node, and uterus (irreversible at the HD) and ovaries and liver, generally occurred at all doses (≥ 30 mg/kg/day).

Methods

Doses:	0, 30, 60 and 100 mg/kg/day (LD, MD, HD)
Frequency of dosing:	Daily for 6 months, 1 month recovery
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	(b) (4) (Batch 14236)
Species/Strain:	Sprague Dawley Rats (Crl:CD[SD]BR)
Number/Sex/Group:	25/sex/group (10/sex/group allocated for recovery (control and HD only); 15/sex/group (LD and MD)
Age:	6 wks
Weight:	♂: 188 to 240 g, ♀: 123 to 169 g
Satellite groups:	None; main study animals were sampled
Unique study design:	No
Deviation from study protocol:	All HD non-recovery ♂ were dosed only 3 wks and sacrificed on D21 due to signs of morbidity. After 3 wks, control and HD males entered the 4-wk recovery period.

Observations and Times

Mortality/Clinical signs:	Daily
Body weights, Food consumption:	Pretest, weekly until wk 15, every 4 wks thereafter; weekly during the recovery period
Ophthalmoscopy:	Pretest, wks 13 and 25
Physical, Hearing Examinations:	Pretest, wks 12, 26 and 30
Hematology, Clinical Chemistry:	Pretest, wks 3, 7 (control and HD♂) or 14, 26, and 30 (all others)
Urinalysis:	Pretest, wks 13 and 25, wk 16 recovery
Gross Pathology, Organ Weights, Histopathology:	At necropsy*
TK (right orbital sinus):	Wks 5 and 20: predose and at 1, 2,4, 8 and 24 hrs postdose

* Necropsy was conducted on D21 (control and HD♂ non-recovery), wk 7 (control and HD♂ recovery), wk 26 (all other non-recovery), wk 30 (all other recovery). All tissues were examined microscopically for control non-recovery animals, MD♂ and HD♂+♀. Only gross lesions and organs of toxicity were examined for other groups.

Results

Mortality

Due to poor physical condition and reduced body weight gain, all surviving non-recovery HD♂ were necropsied on D21.

Table 25 Mortality in Rats – 26-Week Toxicity Study

(Excerpted from the study report)

Summary mortality

Sex:	Males				Females			
Group No.:	1	2	3	4	1	2	3	4
Dose (mg/kg)	0	30	60	100	0	30	60	100
No. of rats:	25	15	15	25	25	15	15	25
Type of death								
Found dead	0	2[a]	1	3	1[a]	0	0	1
Sacrificed moribund	0	0	0	1	0	0	0	1
Terminal sacrifice (week 3)	0	0	0	13	0	0	0	0
Recovery sacrifice (week 8)	10	0	0	8	0	0	0	0
Terminal sacrifice (week 27)	15	13	14	0	14	15	15	14
Recovery sacrifice (week 31)	0	0	0	0	10	0	0	9
Percent survival at 27 weeks	100	87	93	0	93	100	100	93

[a]Died as a result of the bleeding procedure.

Animal No.	Pathology in animals found dead or sacrificed moribund on days 19 and 21 (added by reviewer):
63♂	pale liver, lung, and skeletal muscle; mild plasmacytosis of submaxillary LN; moderate neck hemorrhage; minimal glandular stomach hemorrhage; moderate thymus atrophy (probable cause of death)
70♂	moderate multifocal hemorrhage and erythropoietic hypocellularity of the bone marrow; mild spermatid debris in epididymis; minimal acinar necrosis of the lacrimal gland; moderate lymphoid depletion of the mesenteric LN and spleen; mild bacterial rods in the nonglandular stomach; minimal tubular degeneration of the testes and minimal number of spermatocytes in Stage 7; moderate thymus atrophy (probable cause of death); autolysis of cecum, colon, ileum, and urinary bladder
75♂	minimal cortical hypertrophy of the adrenal gland; moderate multifocal hemorrhage and erythropoietic hypocellularity of the bone marrow; mild to moderate epithelial hyperplasia of the small and large intestine; minimal to moderate dilatation and edema of the large intestine; moderate vacuolation and mild hypocellularity of lamina propria of the small intestine; minimal to mild acinar necrosis of the lacrimal or salivary glands; mild acinar atrophy of the pancreas; moderate lymphoid depletion of the spleen; minimal epithelial hyperplasia and hyperkeratosis of the nonglandular stomach; moderate atrophy of the glandular stomach; severe atrophy of the thymus (probable cause of death)
76♀	mild cortical hypertrophy of the adrenal gland; mild epithelial hyperplasia of the small and large intestine; minimal to mild dilatation and edema of the large intestine; minimal to mild vacuolation and hypocellularity of lamina propria with mild increased mitotic activity of the small intestine; mild erythrophagocytosis/hemosiderosis of the mesenteric LN; mild plasmacytosis of the submaxillary gland; minimal acinar atrophy; mild decreased acidophilic granules and mild acinar vacuolation of the pancreas; mild bacterial rods in the nonglandular stomach; severe atrophy of the thymus (probable cause of death)

- One control ♀, not listed in the above table, died on D135 as a result of the bleeding procedure.
- It is unclear why there are more early deaths in males than females since there are no major differences in Cmax and AUC exposures in the study. Of note, metabolism can be more rapid in male rats. There was also one hydroxylation metabolite (34.5) present in male rats that is not present in females (1.7% of AUC following a single 10 mg/kg oral dose and 8.3% of AUC following a 1 mg/kg

single IV dose)(Study Nos. DMPK-R0201773 and DMPK-R0900142); however, the toxicological relevance of this difference is uncertain.

Clinical Signs

Clinical signs appeared after the 1st week of dosing.

Table 26 Summary of Clinical Signs in 26-Week Rat Study

Dose Level	Clinical Signs
HD♂+♀:	reversible abdominal distension, diarrhea (♂ only), hairloss (↑ incidence in ♀ only), perineal stains (also observed at very low incidence at lower doses)
HD♂:	skin loss of elasticity/dehydration, pale, emaciated prior to 3 wk sacrifice
MD♂:	reversible abdominal distension
All doses/ both sexes:	salivation, but generally with earlier onset and higher incidence with higher doses

Body Weights

Decreased BW gain was observed in HD♂+♀ and MD♂.

Table 27 Summary of Body Weights in 26-Week Rat Study

Dose Level	Body Weights
HD♂:	↓18 to 25% (D15 to 21) and overall 20%↓ at wk 27; statistically significant decreases were observed beginning on D8 and between D29 to 50 during recovery
HD♀:	↓15% (D8), ↓8 to 14% (D92 to 183) and overall ~↓16%, statistically significant compared to controls on wk 27
MD♂:	↓10 to 14% (D43 to D183); statistically significant decreases were observed beginning on D15

Food Consumption

HD♂: ↓8 to 16% (D8 to 21) with significant increase during the first week of recovery
HD♀: ↓10% (D8); ↑19 to 23% (D21 to 29)

Ophthalmoscopy, Hearing Examinations,

Unremarkable

Hematology

Table 28 Summary of Hematology Changes in 26-Week Rat Study

Changes (%)compared to the Control								
Dose (mg/kg/day)		30		60		100		
Day	Parameter	♂	♀	♂	♀	♂	♀	
13	RBC(10^6/μL)	--	--	--	↓11**	↓8**	↓11**	
	Hb (g/dL)	--	--	--	↓10**	--	↓10**	
	HCT(%)	--	--	↓5**	↓8**	↓10**	↓9**	
	WBC(10^3/μL)	--	--	↓29	↓23*	↓21	↓22*	
	LYM(10^3/μL)	--	--	↓30*	↓24*	↓22*	↓30*	
	EOS(10^3/μL)	--	↓16*	↓23*	↓11**	↓41*	↓79**	
	PLT(10^3/μL)	↑32**	↑6*	↑33**	↑24*	↑19**	↑23*	
93	RBC(10^6/μL)	↓9**	↓8**	↓11**	↓12**	NA[c]	↓14**	

Changes (%)compared to the Control							
Dose (mg/kg/day)		30		60		100	
Day	Parameter	♂	♀	♂	♀	♂	♀
	Hb (g/dL)	↓6**	↓7**	↓8**	↓9**	NA	↓14**
	HCT(%)	↓6**	↓8**	--	↓9**	NA	↓10**
	WBC(10^3/µL)	↓14	--	↓25*	↓17	NA	↓21
	LYM(10^3/µL)	↓22*	↓10	↓34**	↓22	NA	↓16
	EOS(10^3/µL)	↓36*	↓17*	↓36*	↑72*	NA	↑11*
	PLT(10^3/µL)	↑21	↑11	↑19*	↑26	NA	↑45

* = p < 0.05; ** = p < 0.01; "--" = changes ≤ 5%; NA = no animal alive at this time point; RBC=red blood cells; Hb=hemoglobin; HCT=hematocrit; WBC= white blood cells; LYM=lymphocytes; EOS=eosinophils; PLT=platelets

- Generally, hematology changes persisted through D177, but the RBC, Hb, and Hct of HD♂ and RBCs in HD♀ remained slightly lower than their concurrent values at the end of their recovery period (D206).
- Applicant notes ↑ PMN in both sexes at ≥ 60 mg/kg/day.
- Note: APTT/coagulation was only documented on D177.

Clinical Chemistry

Table 29 Summary of Clinical Chemistry Changes in 26-Week Rat Study

Changes (%)compared to the Control							
Dose (mg/kg/day)		30		60		100	
Week	Parameter	♂	♀	♂	♀	♂	♀
93	ALT (U/L)	↑242**	↑87**	↑298*	↑235*	NA	↑294*
	AST (U/L)	↑36*	↑19	↑91*	↑62*	NA	↑46*
	ALB (g/dL)	↓6	↓7*	--	↓11**	NA	↓17**
	Total Protein (g/dL)		--	↓5*	↓8*	NA	↓9*
177	ALT (U/L)	↑135*	↑32	↑230*	↑73*	NA	↑144*
	AST (U/L)	↑54*	↑8	↑75*	↑10	NA	↑58
	ALB (g/dL)	↓7	↓8**	--	↓8**	NA	↓9**
	Total Protein (g/dL)	--	--	--	↓8*	NA	↓9*

* = p < 0.05; ** = p < 0.01; "--" = changes ≤ 5%; NA = no animal alive at this time point; ALT=alanine aminotransferase; AST=aspartate aminotransferase; ALB: albumin

- HD♀: ↑ globulin 27% (p< 0.01)
- MD♂+MD/HD♀: ↑3-13% in magnesium
- Albumin, globulin, and magnesium concentrations in HD♀ did not fully recover.
- Some animals with ALT elevations also had hepatocellular vacuolation.

Urinalysis

All dose levels/both sexes: ↓ urine specific gravity, which did not fully recover in HD♀ (statistically significant 1 to 2% decrease).

Table 30 Summary of Urinalysis Changes in 26-Week Rat Study

Changes (%)compared to the Control							
Dose (mg/kg/day)		30		60		100	
Week	Parameter	♂	♀	♂	♀	♂	♀
93	Specific gravity	↓2**	↓2*	↓2**	↓2*	NA	↓3*
177	Specific gravity	↓2**	--	↓2**	--	NA	--

* = p < 0.05; ** = p < 0.01; "--" = changes ≤ 5%; NA = no animal alive at this time point

Gross Pathology

Table 31 Summary of Gross Observations in the 26-Week Rat Study

Dose Level	Gross Pathology
HD♂+♀:	fluid retention in large intestine correlated microscopically with luminal dilation
HD♂+♀:	uterine distension correlated microscopically with uterine luminal dilation
All doses/ both sexes:	dark mesenteric LN correlated microscopically with mesenteric LN erythrophagocytosis/hemosiderosis

Organ Weights

Table 32 Summary of Organ Weights in the 26-Week Rat Study

Dose Level	Organ Weights
MD+HD♀:	↑ mean liver weight (did not correlate with ↑ liver enzymes on an individual level), ↑ mean lung weights, irreversible↓ thymus weights correlated with thymic atrophy
All doses/ both sexes:	↓ mean relative thymus weights in ♂ at all doses (11-23%), ↑ spleen weights in ♀ at all doses (21 to 29%) and in MD♂ groups

Histopathology

Adequate Battery Yes

Peer Review Yes, not signed

Histological Findings

Table 33 Summary of Incidence of Histopathology in 26-Week Rat Study

(Excerpted from Study No. 956016)

	Males				Recovery males		Females				Recovery females	
Group:	1	2	3	4	1	4	1	2	3	4	1	4
Dose (mg/kg):	0	30	60	100	0	100	0	30	60	100	0	100
Approximate duration of dosing (wks):	26	26	26	3	3+4	3+4	26	26	26	26	26+4	26+4
Small intestine - mucosal alterations	0/15	0/15	1/15	12/17	0/10	0/8	0/15	1/15	0/15	3/16	0/10	0/9
Large intestine - mucosal alterations	0/15	1/15	1/15	9/17	0/10	0/8	--	--	--	--	--	--
Bone marrow - hypocellularity	0/15	0/15	0/15	5/17	0/10	0/8	--	--	--	--	--	--
- hemorrhage	0/15	0/15	0/15	4/17	0/10	0/8	--	--	--	--	--	--
Testes - degeneration, tubules	0/15	0/15	0/15	5/17	0/10	0/8	--	--	--	--	--	--
Uterus - luminal dilation	--	--	--	--	--	--	2/15	2/15	5/15	7/16	0/10	4/9
Ovaries - follicular distension	--	--	--	--	--	--	1/15	5/15	4/15	5/16	2/10	2/9
Spleen - lymphoid depletion	0/15	0/15	0/15	6/17	0/10	0/8	--	--	--	--	--	--
Mesenteric lymph node												
- lymphoid depletion	0/15	0/15	0/15	6/17	0/10	0/8	--	--	--	--	--	--
- hemosiderosis	2/15	10/15	15/15	10/17	0/10	1/8	2/15	9/15	11/15	14/16	1/10	5/9
- erythrophagocytosis	1/15	12/15	14/15	10/17	0/10	1/8	0/15	5/15	12/15	14/16	1/10	3/9
Thymus - atrophy	0/15	1/15	3/14	11/17	0/10	0/8	0/15	4/15	6/15	7/16	3/10	2/8
Lacrimal glands - necrosis, acinar	0/15	0/15	0/15	6/16*	0/10	0/8	--	--	--	--	--	--
Salivary glands - necrosis, acinar	0/15	0/15	1/15	3/17*	0/10	0/8	--	--	--	--	--	--
Stomach - bacterial rods	0/15	2/15	0/15	14/17*	0/10	0/8	--	--	--	--	--	--
Adrenal glands - cortical hypertrophy	0/15	0/15	0/15	6/17*	0/10	0/8	--	--	--	--	--	--
Pancreas - diffuse acinar atrophy	1/15	0/15	0/15	7/17*	0/10	0/8	--	--	--	--	--	--
- degranulation	0/15	0/15	0/15	7/17*	0/10	0/8	--	--	--	--	--	--

*These changes are secondary to mortality/moribundity and/or the general poor condition of the animals at the 21 day sacrifice

Dose Level	Detailed Descriptions of Microscopic Findings (from findings in the table above and others with a dose-response that were not included in the Applicant's table above)
HD♂+♀:	• mucosal alterations in **small intestine** included minimal to moderate hyperplasia and/or minimal to mild vacuolation of surface epithelium, increased numbers of mitotic figures in the crypt epithelium, and mild hypocellularity of lamina propria; and, **large intestine** included mild to moderate hyperplasia and dilation of lymphatics and/or edema • Minimal to moderate luminal dilation with gross findings of fluid retention in **small and large intestine** • Mild to moderate acinar vacuolation of the **pancreas** (5/17♂ and 1/16♀) • Mild plasmacytosis of the **submaxillary gland** (8/17♂and 5/16♀); also 9/15 MD♂
HD♂:	minimal to moderate focal degeneration of **seminiferous tubules**; mild hypercellularity of **bone marrow** with concomitant mild hemorrhage; **thymus** atrophy (more diffuse and severe than other groups); mild to moderate lymphoid depletion of **spleen** and **mesenteric LN**; minimal interstitial edema (2/17) of the **pancreas**; severe hypoplasia of the **epididymis** (1/17) or **testes** (1/17); mild to moderate spermatid debris (5/17) of the **epididymis**; minimal syncytial giant cells and mild multifocal Sertoli cell vacuolation of the **testes** (2/17); moderate dilatation of the **urinary bladder** (4/17)
HD♀:	Minimal to mild myocarditis of the **heart** (3♀) and myocardial degeneration of the heart (1♀). Focal/multifocal atrophy of acini in the **pancreas** occurred randomly in ♂, but, in ♀, was present in one control and five 100 mg/kg rats. The Applicant notes this pattern of atrophy is a common spontaneous finding in rats.
All doses/ both sexes:	mild to severe erythrophagocytosis/ mild to moderate hemosiderosis of the **mesenteric LN** – also present in recovery HD♀; **thymus** atrophy - minimal to severe occurring in recovery animals (considered by Applicant as spontaneous, reflecting involution in aging)
All doses ♀:	follicular distension of the **ovaries** with reduced number of corpora lutea with no evidence of oocyte necrosis
MD+HD♀:	interstitial cell hyperplasia/vacuolation of the **ovaries** (3/15 MD and 5/16 HD); luminal dilation of the **uterus**, present during recovery in HD♀. The uterine changes suggest abnormalities of estrous cycle (delayed ovulation, anovulation, prolonged proestrus and uterine fluid accumulation).
1 LD+3 HD♀	multifocal coagulative necrosis of the **liver**; mild centrilobular degeneration in one HD♂

Toxicokinetics

The exposure increased less than proportionally with increasing dose, decreased by 15% from D30 to D135 and was 16% lower for ♂ than ♀.

Table 34 TK Data from the 26-Week Rat Study

(Excerpted from Study No. 956016)

Day	Dose [mg/kg]	Rat Sex	Rat N	Time after dosing [h] 0	1	2 ~	4	8	24	C_{max} [nmol/L]	t_{max} [h]	AUC [h·nmol/L]
				CGP 41251 in plasma [nmol/L]								
30	30	M	14	12	517	489	473	566	0	566	8	8337
		F	15	44	530	671	856	560	5	856	4	9760
	60	M	15	102	681	597	997	847	23	997	4	13276
		F	15	193	1206	1142	992	827	67	1206	1	14800
	100	F	14	148	856	1001	1264	1180	173	1264	4	19404
135	30	M	14	62	319	409	576	469	18	576	4	7523
		F	15	40	575	729	666	444	45	729	2	8488
	60	M	14	38	432	374	633	511	40	633	4	8336
		F	15	85	885	974	914	617	66	974	2	11829
	100	F	14	169	1186	1468	1260	1200	114	1468	2	20167

Dosing Solution Analysis

Dosing solutions were within the limits specified in the protocol (± 10% of the target concentrations). CGP41251 was not detected in any of the analyzed control samples.

Note: Dr. Hua Zheng reviewed a 6/12 month rat study during the 30-Day safety review period that used the less clinically relevant (b) (4) *formulation. Only toxicities in this study that were not observed in the 26-week study are described in this review in* Table 75.

Study title: CGP41251: 3-Month Oral Toxicity Study in Dogs

Study no.: 926041
Study report location: 4.2.3.2.
Conducting laboratory and location: (b) (4)
Date of study initiation: July 14, 1992
GLP compliance: Yes, signed
QA statement: Yes, signed
Drug, lot #, and % purity: PKC412 ((b) (4) Batch 1058/5 and 1058/6); Purity: ~100%

Key Study Findings

- At 30 mg/kg/day, toxicities to the heart (↓ heart rate, P-Q interval prolongation, clotted blood:heart inflammation), lungs (bronchopneumonia), GI tract (hemorrhage, congestion, emesis), liver (multicellular necrosis, ↑liver weight), urinary bladder (congestion), and gland toxicity (thyroid and lacrimal atrophy, adrenal hypertrophy) were observed.
- At doses ≥ 10 mg/kg/day, ↓segmented neutrophils and albumin.
- At doses ≥ 3 mg/kg/day, toxicity to male reproductive organs (oligospermia, ↓organ weights), hematological toxicity (↓thymus weight (females), ↓ RBC parameters (slow to recover)), and kidney toxicity (pyelitis) were observed.

Methods

Doses:	0, 3, 10 and 30 mg/kg/day (LD, MD, HD)
Frequency of dosing:	Daily for 3 months, 1 month recovery
Route of administration:	Oral gelatin capsules
Dose volume:	N/A
Formulation/Vehicle:	(b) (4)
Species/Strain:	Beagle dogs
Number/Sex/Group:	3/sex/group, 3/sex/recovery group (HD only)
Age:	10 to 12 months
Weight:	8 to 13 kg
Satellite groups:	N/A
Unique study design:	No
Deviation from study protocol:	None that were considered by the Study Director to affect integrity. Of note, wk 17 ophthalmic and hearing assessments were missed.

Observations and Times

Mortality/Clinical signs:	Daily
Body weights:	Pretest, 3 times weekly
Food consumption:	Pretest, daily
Ophthalmoscopy:	Pretest, wks 6, 13 and 17 (controls and HD only)
ECG:	Pretest, wk 6 (measured HR, PQ, QRS, and QT)
Neurology:	Pretest, wks 6, 13 and 17 (measured landing or support response, extensor strength, tonic neck reflexes, hopping response, flexor reflex, head position in lateral recumbent position, head position in righting from lateral recumbent position, acoustic startle response, placing reactions, tactile and visual, standing on a straight line, pupil reaction to light, gait observations (activity, muscle coordination, wide stance of hind legs))
Hematology, Clinical Chemistry, Urinalysis	Pretest, wks 5, 9 or 13 and 17 for different groups
Gross Pathology, Organ Weights, Histopathology:	At necropsy on Day 93 (only in HD animals during recovery on Day 123, so could not compare to controls or pretest)

Results

Mortality, Clinical Signs, Body Weights, Food Consumption, Ophthalmoscopy, Neurology, Urinalysis

No mortality. HD: clinical signs of emesis (around 1 hour or later postdose), diarrhea, and salivation. Other endpoints were unremarkable.

ECG

HD and MD: ↓ heart rate (32% relative to pretest in ♂ at wk 13 and during recovery period (HD only)) and prolongation of P-Q intervals ~11% relative to pretest). Sporadic

atrioventricular blocks were noted in one HD♂, showing a marked decrease in heart rate (145 to 75 beats/min).

Hematology

Table 35 Summary of Hematology Changes in 3-Month Dog Toxicity Study

Changes (%) in hematology in HD compared to the Control			
Week	Parameter	♂	♀
	RBC	↓6	--
	Hb	↓8	↓5
	Hct	↓7	↓6
	WBC		
9	RBC	↓7	↓8
	Hb	↓9	↓13
	Hct	↓8	↓12
	MCV	--	↓5
	WBC	↓24	--
13	RBC	↓13	↓14
	Hb	↓15	↓18
	Hct	↓15	↓19
	MCV	--	↓5
	WBC	↓46	--

"--" = changes ≤ 5% or were within the pretest range of variability; RBC=red blood cells; Hb=hemoglobin; Hct=hematocrit; MCV=mean corpuscular volume; WBC= white blood cells

- LD and MD dogs also displayed the decreases in RBC parameters and total WBCs noted in HD animals above, but overall smaller in magnitude.
- MD and HD♂s: decreases in segmented neutrophils.
- Overall, findings were reversible compared to pretest values, except RBC, hematocrit, and MCV decreases persisted during recovery.

Clinical Chemistry

Table 36 Summary of Clinical Chemistry Changes in 3-Month Dog Toxicity Study

Changes (%) in clinical chemistry in HD compared to the Control			
Week	Parameter	♂	♀
5	A2-golbulin	↑39	--
	B1-golbulin	↑10	--
	SB-globulin	--	--
	Albumin	↓15	--
9	A2-golbulin	↑56	--
	B1-golbulin	↑17	--
	SB-globulin	↑17	--
	Albumin	↓20	↓13
	Hb-serum	↑15	↑54
	Glucose	↑12	↑21
13	A2-golbulin	↑46	--
	B1-golbulin	↑13	--
	SB-globulin	↑10	--
	Albumin	↓18	↓15

"--"=changes ≤ 10% or were within the pretest range of variability; globulin levels noted above are relative (to what appears to be a loading control; electrophoresis)

- Clinical chemistry findings were reversible compared to pretest values.
- Similar decreases in albumin in MD compared to HD♀s were also observed.
- Small decreases (<10%) in total protein were also observed in MD and HD dogs as early as wk 9.

Gross Pathology

The following macroscopic findings correlated with the following microscopic findings:

- Hemorrhage or congested margin: spleen congestion
- Small prostate size: atrophy
- Enlarged right auricle with hemorrhagic, clotted blood:heart inflammation
- Red discoloration of the ileum, duodenum, or urinary bladder mucosa: hemorrhage or congestion
- Grey, white foci in the lungs:bronchopnemonia
- Granulated contents of gall bladder: inspisated bile
- Left auricle, irregular grey white surface had no microscopic correlate, but was observed in one HD ♀.

Organ Weights

Table 37 Organ Weight Changes in the 3-Month Dog Study

Sex	Males				Females			
Dose (mg/kg/day)	0	3	10	30	0	3	10	30
Organ	mean	%change	%change	%change	mean	%change	%change	%change
Testis (g)								
absolute	20	11↓	14↓	9↓	NA	NA	NA	NA
rel BW	0.16	--	17↓	--	NA	NA	NA	NA
rel BrW	24	--	13↓	--	NA	NA	NA	NA
Epididymis								
absolute	4.8	23↓	--	26↓	NA	NA	NA	NA
rel BW	0.04	8↓	--	16↓	NA	NA	NA	NA
rel BrW	6	17↓	--	22↓	NA	NA	NA	NA
Prostate								
absolute	11	33↓	32↓	59↓	NA	NA	NA	NA
rel BW	0.09	25↓	34↓	54↓	NA	NA	NA	NA
rel BrW	13	27↓	31↓	57↓	NA	NA	NA	NA
Thymus								
absolute	6	20↓	8↑	11	11	36↓	36↓	45↓
rel BW	0.05	8↓	6↑	27	0.1	36↓	35↓	42↓
rel BrW	7	13↓	9↑	15	13	34↓	31↓	45↓
Liver								
absolute	368	--	--	--	313	24↑	10↑	8↑
rel BW	3	19↑	--	12↑	3	23↑	10↑	13↑
rel BrW	423	7↑	--	--	382	26↑	16↑	8↑
Spleen								
absolute	28	19↓	8↓	23↓	28	8↓	20↓	24↓
rel BW	0.23	--	11↓	14↓	0.25	7↓	20↓	20↓
rel BrW	34	13↓	6↓	21↓	34	7↓	15↓	22↓

NA=organ not present in sex; "--"=changes ≤ 5%; rel=relative; BW=body weight; BrW=brain weight

- Changes were not present during recovery except increased liver weights (↑25% in recovery HD♀ compared to Day 93 controls). The female with multicellular necrosis also had elevated liver weight.

Histopathology

Adequate Battery Yes

Peer Review Yes

Histological Findings

Table 38 Microscopic Findings in 3-Month Toxicity Study in Dogs

Sex	Males				Females			
Dose (mg/kg/day)	0	3	10	30	0	3	10	30
No. of Animals Examined	3/-	3/-	3/-	3/3	3/-	3/-	3/-	3/3
Microscopic Finding								
Testes								
Hypospermatogenesis	0	2	3	3/1	NA	NA	NA	NA
Grade 1	0	1	1	1/1	NA	NA	NA	NA
Grade 2	0	1	3	1/0	NA	NA	NA	NA
Grade 3	0	0	0	1/0	NA	NA	NA	NA
Epididymis								
Aspermia	0	0	0	1/0	NA	NA	NA	NA
Oligospermia	0	2	1	2/0	NA	NA	NA	NA
Grade 1	0	1	1	1/0	NA	NA	NA	NA
Grade 2	0	1	0	1/0	NA	NA	NA	NA
Spermatic debris	0	0	0	1/0	NA	NA	NA	NA
Grade 2	0	0	0	1/0	NA	NA	NA	NA
Prostate								
Atrophy	0	0	1	2/0	NA	NA	NA	NA
Grade 2	0	0	1	0/0	NA	NA	NA	NA
Grade 3	0	0	0	2/0	NA	NA	NA	NA
Ax. Lymph Nodes								
Lymphoid depletion	0	0	0	0/0	0	0	1	1/0
Grade 2	0	0	0	0/0	0	0	1	1/0
Pigmentation	1	0	1	0/0	0	0	0	2/0
Grade 1	1	0	1	0/0	0	0	0	2/0
Retropharyngeal Lymph Nodes								
Lymphoid depletion	0	0	0	0/1	0	0	0	1/0
Grade 2	0	0	0	0/0	0	0	0	1/0
Grade 3	0	0	0	0/1	0	0	0	0/0
Mesenteric lymph node								
Erythrophagocytosis	2	3	1	2/1	1	2	3	3/1
Grade 1	1	2	0	0/1	0	1	1	1/0
Grade 2	1	1	1	2/0	1	1	2	2/1
Kidneys								
Pyelitis/suppurative	0	3	2	2/2	0	2	1	0/0
Grade 1	0	1	1	2/2	0	1	0	0/0
Grade 2	0	2	1	0/0	0	1	1	0/0
Nephritis/suppurative	0	1	0	0/0	0	0	0	0/0
Grade 2	0	1	0	0/0	0	0	0	0/0

Sex	Males				Females			
Dose (mg/kg/day)	0	3	10	30	0	3	10	30
No. of Animals Examined	3/-	3/-	3/-	3/3	3/-	3/-	3/-	3/3
Microscopic Finding								
Urothel. Hyperplasia	0	1	0	0/0	0	0	0	0/1
Grade 1	0	1	0	0/0	0	0	0	0/1
Stomach								
Lymphoid hyperplasia	0	0	2	2/0	0	0	1	1/1
Grade 1	0	0	2	2/0	0	0	1	1/1
Thymus								
Atrophy	0	0	0	0/0	0	1	1	1/0
Grade 2	0	0	0	0/0	0	1	1	0/0
Grade 3	0	0	0	0/0	0	0	0	1/0
Spleen								
Siderotic plaque	0	0	0	0/1	0	1	0	0/0
Grade 1	0	0	0	0/1	0	1	0	0/0
Congestion	1	0	1	0/1	1	0	2	0/1
Grade 1	1	0	1	0/1	1	0	1	0/1
Grade 2	0	0	0	0/0	0	0	1	0/0
Duodenum								
Congestion, acute	0	0	0	1/0	0	0	0	0/2
Grade 1	0	0	0	1/0	0	0	0	0/2
Colon								
Hemorrhage, focal	0	1	0	1/0	0	0	0	0/0
Grade 1	0	1	0	1/0	0	0	0	0/0
Gallbladder								
Inspisated bile	0	0	0	1/0	0	0	0	0/0
Ileum								
Hemorrhage	0	0	0	0/0	0	0	0	1/0
Grade 1	0	0	0	0/0	0	0	0	1/0
Rectum								
Hemorrhage	0	2	0	0/1	0	0	0	1/0
Grade 1	0	2	0	0/1	0	0	0	1/0
Eyes								
Inflammatory focus	0	1	0	1/0	0	0	0	0/0
Grade 1	0	1	0	1/0	0	0	0	0/0
Nictitating Membrane								
React. Hyperplasia	0	2	1	2/0	1	1	1	0/0
Grade 1	0	1	1	1/0	1	1	1	0/0
Grade 2	0	1	0	1/0	0	0	0	0/0
Conjunctivitis	0	0	0	0/0	0	0	0	1/0
Grade 1	0	0	0	0/0	0	0	0	1/0
Lacrimal glands								
Atrophy, diffuse	0	0	0	0/0	0	0	0	1/0
Grade 3	0	0	0	0/0	0	0	0	1/0
Adrenal gland								
Hypertrophy, cortical	0	0	0	1/0	0	1	0	0/0
Grade 1	0	0	0	0/0	0	1	0	0/0
Grade 2	0	0	0	1/0	0	0	0	0/0
Heart								
Inflammation	0	0	0	0/0	0	0	0	1/1
Grade 2	0	0	0	0/0	0	0	0	1/1
Liver								

Sex	Males				Females			
Dose (mg/kg/day)	0	3	10	30	0	3	10	30
No. of Animals Examined	3/-	3/-	3/-	3/3	3/-	3/-	3/-	3/3
Microscopic Finding								
Multicellular necrosis	0	0	0	0/0	0	0	0	1/0
Grade 1	0	0	0	0/0	0	0	0	1/0
Lungs								
Bronchopneumonia	1	2	1	1/0	1	1	1	2/3
Grade 1	1	2	1	1/0	1	1	1	2/3
Hemorrhage/alveolar	0	0	0	0/0	0	0	0	0/1
Grade 1	0	0	0	0/0	0	0	0	0/1
Thyroid gland								
Atrophy, follicular	0	0	0	0/0	0	0	0	1/0
Grade 2	0	0	0	0/0	0	0	0	1/0
Ultimobran. Remnants	0	2	0	0/0	0	0	1	1/1
Grade 1	0	1	0	0/0	0	0	1	1/1
Grade 2	0	1	0	0/0	0	0	0	0/0
Parathyroid glands								
Developmental cyst	0	0	0	0/1	0	0	0	2/1
Grade 1	0	0	0	0/0	0	0	0	1/1
Grade 2	0	0	0	0/1	0	0	0	1/0
Sternum/bone marrow								
Fibr. Osteodystophy	0	0	0	0/0	0	0	0	0/1
Grade 1	0	0	0	0/0	0	0	0	0/1
Urinary bladder								
Congestion	0	0	0	0/0	0	0	0	0/1
Grade 1	0	0	0	0/0	0	0	0	0/1

NA=organ not present in animals; "-" = control, LD, and MD animals were not examined during recovery

Toxicokinetics

TK analysis was submitted as a separate study (Study No. PCS-R91993), but was conducted on the dogs in the 3-month study. The study evaluated 3 dogs/sex/group for LD and MD; 6 animals/sex/group for HD. Plasma concentrations were measured from each animal on Days 1 and 92 predose, and 1, 2, 3, 4, 6, 8, 12 and 24 hrs postdose. Controls were sampled only 4 hrs postdose.

Individual Cmax and AUC values were highly variable, but mean values were generally proportional to dose. Median Tmax = 2 hours. Dose-normalized AUC was independent of dose and the shape of the mean concentration-time curve changed little from Day 1 to 92, indicating linear kinetics. There was accumulation between Days 1 and 92 and not sex related differences.

Table 39 TK Data for 3-Months Dosing in Dogs

	Mean Cmax (nM)		Ratio Day 92/Day 1 C_{max}		Mean AUC (nM.hr)		Ratio Day 92/Day 1 AUC	
Sex	♂	♀	♂	♀	♂	♀	♂	♀
Dose (mg/kg)								
Day 1								
3	135	79	NA	NA	979	660	NA	NA
10	269	444	NA	NA	2249	3899	NA	NA
30	948	778	NA	NA	5123	5173	NA	NA
Day 92								
3	119	117	0.93	1.47	858	1007	1.13	1.53
10	505	528	1.9	1.23	5088	5123	2.28	1.37
30	1423	1521	1.76	2.27	10473	11854	2.25	3.38

Note: Day 92/Day1 ratios are based on dose normalized Cmax and AUC values (not shown)

Study title: CGP41251: 6-12 Month Oral Toxicity Study in Dogs

Study no.: PCS-R946003
Study report location: 4.2.3.2.
Conducting laboratory and location: (b) (4)
Date of study initiation: January 12, 1995
GLP compliance: Yes, signed
QA statement: Yes, signed
Drug, lot #, and % purity: PKC412 ((b) (4) ; Batches 1059/11); Purity: 100%

Key Study Findings

- At doses ≥ 3 mg/kg/day (MD): cardiotoxicity (prolonged PQ interval), kidney toxicity (↓ total protein, hypoalbuminemia, ↓urea, electrolyte imbalances), hematological toxicity (anemia, ↓segmented neutrophils, ↓lymphocytes, thymus atrophy, spleen congestion and hyperplasia (HD only), bone marrow hypocellularity, erythrophagocytosis of the mesenteric LN (HD only)), liver toxicity (HD only: ↑AST, ↑liver weight, tension lipidosis), lung toxicity (bronchopneumonia), adrenal toxicity (HD only: vacuolation), pituitary cysts (HD only), reduced spermatogenesis, and inflammatory foci in brain were observed.
- At all doses there was evidence of kidney, lung, thymus and bone marrow toxicity.

Methods

Doses:	0, 1, 3, 10 mg/kg/day (LD, MD, HD)
Frequency of dosing:	Daily for 6-12 months, 1 month recovery
Route of administration:	Gelatin capsule (perorally)
Formulation/Vehicle:	(b) (4) (Batch C11828)
Species/Strain:	Beagle dog
Number/Sex/Group:	4/sex/group (12 months); 2/sex/group (6 months); 2/sex/group (recovery)
Age:	7 to 14.5 months
Weight:	8 to 15 kg
Satellite groups:	None; main study animals were sampled
Unique study design:	No
Deviation from study protocol:	None considered by the Study Director to affect the interpretation of the data or the integrity of the study.

Observations and Times

Mortality:	Daily
Clinical signs:	Pretest, daily
Body weights:	Pretest, 3 times weekly
Food consumption:	Pretest, daily
Water Consumption:	Pretest, wks 12, 26, 52, and 56 (control and HD)
Ophthalmoscopy:	Pretest (all groups), wk 13, (control and HD only), wks 26, 52 and 56 (all groups)
ECG:	Pretest (all groups); approximately 3 to 4 hours after dosing: wk 13 (control and HD), 26 and 27 (control, MD, HD), 52 and 56 (control and HD)
Neurology:	Pretest, wk 13 (all groups), wks 26, 52 and 56 (control and HD). Measurements: acoustic startle response, extensor strength, flexor reflex, head position in lateral, recumbent position, landing or support response, placing reactions, tactile and visual, pupil reaction to light, standing on a straight line, head position in righting from, lateral recumbent position, hopping response, tonic neck reflexes.
Hematology, Clinical Chemistry, Urinalysis:	Pretest, wks 13, 26, 39, 52, and 56
Gross Pathology, Organ Weights, Histopathology:	At necropsy*
TK (right orbital sinus):	Day 1 and at 6/12 months (3 animals/sex LD and HD): before and 1, 2, 3, 4, 6, 8, 12, and 24 hrs postdose

* Necropsy was conducted after wks 26, 52, and 56 (recovery).

Reference ID: 4051584

Results

Mortality, Clinical Signs, Body Weights, Food and Water Consumption, Ophthalmoscopy, Neurology

One control male dog (main study) died due to bite wounds on day 265 of the study (individually housed). All other endpoints were unremarkable; however, no raw data was provided for neurology endpoints.

ECG

- 1 control♂, 1MD♂, 1 HD♂, 1 HD♀: prolonged PQ interval (150 to 160 ms, with a difference of 20 to 50 ms between the maximum and minimum duration at 26 wks (note: HD♀ variable PQ intervals were followed by series of atrioventricular blocks (8 within 43 sec) while the heart rate was 85 beats/min; the curve being recorded about 4 hours after dosing. 24 hours after dosing, with a heart rate of 135 beats/min, the PQ-interval was normal again).
- A different HD♂ had a slightly prolonged PQ interval (difference of 30 ms between minimum and maximum duration) about 4 hours after dosing that was reduced by 24 hours postdose.

Hematology

Table 40 Summary of Hematology Changes in 6/12 Month Dog Toxicity Study

				Percent Change from Control					
Dose (mg/kg/day)		Control (mean)		1		3		10	
	Week	♂	♀	♂	♀	♂	♀	♂	♀
Hb (mmol/L)	13	9.3	9.7	--	--	--	--	11↓	9↓
	26	9.5	10.2	--	--	8↓*	--	12↓*	16↓*
	39	9.5	10	--	--	--	--	14↓*	11↓
	52	9.9	9.8	--	7↑	--	--	12↓	--
RBC (T/L)	13	6.58	6.56	--	--	8↓	--	10↓*	7↓
	26	6.85	7.08	--	--	10↓*	--	11↓*	13↓*
	39	6.61	6.69	--	--	7↓*	--	12↓	7↓
	52	6.74	6.5	--	7↑	8↓	7↑	12↓	--
HCT (L/L)	13	0.45	0.46	--	--	7↓	--	9↓	9↓
	26	0.45	0.48	--	--	9↓*	--	9↓	15↓*
	39	0.43	0.46	--	--	--	--	12↓	9↓
	52	0.44	0.44	--	7↑	7↓	--	11↓	--
Retic (T/L)	13	0.06	0.06	--	--	20↓	--	28↓	21↓
	26	0.06	0.07	26↓	23↓	29↓*	30↓	34↓*	30↓
	39	0.06	0.07	23↑	31↓	--	--	25↓	--
	52	0.07	0.05	--	29↑	--	53↑	41↓	20↑
WBC (g/L)	13	9.6	9.7	--	--	--	--	22↓	24↓
	26	10.7	10.1	--	--	--	--	37↓*	26↓*
	39	8.8	9.6	--	--	--	--	42↓	27↓
	52	9.1	8.8	--	--	--	--	35↓	26↓

				Percent Change from Control					
Dose (mg/kg/day)		Control (mean)		1		3		10	
	Week	♂	♀	♂	♀	♂	♀	♂	♀
SegNeu (g/L)	26	7	6.3	--	--	23↓	--	34↓*	21↓
	39	5.7	5.9	--	--	--	--	42↓	--
	52	5.8	5.4	--	--	--	--	28↓	24↓
LYM (g/L)	13	2.8	2.6	--	--	--	--	32↓	31↓
	26	2.8	2.8	--	--	--	--	43↓*	36↓
	39	2.2	3.1	--	--	--	26↓	45↓	58↓*
	52	2.1	2.5	--	--	--	--	38↓	32↓

* = $p < 0.05$; "--" = changes ≤ 5% for RBC, Hb, and Hct; ≤ 20% for all others; RBC=red blood cells; Hb=hemoglobin; HCT=hematocrit; Retic=reticulocytes; WBC= white blood cells; SegNeu=segmented neutrophils; LYM=lymphocytes

- In some HD dogs, erythrocytes were hypochromic.
- Changes were full or partially reversible by the end of the recovery period.

Clinical Chemistry

Table 41 Summary of Clinical Chemistry Changes in 6/12 Month Dog Study

				Percent Change from Control					
Dose (mg/kg/day)		Control (mean)		1		3		10	
	Week	♂	♀	♂	♀	♂	♀	♂	♀
AST (U/L)	13	29	27	--	--	--	--	--	--
	26	28	29	--	--	--	--	--	--
	39	30	31	--	--	--	--	57↑*	--
	52	36	29	--	--	--	--	50↑	--
TP (g/L)	13	54.6	60.4	--	--	--	--	5↓**	--
	26	57.9	60.8	--	--	--	--	5↓	--
	39	56.2	61.6	--	--	--	--	6↓	--
	52	60.4	62.7	--	--	--	6↓*	9↓**	5↓
ALB (g/L)	13	29.2	35.4	--	--	--	--	11↓**	13↓**
	26	30	35.2	--	--	--	5↓	12↓*	12↓*
	39	29.8	35.3	--	--	--	6↓	17↓*	10↓
	52	33.2	35.4	--	--	7↓	--	23↓*	6↓
Urea (mmol/L)	13	5.44	6.13	--	8↓	13↓*	--	24↓*	24↓**
	26	5.06	5.79	8↓	19↓*	--	9↓	30↓**	25↓**
	39	5.6	6.22	21↓*	24↓	20↓	18↓	21↓*	16↓
	52	4.33	5.7	--	19↓*	7↑	8↓	16↓	20↓*

*: $p < 0.05$; **: $p < 0.01$; "--": changes < 5% (except for AST, changes < 50%); AST=aspartate aminotransferase; TP=total protein; ALB=albumin

- Transient ↓ Ca^{2+}, ↓Mg^{2+} (HD only) and ↑Cl- (all doses) were observed.
- Changes were full or partially reversible by the end of the recovery period.

Urinalysis

Unremarkable

Gross Pathology

Grey-white foci of the lung correlated with microscopic findings of minimal to moderate chronic purulent bronchopneumonia with deposition of brownish pigment in macrophages.

Organ Weights

Compared to controls, the following changes in absolute and relative (to body and brain) weights are noted:

- HD♂, MD and HD♀: 17 to 30%↓ spleen weight at 26 wks (only HD♂ at 12 months)
- HD♂: >12 and <27%↑ in liver weight at 26 wks, 12 months and recovery
- HD♀: 2-fold ↑ovary weights,18 to 31%↑ adrenal weights at 26 wks; ovary weights were lower than controls and adrenal weights were still elevated at 12 months

Histopathology

Adequate Battery Yes

Peer Review Yes, not signed

Histological Findings

Table 42 Microscopic Findings in Dogs after 6-Months Treatment

(Excerpted from the study report)

Males	Groups	01	02	03	04
Organ/ Finding	Dose (mg/kg)	0	1	3	10
	No. of Animals	2	2	2	2
Thymus	No. Exam.	2	2	2	2
Atrophy			2	1	2
	Av. Grading		1.5	2.0	1.5

Females	Groups	01	02	03	04
Organ/ Finding	Dose (mg/kg)	0	1	3	10
	No. of Animals	2	2	2	2
Lung	No. Exam.	2	2	2	2
Bronchopneumonia, chronic			1	2	1
	Av. Grading		2.0	2.0	2.0
Thymus	No. Exam.	2	2	2	2
Atrophy		1			1
	Av. Grading	1.0			1.0

Table 43 Microscopic Findings in Dogs after 12-Months Treatment

(Excerpted from the study report)

Males	Groups	01	02	03	04
Organ/ Finding	Dose (mg/kg)	0	1	3	10
	No. of Animals	3	4	4	4
Testes	No. Exam.	3	4	4	4
Reduced spermatogenesis					2
	Av. Grading				1.0
Epithelial vacuolation					1
	Av. Grading				1.0
Epididymides	No. Exam.	3	4	4	4
Oligospermia					2
	Av. Grading				1.5
Cellular debris					1
	Av. Grading				2.0
Thymus	No. Exam.	3	4	3	4
Atrophy		1	2	2	4
	Av. Grading	4.0	2.0	2.5	3.3

Females	Groups	01	02	03	04
Organ/ Finding	Dose (mg/kg)	0	1	3	10
	No. of Animals	4	4	4	4
Thymus	No. Exam.	4	4	4	4
Atrophy		1	3	2	3
	Av. Grading	1.0	2.3	1.5	2.0

Table 44 Microscopic Findings in Dogs after 12-Months Treatment (Recovery)

(Excerpted from the study report)

Males	Groups	01	02	03	04
Organ/ Finding	Dose (mg/kg)	0	1	3	10
	No. of Animals	2	2	2	2
Thymus	No. Exam.	2	2	2	2
Atrophy		1	1	1	2
	Av. Grading	2.0	2.0	1.0	2.0

Females	Groups	01	02	03	04
Organ/ Finding	Dose (mg/kg)	0	1	3	10
	No. of Animals	2	2	2	2
Thymus	No. Exam.	2	2	2	2
Atrophy		1		1	
	Av. Grading	2.0		2.0	

Codes and symbols used at:

- finding level:

GRADE 1	= minimal	/ very few	/ very small
GRADE 2	= slight	/ few	/ small
GRADE 3	= moderate	/ moderate number	/ moderate size
GRADE 4	= marked	/ many	/ large
GRADE 5	= massive	/ extensive number	/ extensive size

Other noted microscopic findings at 26 weeks:

- 2/2HD♂: minimal to mild ↑adrenal vacuolation
- 1/2HD♀: minimal inflammatory focus of the liver, minimal erythrophagocytosis of the mesenteric LN (also present mildly in 1/2 HD♂)

- 1/2 MD and 1/2 HD♀: minimal inflammatory focus of the brain, mild megakaryocyte hyperplasia of the spleen (HD only)

Other noted microscopic findings at 12 months:

- 4/4 HD♂ and 2/4♀: pituitary cyst
- 2/4 HD♂ and ♀: minimal to mild diffuse congestion of the spleen
- 1 to 2/4 HD♀: tension or minimal inflammatory focus of the liver
- 2/4 HD♀: minimal to mild erythrophagocytosis of the mesenteric LN
- Females had minimal to mild hypercellularity of bone marrow at all doses (1 animal/dose)

Other noted microscopic findings at recovery:

- 1/2HD♂: detachment, fold, and degeneration of the retina (likely not test article-related. This animal also had multiple grey spots of the retina pretest and postdose in the ophthalmology examination).

Toxicokinetics

TK analysis was submitted as a separate study (Study No. DMPK-R1996089), but was conducted on the dogs in the 6/12 month study. Exposure was roughly dose proportional, slighter higher in males versus females, and with slight accumulation with repeat dosing. The plasma concentration-time curves did not show the extended exponential elimination phase necessary for the determination of terminal elimination rate.

Table 45 Summary of TK Parameters from 6/12 Month Dog Study

(Excerpted from the TK report)

Day	Dose [mg/kg]	Sex	C_{max} [nmol/L]	t_{max} [h]	AUC(0-24h) [h·nmol/L]	Dose [mg/kg]	Sex	C_{max} [nmol/L]	t_{max} [h]	AUC(0-24h) [h·nmol/L]
1	1	M	43	2	443	10	M	752	3	7047
1	1	F	46	3	397	10	F	315	2	1984
176	1	M	53	3	756	10	M	513	3	6809
176	1	F	39	2	457	10	F	593	3	7325
361	1	M	56	3	741	10	M	817	2	9999
361	1	F	51	3	591	10	F	898	3	8190

Additionally, plasma samples from HD dogs were measured for metabolite exposure. Animals were exposed to CGP52421 epimer 1 (P6) at similar levels as the parent.

Table 46 Concentrations of CGP41251 and Metabolites in 10 mg/kg Dogs (6/12 Month Study)

(Excerpted from Study No. DMPK-R1997521)

Male dogs, 6 months treatment:

Plasma collection time [h]	Concentration and standard deviation (SD)							
	CGP 52421, P6		CGP 62221, P8		CGP 52421, P7		CGP 41251	
	[nmol/L]	(SD)	[nmol/L]	(SD)	[nmol/L]	(SD)	[nmol/L]	(SD)
2 *)	293	(24)	61	(16)	32	(19)	393	(90)
8	295	(179)	61	(21)	15	(12)	240	(20)
24	228	(207)	52	(51)	10	(13)	183	(213)

*) plasma samples from only 2 dogs

Male dogs, 12 months treatment:

Plasma collection time [h]	Concentration and standard deviation (SD)							
	CGP 52421, P6		CGP 62221, P8		CGP 52421, P7		CGP 41251	
	[nmol/L]	(SD)	[nmol/L]	(SD)	[nmol/L]	(SD)	[nmol/L]	(SD)
2	676	(348)	120	(45)	44	(13)	731	(63)
8	614	(294)	109	(27)	24	(10)	425	(24)
24	336	(260)	67	(60)	10	(11)	238	(239)

Female dogs, 6 months treatment:

Plasma collection time [h]	Concentration and standard deviation (SD)							
	CGP 52421, P6		CGP 62221, P8		CGP 52421, P7		CGP 41251	
	[nmol/L]	(SD)	[nmol/L]	(SD)	[nmol/L]	(SD)	[nmol/L]	(SD)
2	312	(145)	74	(31)	30	(10)	501	(194)
8	329	(141)	75	(41)	20	(11)	342	(154)
24	207	(224)	48	(43)	9	(12)	165	(141)

Female dogs, 12 months treatment:

Plasma collection time [h]	Concentration and standard deviation (SD)							
	CGP 52421, P6		CGP 62221, P8		CGP 52421, P7		CGP 41251	
	[nmol/L]	(SD)	[nmol/L]	(SD)	[nmol/L]	(SD)	[nmol/L]	(SD)
2	354	(143)	78	(35)	40	(16)	694	(373)
8	336	(184)	92	(49)	17	(11)	337	(211)
24	204	(173)	52	(43)	9	(11)	174	(168)

Dosing Solution Analysis

Based on the expiry date and analytical reference data it is concluded that the test article was stable and uniformly distributed in the vehicle.

Note: Dr. Hua Zheng also reviewed a 6/12 month dog study during the 30-Day safety review period; however, it appears to be a different study (same doses, different batches of test article and vehicle, and number of dogs used) than PCS-R946003. Notable findings in addition to the 6/12 month study reviewed above are emesis within the first few days, prostatic atrophy in MD and HD♂, and follicular lymphoid hyperplasia in the stomach at MD and HD.

7 Genetic Toxicology

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

Study title: Salmonella and Escherichia/Liver-Microsome Test

Study no.:	926113
Study report location:	4.2.3.3.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 27, 1992
GLP compliance:	Yes
QA statement:	Yes, signed
Drug, lot #, and % purity:	CGP41251; Batch No. 800591; Purity: 98.5%

Key Study Findings

- CGP41251 is negative in the in vitro reverse mutation assay in bacterial cells (Ames) under the conditions tested. A negative result was also obtained from a prior non-GLP study (Study No. AFP59).

Methods

Strains:	S. typhimurium TA98, TA100, TA1535, TA1537, and E. coli WP2 uvrA
Concentrations in definitive study:	0, 312.5, 625, 1250, 2500, 5000 µg/plate
Basis of concentration selection:	Range-finding test determined highest concentration with four lower concentrations decreasing by a factor of 2.
Negative control:	DMSO
Positive control:	

A) Experiment without metabolic activation:

Strain	Mutagen	Solvent	Concentration
TA 100	sodium azide	bidist. water	5.0 µg/ plate
TA 1535	sodium azide	bidist. water	5.0 µg/ plate
WP2 uvrA	4-nitroquinoline-N-oxide	DMSO	2.0 µg/ plate
TA 98	2-nitrofluorene	DMSO	20.0 µg/ plate
TA 1537	9(5)-aminoacridine	DMSO	150.0 µg/ plate

B) Experiment with metabolic activation:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/ plate
TA 1535	cyclophosphamide·H_2O	bidist. water	400.0 µg/ plate
WP2 uvrA	2-aminoanthracene	DMSO	50.0 µg/ plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/ plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/ plate

Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plate incorporation for 48 hrs

Study Validity

The study is valid.

Reference ID: 4051584

Results

Table 47 Summary of Ames Test Results – GLP

(Excerpted from the Toxicology Tabulated Summary)

Experiment 1 (plate incorporation):

Strain:		TA98		TA100		TA1535		TA1537		WP2 uvrA	
S9:		-	+	-	+	-	+	-	+	-	+
Dose (µg/ plate):	0	22.3	34.3	103.0	114.3	11.7	11.0	4.7	5.3	14.0	15.0
	312.5	16.7	29.7	98.0	101.0	8.3	13.3	3.3	6.0	12.3	17.0
	625	16.0	34.0	102.7	106.3	9.3	11.7	4.3	5.7	10.7	16.7
	1,250	16.0	28.3	109.7	60.3	10.7	7.0	5.7	3.7	13.7	9.7
	2,500	11.7	26.7	96.3	73.3	11.0	5.7	4.0	2.7	17.7	8.0
	5,000	6.7	23.3	92.7	85.7	6.3	3.7	1.0	2.7	6.3	8.0

DMSO = Dimethylsulfoxide; NR = Not reported; S9 = Externally-added rat metabolic activating system.

Experiment 2 (plate incorporation):

Strain:		TA98		TA100		TA1535		TA1537		WP2 uvrA	
S9:		-	+	-	+	-	+	-	+	-	+
Dose (µg/ plate):	0	14.7	34.3	117.0	152.0	11.0	13.0	5.7	9.7	15.3	17.0
	312.5	14.7	36.3	111.0	133.0	10.7	11.0	6.7	8.7	14.3	19.7
	625	12.7	33.3	125.3	146.0	10.7	13.7	7.0	5.7	15.3	25.7
	1250	12.3	16.3	120.7	125.7	11.0	12.0	3.7	8.7	16.0	16.3
	2500	10.0	13.3	103.7	115.0	8.3	12.7	3.3	3.7	14.7	15.3
	5000	5.0	17.3	104.0	106.7	6.3	12.0	2.0	2.0	12.0	11.3

S9 = Externally-added rat metabolic activating system.

Positive controls:
Without S9: Sodium azide (5.0 µg/plate), 4-nitroquinoline-N-oxide (2.0 µg/plate), 2-nitrofluorene (20.0 µg/plate), and 9(5)-aminoacridine (150.0 µg/plate)
With S9: Cyclophosphamide·H_2O (400.0 µg/plate) and 2-aminoanthracine (2.5 or 50.0 µg/plate)
Effects of positive controls: Clearly genotoxic

7.2 *In Vitro* Assays in Mammalian Cells

Study title: Gene Mutation Test with Chinese Hamster Cells V79 In Vitro

Study no.:	926298
Study report location:	4.2.3.3.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 2, 1992
GLP compliance:	Yes
QA statement:	Yes, signed
Drug, lot #, and % purity:	CGP41251; Batch No. 800591; Purity: 98.5%

Key Study Findings

- CGP41251 was negative for mutagenicity in V79 Chinese hamster cells based on the conditions tested.

Methods

Cell line:	V79 Chinese hamster cells
Concentrations in definitive study:	0.0023, 0.0094, 0.0375, 0.1500 µg/mL
Basis of concentration selection:	Cytotoxicity test determined highest concentration +S9 to be <312.5 µg/mL in which non acceptable precipitates were observed; and, –S9 to be 0.15 µg/mL, with mean growth inhibition <50% and three

	lower concentrations decreasing by a factor of 4.
Negative control:	DMSO
Positive control:	-S9: Ethyl methansulfonate (EMS) +S9: N-Nitrosodimethylamine (DMN)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	5 hr with metabolic activation, 21 hr without metabolic activation

Study Validity

The study is valid.

Results

Table 48 Summary of Results from Gene Mutation Test with Chinese Hamster Cells V79

(Excerpted from the Toxicology Tabulated Summary)

Metabolic activation	Test article	Concentration (µg/mL)	Mean Cytotoxicity[a] after treatment (% of control)	Mean Cytotoxicity after expression (% of control)	Mean Mutant frequency ($x10^{-6}$)
Experiment 1: 21-h Treatment					
-S9	DMSO	0	NA	NA	0.76
	PKC412	0.0037	10.6	-	2.85
		0.0111	10.6	-	1.63
		0.0333	11.5	-	2.27
		0.1000	34.2	-	2.56
	EMS	300 nL/mL	64.3	58.9	1692.18
Experiment 1: 5-h Treatment					
+S9	DMSO	0	NA	NA	1.21
	PKC412	7.4074	18.4	-	1.67
		22.2222	15.1	-	2.43
		66.6667	32.6	-	2.14
		200.0000	22.4	16.3	2.56
	DMN	1	19.8	28.8	149.65
Experiment 2: 21-h Treatment (confirmatory)					
-S9	DMSO	0	NA	NA	2.54
	PKC412	0.0023	-	-	1.56
		0.0094	-	21.4	2.63
		0.0375	-	10.2	1.65
		0.1500	48.6	20.9	2.48
	EMS	300 nL/mL	50.3	50.8	1577.25
Experiment 2: 5-h Treatment (confirmatory)					
+S9	DMSO	0	NA	NA	3.80
	PKC412	7.4074	3.22[b]	6.61[b]	23.23
		22.2222	-	-	1.63
		66.6667	-	21.2	1.45
		200.0000	5.12[b]	15.0	1.73
	DMN	1	10.5	21.5	141.77

- = No noteworthy findings; DMN = N-Nitrosodimethylamine; DMSO = Dimethylsulfoxide; EMS = Ethyl methansulfonate; h = Hours; NA = Not applicable; S9 = Externally-added rat metabolic activation system.

[a] = Based on the number of viable cells.

[b] = Cytotoxicity observed in only one of the duplicate cultures.

Study title: Cytogenetic Test on Chinese Hamster Cells In Vitro (EC Conform)

Study no.:	926300
Study report location:	4.2.3.3.1.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 3, 1992
GLP compliance:	Yes
QA statement:	Yes, signed
Drug, lot #, and % purity:	CGP41251; Batch No. 800591; Purity: 98.5%

Key Study Findings

- CGP41251 was negative for inducing chromosome aberrations (not greater than historical control range for negative controls); however, a concentration dependent increase in the frequency of polyploidy metaphases was observed, suggesting strong inhibition of cell division without affecting cell cycle kinetics, which is expected from the pharmacological activity of CGP41251.

Methods

Cell line:	Chinese hamster ovary cells
Concentrations in definitive study:	See Table 49 below
Basis of concentration selection:	Based on the cytotoxicity test, the highest concentration used or the lowest concentration which suppresses mitotic activity by approximately 50-80% compared to the control group was selected as the highest for the analysis of chromosome aberrations together with two lower concentrations in succession.
Negative control:	DMSO
Positive control:	-S9: Mitomycin C (MMC) +S9: Cyclophosphamide (CP)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	18 or 42h (-S9); 3 hrs with a 15 or 39 hrs recovery period (+S9)

Study Validity

The study is valid.

Results

Table 49 Summary of Clastogenicity in Chinese Hamster Ovary Cells

(Excerpted from the Toxicology Tabulated Summary)

Experiment 1 (Original):

Metabolic activation	Test article	Concentration (µg/mL)	Cytotoxicity[a] (% of control)	Treatment (hours)	Recovery (hours)	Cells with aberrations (%)	Total polyploid cells	Cells >G_2+M (resulting in polyploidy) (%)[b]
-S9	DMSO	0	100	18	0	0	1	4.8[c]
	PKC412	0.10	83.69	18	0	0.5	12	12.6[c]
		0.20	50.35	18	0	1.0	154	73.2[d]
		0.39	68.79	18	0	1.5*[c]	317	69.3[d]
	MMC	0.2	NR	18	0	34.0***	1	NA

DMSO = Dimethylsulfoxide; h = Hours; MMC = Mitomycin C; NA = Not applicable; NR = Not reported; S9 = Externally-added rat metabolic activating system

* 0.05 ≥ p > 0.01; *** p ≤ 0.001

[a] = Based on mitotic indices.

[b] = Investigation of cytotoxicity *via* monitoring of the cell cycle by flow cytometry.

[c] = The frequency is below the limit required for a positive response (6%).

[d] = Estimated values.

Experiment 2 (Original):

Metabolic activation	Test article	Concentration (µg/mL)	Cytotoxicity[a] (% of control)	Treatment (hours)	Recovery (hours)	Cells with aberrations (%)	Total polyploid cells	Cells >G_2+M (resulting in polyploidy) (%)
+S9	DMSO	0	100	3	15	0.5	4	5.1[b]
	PKC412	6.25	71.37	3	15	0	21	7.5[b]
		12.5	70.12	3	15	1.5	33	15.5[b]
		25.0	38.17	3	15	0	25	57.7[b]
	CP	20	NR	3	15	24.0***	5	NA

CP = Cyclophosphamide; DMSO = Dimethylsulfoxide; NA = not applicable; NR = Not reported; S9 = Externally-added rat metabolic activating system

*** p ≤ 0.001

[a] = Based on mitotic indices.

[b] = Estimated values.

Experiment 1 (Confirmatory):

Metabolic activation	Test article	Concentration (µg/mL)	Cytotoxicity[a] (% of control)	Treatment (hours)	Recovery (hours)	Cells with aberrations (%)	Total polyploid cells	Cells >G_2+M (resulting in polyploidy) (%)
-S9	DMSO	0	NR	18	0	2.0	9	ND
	PKC412	0.10	NR	18	0	2.0	21	ND
		0.20	NR	18	0	4.5	160	ND
		0.39	NR	18	0	1.0	177	ND
	MMC	0.2	NR	18	0	62.0***	1	ND

DMSO = Dimethylsulfoxide; MMC = Mitomycin C; ND = Not done; NR = Not reported; S9 = Externally-added rat metabolic activating system

*** p ≤ 0.001

[a] = Based on mitotic indices.

Experiment 2 (Confirmatory):

Metabolic activation	Test article	Concentration (µg/mL)	Cytotoxicity[a] (% of control)	Treatment (hours)	Recovery (hours)	Cells with aberrations (%)	Total polyploid cells	Cells >G_2+M (resulting in polyploidy) (%)
+S9	DMSO	0	NR	3	15	4.0	8	ND
	PKC412	6.25	NR	3	15	2.0	23	ND
		12.5	NR	3	15	0	17	ND
		25.0	NR	3	15	3.5	24	ND
	CP	20	NR	3	15	50.0***	1	ND

CP = Cyclophosphamide; DMSO = Dimethylsulfoxide; ND = Not done; NR = Not reported; S9 = Externally-added rat metabolic activating system

*** p ≤ 0.001

[a] = Based on mitotic indices.

Experiment 3 (Confirmatory):

Metabolic activation	Test article	Concentration (µg/mL)	Cytotoxicity[a] (% of control)	Treatment (hours)	Recovery (hours)	Cells with aberrations (%)	Total polyploid cells	Cells >G_2+M (resulting in polyploidy) (%)
-S9	DMSO	0	100	42	0	1.5	5	ND
	PKC412	0.02	91.94	ND	ND	ND	ND	ND
		0.025	ND	42	0	0.5	11	ND
		0.05	131.45	42	0	3.0	8	ND
		0.10	83.06	42	0	3.0	21	ND

DMSO = Dimethylsulfoxide; ND = Not done; S9 = Externally-added rat metabolic activating system

[a] = Based on mitotic indices.

Experiment 4 (Confirmatory):

Metabolic activation	Test article	Concentration (µg/mL)	Cytotoxicity[a] (% of control)	Treatment (hours)	Recovery (hours)	Cells with aberrations (%)	Total polyploid cells	Cells >G_2+M (resulting in polyploidy) (%)
+S9	DMSO	0	100	3	39	1.0	6	ND
	PKC412	1.56	77.40	3	39	1.0	17	ND
		3.13	68.36	3	39	1.5	25	ND
		6.25	59.89	3	39	2.5	75	ND

DMSO = Dimethylsulfoxide; ND = Not done; S9 = Externally-added rat metabolic activating system

[a] = Based on mitotic indices.

Note: Historical Control Range for No. of cells with polyploidy: 1 to 16 (+S9); 1 to 13 (-S9)

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

Study title: Micronucleus Test, Rat (OECD conform) In Vivo Study

Study no:	926299
Study report location:	4.2.3.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 31, 1993
GLP compliance:	Yes
QA statement:	Yes, signed
Drug, lot #, and % purity:	CGP41251; Batch No. 1058/6; Purity: 97.6%

Key Study Findings

- CGP41251 was negative in the rat bone marrow micronucleus assay up to the MTD of 200 mg/kg.

Methods

Doses in definitive study:	0, 50 (LD), 100 (ID), 200 (HD) mg/kg
Frequency of dosing:	Single Dose
Route of administration:	CGP41251 (PO); CP (IP)
Dose volume:	10 mL/kg
Formulation/Vehicle:	Bidistilled water
Species/Strain:	Rat/Tif: RAIf(SPF)
Number/Sex/Group:	15 sex/group (control and HD); 5/sex/group (all other groups)

Preparation of bone marrow after treatment	HD	ID	LD	Positive control	Negative control (vehicle)
16 hours	5m + 5f	-	-	-	5m + 5f
24 hours	5m + 5f	5m + 5f	5m + 5f	5m + 5f	5m + 5f
48 hours	5m + 5f	-	-	-	5m + 5f

Satellite groups:	None
Basis of dose selection:	200 mg/kg was highest dose without death or severe toxicity
Negative control:	Bidistilled water
Positive control:	Cyclophosphamide 20mg/kg (CP)

Study Validity

The study is valid.

Results

Table 50 Summary of Results from In Vivo Rat Bone Marrow Micronucleus Assay

	Dose (mg/kg)	No. of Animals	Ratio PCE/NCE	%MN-PCEs
Animals sacrificed 16h postdose				
Vehicle: Bi-distilled water	0	5M	0.78	0.09
		5F	0.6	0.07
CGP41251	200	5M	0.43	0.13
		5F	0.31	0.12
Animals sacrificed 24h postdose				
Vehicle: Bi-distilled water	0	5M	0.35	0.08
		5F	0.34	0.05
CGP41251	50	5M	0.3	0.05
		5F	0.47	0.04
	100	5M	0.47	0.03
		5F	0.36	0.09
	200	5M	0.37	0.13
		5F	0.46	0.06
CP	20	5M	0.36	1.01*
		5F	0.24	1.22*
Animals sacrificed 24h postdose				
Vehicle: Bi-distilled water	0	5M	0.53	0.05
		5F	0.49	0.06
CGP41251	200	5M	0.31	0.15*
		5F	0.53	0.09

* p≤0.05; Laboratory Historical Control Range for % MN-PCEs is 0 to 0.18%; MN-PCE = Micronucleated polychromatic erythrocyte; PCE = Polychromatic erythrocyte; NCE = Normochromatic erythrocyte

7.4 Other Genetic Toxicity Studies

The genotoxicity of the metabolites and impurity (b) (4) are reviewed under Section 10 since they were submitted under Module 4.2.3.7.5.

8 Carcinogenicity

No carcinogenicity studies were submitted nor are required for the proposed indications.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: An Oral Study for Effects on Fertility and Early Embryonic Development in Rats

Study no.:	PCS-964123
Study report location:	4.2.3.5.1.
Conducting laboratory and location:	Novartis, New Jersey, USA
Date of study initiation:	October 25, 1996
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	CGP41251 ((b) (4) (Lot 800292) (b) (4) ; Batch No.1058/13); Purity: 99%

Key Study Findings

- Mortality was observed in males at 60 mg/kg/day.
- BW loss was observed in males at ≥ 30 mg/kg/day.
- Sperm motility and pregnancy rate were reduced at 60 mg/kg/day.
- All doses ♂: microscopic lesions of testicular tubular degeneration and atrophy.
- At 60 mg/kg ♀: ↑resorptions, ↓no. of implantation sites, ↑pre and post-implantation losses were observed in females in the absence of a treatment-related effect on corpora lutea, statistically significant ↓BW gain and gravid uterine weights.

Methods

Doses:	0, 10, 30, 60 mg/kg/day
Frequency of dosing:	♂: 70 days prior to mating, during the 2 wk mating period and for 11 to 15 days thereafter; ♀: 14 days prior to mating, during mating and until presumed GD6.
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) in purified water BP
Species/Strain:	Sprague Dawley Rats (Crl:COBS CD[SD]BR)
Number/Sex/Group:	24/sex/group
Age:	♂: 12 wks; ♀: 11 wks
Weight:	♂: 372 to 498 g; ♀: 212 to 297 g
Satellite groups:	No TK sampling
Deviation from study protocol:	None considered by the Study Director to affect the integrity of the study or the interpretation of results

Observations and Times

Vaginal Cytology:	Pretest, daily until mating
Mortality:	Once daily
Clinical Signs:	Daily
Body Weight:	Every 3 to 4 days
Food Consumption:	♂: weekly; ♀: D 0, 7, and 14 premating, GD 0, 3, 6,9, and 13
Breeding procedures:	Treated ♂ were paired with treated ♀ on a 1:1 basis within each treatment group following 70 days of dosing for the ♂
Spermatogenic Endpoint Evaluations:	At necropsy*
Gross pathology:	At necropsy*
Gestation Laparohysterectomy:	At necropsy*
Organ weights and histopathology (testis and epididymis only):	At necropsy*

* Necropsy: ♂ on D98-102; ♀ GD13

Results

Mortality

Table 51 Summary of Mortality in Rats in a Fertility Study

Dose of PCK412 (mg/kg/day)	Animal #	Found Dead or Euthanized	Day	Clinical Signs/Macroscopic Findings
60 (HD♂)	78	Found Dead	D29	Weight loss, stained forelimbs and mouth, ↓stool, unthrifty, salivation after dosing, diarrhea day prior to death
	81	Found Dead	D38	Same as Animal # 78, except also emaciation and lethargic prior to death
	86	Found Dead	D42	Same as Animal # 78, except also abdomen distended prior to death
	93	Found Dead	D60	Same as Animal # 78 prior to death
	95	Found Dead	D54	Same as Animal # 86 prior to death
	89	Euthanized	D47	Stool changes, diarrhea, salivation, staining on various regions of the body and unthriftiness/distended stomach filled with food

Clinical Signs

Table 52 Summary of Clinical Signs in Male Rats in a Fertility Study

Clinical Signs	No. of Observations			
Dose (mg/kg/day)	Control	10	30	60
Males				
Alopecia	0	1	2	3
Chromodacryorrhea	0	0	0	1
Distended abdomen	0	0	0	6

Clinical Signs	No. of Observations			
Dose (mg/kg/day)	Control	10	30	60
Diarrhea	0	0	0	6
Emaciation	0	0	0	2
Lethargic	0	0	0	1
Salivation	0	20	24	24
Staining (mouth, pubic abdomen, forelegs)	0	0	0	11
Stool				
Decreased	0	0	1	9
Soft	0	0	0	8
Unthriftiness	0	0	0	9
Females				
Discharge - vaginal bloody	0	0	0	1
Salivation	0	5	17	20

Body Weight and Food Consumption

Table 53 Summary of Body Weight (BW) and Food Consumption (FC) Changes in a Rat Fertility Study

	Percent Change from Control			
Dose (mg/kg/day)	Control (mean)	10	30	60
Males				
BW at premating D59 (g)	616	--	↓5.0*	↓12.9**
BW at premating D70 (g)	636	--	↓5.2*	↓10.5**
Peak BW gain ↓ (g)	12.8	↓6.3	↓41**	↓167**
FC at premating D0-7 (g/day)	29.5	--	--	↓8.5**
Females				
BW at premating D14 (g)	283	--	--	--
FC at premating D7-14 (g/day)	23.1	--	↑11.26*	↑6.5*
BW at GD13 (g)	368	--	--	↑6.49*
BW gain at GD6-9	12.9	--	↓6	↓30
BW gain at GD13 corrected (g)	67.4	↓13.8	↓9.3	↓9.1
FC at GD 0-3 (g/day)	27.7	↑6.1	↑6.5	↑15.2**
FC at GD9-13 (g/day)	32	--	--	↑9.06*

* p<0.05, ** p<0.01, *** p<0.001; "--", changes< 5%.

- HD♂: statistically significant ↓ in BW were observed beginning on premating D17.
- MD♂:statistically significant ↓ in BW were observed beginning on premating D42.
- ↓BW persisted through the last reported timepoint (D94).
- Gravid uterine weights were lower for HD♀.

Toxicokinetics

Not conducted

Dosing Solution Analysis

Analyses of the compound [redacted] (b) (4) prepared for use during wks 1, 5, 8 and 12 revealed the concentrations of the [redacted] (b) (4) to be 93% to 105% of the expected values based on average results obtained from twelve samples tested, and therefore,

were within the limits specified in the protocol (± 10% of the target concentrations). CGP41251 was not detected in any of the analyzed control samples.

Necropsy

Macroscopic:
1 HD♂: pale blue testes; 3 MD♂: hollow kidneys

Microscopic:

Table 54 Microscopic Findings in Male Rats in a Fertility Study

(Excerpted from the Toxicology Written Summary)

Daily dose (mg/kg)	0 (Control)	10	30	60
Histopathology (testis and epididymis)				
No. examined	24	24	24	18
Testis				
Degeneration, seminiferous tubules	0	2	0	4
Atrophy, seminiferous tubules	0	2	1	4
Epididymis				
Aspermia	0	0	0	3
Stasis, spermatid	0	0	0	2
Oligospermia	0	1	0	1

Also observed:

- 3 LD♂, 1 HD♂: spermatid debris of the epididymis
- 1 control ♂, 3 LD♂, 4 MD♂, 3 HD♂: intraluminal spermatid debris of the testis
- 1 HD♂: edema of the testis; 1 HD♂: tubular mineralization of the testis
- 1 LD♂, 1 MD♂, 1 HD♂: sloughing, epithelial, seminiferous, testis

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

Table 55 Summary of Fertility Parameters in Rats in a Fertility Study

Dose group		1	2	3	4	Historical Control	
Dose (mg/kg/day)		0 (vehicle)	10	30	60	Mean	Range
No. ♂ cohoused	N	24	24	24	18		
No. ♀ cohoused	N	24	24	24	24		
♀ inseminated	N	23	24	23	24		
	%	96	100	96	100		
♀ pregnant	N	23	23	23	20		
	%	96	96	96	83	94.7	80.8-100
Testicular sperm count ($x10^6$)(No. sperm/g testis)	Mean	74.4	67.6	73.7	63.3		
	%Δ	0	↓9.1	--	↓14.9		
% motility	Mean	76.2	73.1	80.8	62.9		
	%Δ	0	--	↑6	↓17.5		
Testes wt	g	3.8	3.9	3.8	3.3		
	%Δ	0	--	--	↓11.9		
Epididymis wt	g	1.7	1.6	1.6	1.4**		
	%Δ	0	--	--	↓15**		

Dose group		1	2	3	4	Historical Control	
Dose (mg/kg/day)		0 (vehicle)	10	30	60	Mean	Range
♀ pregnant	N	23	23	23	20		
No. w/ total resorption of litter	N	0	0	0	2		
Corpora Lutea	MEAN	18.3	17.9	19.6	18.8	15.9	15-17.9
Implantation sites	MEAN	16.9	16.35	18.9	14.45	15.3	14.4-17.2
Pre-implantation loss	MEAN	1.43	1.57	0.74	4.35**		
	%	7.6	8.9	3.6	25.3**	3.9	1.7-7.2
Litter sizes							
Live fetuses	MEAN	16.1	15.2	17.3	10.15**	14.3	13.5-16.1
No. Resorptions	MEAN	0.78	1.17	1.61	4.3**		
% post-implantation loss	MEAN	0.78	1.17	1.61	4.3**	6.2	2.8-10.3
	%	4.8	7.2	8.85	34.3**		

Historical Control (HC) data came from (b) (4) from SD rats from 485 pregnant dams on GD 13 from 21 studies between 2008 and 2010.

9.2 Embryonic Fetal Development

Study title: CGP41251 Segment II (Teratology) Study in Rats by Oral Administration

Study no.: PCS-936241
Study report location: 4.2.3.5.2.
Conducting laboratory and location: (b) (4)
Date of study initiation: October 20, 1994
GLP compliance: Yes, signed
QA statement: Yes, signed
Drug, lot #, and % purity: CGP41251 ((b) (4) (b) (4) Batch No.1058/7)

Key Study Findings

- An NOAEL could not be defined based on effects on skeletal ossification at the low dose.
- CGP41251 caused embryo-fetal toxicity (↑late resorptions and post-implantation loss, ↓ in fetal weight) and many developmental variations: severe renal pelvic cavitation, widened anterior fontanelle, and reductions in the degree of ossification of skeletal bones.

Methods

Doses:	0, 3, 10, 30 mg/kg/day
Frequency of dosing:	Daily from GD6 to 17
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) in purified water BP
Species/Strain:	Rat/Hsd/Ola: Sprague-Dawley
Number/Sex/Group:	24♀/group
Initial Age (wt):	16 to 23 wks (219 to 285 g)
Satellite groups:	No TK sampling
Deviation from study protocol:	None considered by the Study Director to affect the integrity of the study or the interpretation of results

Observations and Times

Mortality	Twice daily
Clinical Signs	Daily from GD 0 through 20
Body Weight	GD 0, 6-18 (daily), and 20
Food Consumption	GD 0 through 20 (daily)
Cesarean section data	At necropsy (GD20)
Offspring	At necropsy (GD20)

Results

Mortality and Clinical Signs

Unremarkable

Body Weight

All doses, most pronounced at HD: ↑BW gain (statistically significant) compared to controls, but initial body weights for HD♀ were lower than controls in CGP41251 allocated groups between mating and the first day of dosing (GD 0 to 6)

Food Consumption

All doses: ↓ between GD 0 to 6 (HD only); statistically significant ↑8 to 10% in food consumption compared to controls thereafter.

Dosing Solution Analysis

The test article was homogeneous in the vehicle and the actual concentrations of the test article in the vehicle corresponded to the nominal concentrations with deviations of +2.0% to +7.0%.

Reference ID: 4051584

Necropsy

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

Table 56 Summary of Cesarean Section Data in Rats

Dose group		1	2	3	4	Historical Control	
Dose (mg/kg/day)		0 (vehicle)	3	10	30	Mean	Range
Rats tested	N	24	24	24	24		
Pregnant[a]	N	22	21	22	23		
	%	92	87.5	92	96	92.9-93.7	62.5-100
No. Dams with Live Fetuses	N	22	21	22	23	100	
Corpora Lutea	MEAN	15.55	15.95	15.14	14.61	14.1-17.2	13-19.4
Implantation sites	MEAN	12.27	13.10	12.77	13.04	13.2-14.8	9.9-17.2
% pre-implantation loss	MEAN	20.52	17.30	14.95	10.75	3.4-5.4	1.8-18.9
Litter sizes							
Live fetuses	MEAN	11.86	11.95	12.14	11.70	13.7-14.1	11.1-16.3
Dead fetuses	N	0	0	0	0	0	0-0.1
No. Resorptions	MEAN	0.41	1.14	0.64	1.35	0.6-0.9 (0.67)	0.1-1.5 (0.18-0.92)
Early Resorptions	MEAN	0.32	**0.86**	0.32	**0.91**	0.56	0.18-0.8
Late Resorptions	MEAN	0.09	**0.29**	**0.32**	**0.43**	0.07	0-0.11
% post-implantation loss	MEAN	3.39	8.44	6.59	10.55	4.6-5.4 (5.27)	1.4-10.8 (1.3-8.07)
Fetal sex ratios (% males)	MEAN	50.16	46.38	50.50	50.49	49.3-52.1	42-57
Fetal weights (g)	MEAN	3.45	3.51	3.47	**3.24*****	3.72-5.47	3.48-6.25
	%Δ		2↓	0.6↓	6↓		

a No dams died or were sacrificed moribund or aborted or with total resorption of litter; * p<0.05, ** p<0.01, *** p<0.001; (HC data provided by Applicant taken from 126 litters and 1697 fetuses from 6 studies carried out between 1993 and 1994); other HC data came from (b) (4) from SD rats from ~1200 pregnant dams between 2008 and 2010 and ~2000 pregnant dams between 1996 and 2009 or 856 pregnant dams between 1996 and 2010; **bold:** outside of the HC ranges

Offspring (Malformations, Variations, etc.)

Table 57 Summary of Variations in Rat Offspring

Dose group	Control	LD	MD	HD	Historical Control Data	
Dose (mg/kg/day)	0	3	10	30		
External variations						
Number of litters	22	21	22	23	Number affected or % per litter	Range
Number of fetuses examined	261	251	267	269		
Hematoma	L 2 F 2	L 2 F 3	L 1 F 1	L 5 F 5	L 2 (13) F 2 (19)	
% litters	9.09	9.52	4.55	21.74	0.09 (10.43)	0-8 (0-25.53)
% fetuses	0.77	1.2	0.37	2.23	0.01 (1.14)	0-0.54 (0-2.68)
Visceral variations						

Dose group	Control	LD	MD	HD	Historical Control Data	
Dose (mg/kg/day)	0	3	10	30		
Number of litters	22	21	22	23		
Number of fetuses examined	261	251	267	269		
Thyroid reduced	L 0 F 0	L 0 F 0	L 0 F 0	L 1 F 1	L 4 F 4	
% litters	0	0	0	4.35	(2.97)	(0-9.1)
% fetuses	0	0	0	0.79	(0.45)	(0-1.38)
Renal pelvic cavitation severe	L 0 F 0	L 0 F 0	L 0 F 0	L 2 F 3	L 2 F 2	
% litters	0	0	0	**8.70**	(1.57)	(0-5.26)
% fetuses	0	0	0	**2.36**	(0.25)	(0-0.81)
Lateral brain ventricles dilated	L 0 F 0	L 4 F 5	L 3 F 3	L 1 F 1	L 10 F 14	
% litters	0	**19.05**	**13.64**	4.35	(7.86)	(0-13.04)
% fetuses	0	**4.03**	2.33	0.79	(1.61)	(0-2.89)
Ovary and uterus malpositioned (low)	L 0 F 0	L 0 F 0	L 0 F 0	L 1 F 1	L 0 F 0	
% litters	0	0	0	**4.35**	(0)	
% fetuses	0	0	0	**0.79**	(0)	
Skeletal variations						
Number of litters	22	21	22	23	See legend	
Number of fetuses examined	261	251	267	269	See legend	
Incomplete ossification interparietal	L 3 F 4	L 3 F 7	L 4 F 4	L 18 F 37***		
% litters	13.64	14.29	18.18	**78.26**	39 (30.72)	0-76.19 (15.8-58.33)
% fetuses				13.75	12.41 (8.17)	0-30 (2.34-17.2)
Incomplete ossification occipital	L 2 F 2	L 3 F 4	L 0 F 0	L 7 F 7	L 2 F 2	
% litters	**9.09**	**14.29**	0	**30.43**	(1.54)	(0-5.26)
% fetuses	1.45	**3.15**	0	**4.93**	(0.35)	(0-1.56)
Anterior fontanelle – widened	L 0 F 0	L 0 F 0	L 0 F 0	L 1 F 1	L 0 F 0	
% litters	0	0	0	**4.35**	(0)	
% fetuses	0	0	0	**0.70**	(0)	
Vertebral arch – incomplete ossification	L 0 F 0	L 4 F 6	L 0 F 0	L 2 F 2	L 3 F 3	
% litters	0	**19.05**	0	**8.7**	(2.34)	(0-5.88)
% fetuses	0	**4.72**	0	**1.41**	(0.34)	(0-0.87)
Incomplete ossification centra	L 5 F 5	L 7 F 10	L 0 F 0	L 13 F 18	L 31 F 38	
% litters	22.73	**33.33**	0	**56.52**	(25.12)	(16-31.82)
% fetuses	3.62	7.8	0	**12.68**	(4.54)	(2.16-7.83)
Centra not ossified	L 4 F 4	L 4 F 5	L 2 F 4	L 17 F 43***	L 11 F 15	
% litters	18.18	**19.05**	9.09	**73.91**	(9.12)	(0-15.79)

Dose group	Control	LD	MD	HD	Historical Control Data	
Dose (mg/kg/day)	0	3	10	30		
% fetuses	2.9	**3.94**	2.9	**30.28**	(1.76)	(0-3.12)
Centra dumb-bell	L 1 F 1	L 3 L 4	L 3 F 4	L 8 F 8*	L 19 F 23	
% litters	4.55	14.29	13.64	**34.78**	(15.51)	(4.17-26.32)
% fetuses	0.72	3.15	2.9	**5.63**	(2.7)	(0.62-4.58)
Centra – bifid	L 1 F 1	L 1 L 1	L 0 F 0	L 2 F 2	L 4 F 4	
% litters	4.55	4.76	0	**8.7**	(2.96)	(0-8)
% fetuses	0.72	0.79	0	**1.41**	(0.41)	(0-1.08)
Extra rib	L 4 F 4	L 4 L 5	L 8 F 10	L 9 F 15		
% litters	18.18	19.05	36.36	39.13		
% fetuses	2.9	3.94	7.25	10.56		
Sternebra incompletely ossified	L 16 F 41	L 11 F 23	L 15 F 22	L 20 F 81***	L 78 F 147	
% litters	72.73	52.38	68.18	86.96	(62.59)	(47.37-94.12)
% fetuses	29.71	18.11	15.94	**57.04**	(17.05)	(10.94-30.43)
Sternebra not ossified (3 or more)	L 7 F 7	L 7 F 11	L 4 F 5	L 15 F 33***	L 2 F 3	
% litters	**31.82**	**33.33**	**18.18**	**65.22**	(1.36)	(0-4.17)
% fetuses	**5.07**	**8.66**	**3.62**	**23.24**	(0.29)	(0-1.08)
Sternebra – dumb-bell	L 2 F 2	L 2 F 2	L 3 F 3	L 9 F 10*	L 19 F 26	
% litters	9.09	9.52	13.64	**39.13**	(15.35)	(9.1-26.32)
% fetuses	1.45	1.57	2.17	**7.04**	(2.99)	(1.53-4.35)
Caudal vertebrae reduced in number	L 2 F 2	L 5 F 8	L 1 F 1	L 8 F 9	L 2 L 2	
% litters	**9.09**	**23.81**	4.55	**34.78**	0.14	0-4.76
% fetuses	**1.45**	**6.3**	0.72	**6.34**	0.02	0-0.72
Metatarsal - incomplete ossification	L 3 F 4	L 4 F 5	L 0 F 0	L 5 F 8	L 3 F 6	
% litters	**13.64**	**19.05**	0	**21.74**	(2.24)	(0-5.26)
% fetuses	**2.9**	**3.94**	0	**5.63**	(0.68)	(0-2.34)
Metatarsal – not ossified	L 1 F 1	L 3 F 3	L 0 F 0	L 5 F 5	L 4 F 4	
% litters	4.55	**14.29**	0	**21.74**	(3.09)	(0-5.88)
% fetuses	0.72	**2.36**	0	**3.52**	(0.44)	(0-0.87)
Pubis unossified	L 6 F 10	L 3 F 3	L 2 F 2	L 12 F 17	L 5 F 6	
% litters	**27.27**	**14.29**	9.09	**52.17**	0.35	0-9.09
% fetuses	**7.25**	**2.36**	1.45	**11.97**	0.06	0-1.48

* p<0.05, ** p<0.01, *** p<0.001; (HC data provided by Applicant taken from 126 litters and 1697 fetuses from 6 studies carried out between 1993 and 1994); other HC data came from [redacted] (b) (4) from SD rats from ~2220 litters and~15000 fetuses between 2006 and 2009 and from ~1200 litters and ~16000 fetuses between 2008 and 2010.
bold: outside of the HC ranges

- Malformed fetuses had findings slightly higher than historical controls ((b) (4) 2006 to 2010): anophthalmia, occipital bone and ischium not ossified, humerus and femur shortened, curved femur, and scapula shortened and thickened. Since they were not observed in HD fetuses, they are likely not treatment related. The Applicant noted that some of these findings could be related to the sporadic low fetal weight of individual fetuses.
- All doses: dose-response and higher incidence of hematoma, ureter kinked, ureter dilated, innominate artery absent, but were within the laboratory HC range and may not be treatment related. Of note, the higher incidence of hematomas in HD litters was outside of the (b) (4) HC range.

Study title: CGP41251 Segment II (Teratology) Study in Rabbits by Oral Administration

Study no.:	PCS-936243
Study report location:	4.2.3.5.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 4, 1994
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	CGP41251 (b) (4) Batch 1058/7)

Key Study Findings

- CGP41251 was maternally toxic (↓BW gain and food consumption, ↑spontaneous abortions) at all doses (≥ 2 mg/kg/day).
- Fetotoxicity (↓ fetal weight, with reduced gall bladder size and reduced skeletal ossifications) was observed mainly at doses ≥ 10 mg/kg/day.

Methods

Doses:	2, 10, 20 mg/kg
Frequency of dosing:	Daily from GD7 to 20
Dose volume:	2 mL/kg (GD7); 5 mL/kg (GD8-20)
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4) in purified water BP
Species/Strain:	New Zealand White Rabbits
Number/Sex/Group:	20♀/group
Initial Age (Wt):	25-30 wks (3.299 to 4.967 kg)
Satellite groups:	No TK sampling
Deviation from study protocol:	None considered by the Study Director to affect the integrity of the study or the interpretation of results

Observations and Times

Mortality	Twice daily
Clinical Signs	Pretest, twice daily
Body Weight	Reported for GD 0, 7, 10, 14, 21, 25 and 29
Food Consumption	Recorded for the periods of GD 0-7, 7-10, 10-14, 14-21, 21-25 and 25-29
Cesarean section data	At necropsy (GD29)
Offspring	At necropsy (GD29)

Results

Mortality

Table 58 Summary of Mortality in Rabbit Dams

Dose of PCK412 (mg/kg/day)	Animal #	Found Dead or Euthanized	Day	Clinical Signs/Macroscopic Findings
Control	1032	Euthanized *in extremis*	GD29	Suspected misdosing/hemorrhagic lungs and gall bladder, pale heart and liver
	1000	Euthanized	GD16	↓food and water intake, ↓BW and nasal discharge containing blood/ hemorrhagic lungs
10 (MD)	3032	Euthanized	GD28	↓ food and water intake from GD17, blood in urine. Moderate BW loss and marked reduction in food consumption from GD14/ unusual amount of blood was seen in the uterus.
	3030	Euthanized	GD15	Suspected misdosing/ hemorrhagic lungs
20 (HD)	4036	Found Dead	GD24	↓food and water intake and moderate BW loss from the onset of dosing/distended gall bladder, hemorrhagic lungs and empty alimentary tract.
	4030	Euthanized	GD19	Marked reduction in food consumption on GD10-14/nothing abnormal

- One, 2, and 4 LD, MD, and HD♀ aborted. All had slight to marked ↓BW and marked ↓ in food consumption (mainly during wk 1 and 2 of treatment). All aborted between GD26 to 29. 3/4 ♀ had distended gall bladders at necropsy.

Reference ID: 4051584

Clinical Signs

Table 59 Summary of Clinical Signs in Rabbit Dams

Clinical Signs	No. of Observations			
Dose (mg/kg/day)	Control	2	10	20
Defecation				
Decreased	6	13	44	98
Ceased	0	1	5	11
Hard and Dry	3	8	23	47
Water Consumption				
Decreased	6	5	31	64
Hypoactivity	0	0	24	15

Body Weight and Food Consumption

Table 60 Summary of Peak Body Weight (BW) Changes in Rabbit Dams

	Percent Change from Control			
Dose (mg/kg/day)	Control (mean)	2	10	20
Peak BW loss GD21	4.356 kg	↑4	↓2.5**	↓7***
Peak BW Gain Changes GD7-21	125 g	↓77	↓172**	↓306***
Food Consumption GD14-21	999 g/dam	↓11.5	↓41*	↓82***

* p<0.05, ** p<0.01, *** p<0.001

Dosing Solution Analysis

The test article was homogeneous in the vehicle and the actual concentrations of the test article in the vehicle corresponded to the nominal concentrations with deviations of 1.5% to 5.5%.

Necropsy

In dams, distended gall bladder was observed at all doses. Slightly pale kidneys, distended vulva with gelatinous fluid, clear fluid in the pericardium, and hairloss with reddened areas were observed at the MD and HD. At HD, fur balls in stomach and distended and gaseous intestines were also observed.

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

Table 61 Summary of Cesarean Section Data in Rabbits

Dose group		Control	LD	MD	HD	Historical Control	
Dose (mg/kg/day)		0 (vehicle)	2	10	20	Mean	Range
Rabbits tested	N	20	20	20	20	758	
Pregnant	N	18	20	18	20	706	
	%	90	100	90	100	93.6	80-100
No. died or sacrificed moribund	N	2	0	2	2	7	
No. aborted or with total resorptions of litter	N	0	**1**	**2**	**4**	3	
Corpora Lutea	MEAN	**13.25**	**12.32**	**12.21**	**12.00**	9.3	7.8-11
Implantation sites	MEAN	**10.31**	**9.74**	**9.93**	9.5	8.7	7.5-9.6
% pre-implantation loss	MEAN	21.09	20.94	17.14	19.93	6.5	1.6-25.3
Litter sizes							
Live fetuses	MEAN	**9.5**	8.47	9.14	8.5	8.4	7.2-9.4
Dead fetuses	N	0	0	0	0	0	
No. Resorptions	MEAN	**0.81**	**1.26**	**0.79**	**1.00**	0.3	0.1-0.5
Early Resorptions	MEAN	0.38	0.32	0.36	0.29	0.2	0-0.4
Late Resorptions	MEAN	**0.44**	**0.95**	**0.43**	**0.71**	0.1	0-0.2
% post-implantation loss	MEAN	**7.8**	**11.47**	**7.85**	**9.98**	3.2	1.1-5.2
Fetal sex ratios (% males)	MEAN	58.53	41.33**	48.09	50.61	47.5	35.6-60.5
Fetal weights (g)	MEAN	40.86	41.41	**39.12**	**34.64*****	43.14	40.01-45.52
	%Δ		1↓	4↓	15↓		

* p<0.05, ** p<0.01, *** p<0.001; HC data taken from 695 litters from 34 studies carried out between 2008 and 2010 at (b) (4) **bold:** outside of the HC ranges

- An 8%↓ (p<0.05) in male fetal weight was observed at the MD.

Offspring (Malformations, Variations, etc.)

Table 62 Summary of Variations in Rabbit Offspring

Dose (mg/kg/day)	Control	2	10	20	Historical control data	
External variations						
Number of litters	16	19	14	14	Number affected or % per litter	Range
Number of fetuses examined	152	161	128	119		
Placenta pale[a]	L 0 F 0	L 0 F 0	L 1 F 4	L 1 F 8	L 1 F 1	
% litters	0	0	**7.14**	**7.14**	2.63	0-5.26
% fetuses	0	0	**2.13**	**6.72**	0.28	0-0.55
Placenta reduced in size[a]	L 0 F 0	L 0 F 0	L 0 F 0	L 1 F 1	L 0 F 0	
% litters	0	0	0	**7.14**	0	
% fetuses	0	0	0	**0.84**	0	
Placental remains divided and adhered to uterine wall[a]	L 0 F 0	L 0 F 0	L 0 F 0	L 1 F 1	L 0 F 0	
% litters	0	0	0	**7.14**	0	
% fetuses	0	0	0	**0.84**	0	
Visceral variations						
Number of litters	16	19	14	14		
Number of fetuses examined	152	161	128	119		
Gall bladder reduced[b]	L 1 F 1	L 1 F 1	L 2 F 2	L 2 F 3	L 1 F 1	
% litters	**6.25**	**5.26**	**14.29**	**14.29**	0.14	0-5.6
% fetuses	**0.65**	**0.62**	**1.56**	**2.52**	0.02	0-0.6
Innominate artery absent[b]	L 0 F 0	L 0 F 0	L 0 F 0	L 1 F 1	L 3 F 3	
% litters	0	0	0	**7.14**	0.43	0-5.3
% fetuses	0	0	0	**0.84**	0.05	0-0.7
Caudate lobe of liver adhered to small intestine	L 0 F 0	L 0 F 0	L 0 F 0	L 1 F 1	L 0 F 0	
% litters	0	0	0	**7.14**	0	
% fetuses	0	0	0	**0.84**	0	
Skeletal variations						
Number of litters	16	19	14	14		
Number of fetuses examined	152	161	128	119		
Metacarpals not ossified	L 4 F 8	L 4 F 18	L 7 F 28*	L 8 F 24*	L 8 F 17	
% litters	25	21.05	**50**	**57.14**	21.06	0-42.11
% fetuses	5.26	**11.18**	**21.88**	**20.17**	4.65	0-9.29
Mandible(s) incompletely ossified	L 0 F 0	L 0 F 0	L 0 F 0	L 1 F 1	L 0 F 0	
% litters	0	0	0	**7.14**	0	
% fetuses	0	0	0	**1.61**	0	

* p<0.05, ** p<0.01, *** p<0.001; HC data provided by Applicant taken from 28 litters and 256 fetuses from two studies carried out between 1993 and 1994; **bold:** outside of the HC ranges. a Applicant noted while outside of the HC range, findings did not affect the viability of the fetus. b HC data taken from 701 litters and 5897 fetuses from 35 studies conducted by [redacted] (b) (4) between 2008 and 2010.

The following are noted but are unlikely to have toxicological significance since they were not observed in HD fetuses:

- One LD fetus was found to have great vessel malformations associated with an absent aortic arch and a reduced spleen.
- One MD fetus had an absent vertebral arch and rib higher than the HC control range. In another MD fetus, the vertebral arch was displaced and severely reduced in size. A number of associated vertebral column findings were noted for both fetuses, such as displaced centra and vertebral arches and centra unilaterally ossified and enlarged.

In the dose finding rabbit study, the Cmax for the 10 mg/kg dose after repeated dosing daily from GD7-20 was 262 nmol/L and AUC(0-24hr) was 1581 h.nmol/L (Study No. DMPK-R936242).

9.3 Prenatal and Postnatal Development

Study title: PKC412: An Oral (Gavage) Pre and Postnatal Study in the Rat

Study no.:	PCS-770270
Study report location:	4.2.3.5.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 17, 2008
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	PKC412; Batch No. 0424020; Purity: 95%

Key Study Findings

- PKC412 was maternally toxic (↓BW gain and food consumption (FC)) at 30 mg/kg/day. Adverse effects upon maternal performance at 30 mg/kg/day: dystocia and ↓live litter size. NOAEL for maternal performance = 15 mg/kg/day.
- F1 generation: ↓BW, changes in days to complete eye opening and auricular startle ontogeny at 30 mg/kg/day.

Methods

Doses:	0, 5, 15, 30 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	(b) (4)
Species/Strain:	Sprague-Dawley (Crl:CD[SD])
Number/Sex/Group:	24♀/group
Satellite groups:	No TK sampling
Study design:	24 pregnant ♀ were randomly assigned to 4 groups. Test article and control vehicle were administered on GD6-PND 21, 22, or 23.
Parameters and endpoints evaluated:	*For F0 generation rats*: mortality and signs of ill health or reaction to treatment (2x daily, 3x from GD20-paturition); clinical signs, body weights, food evaluation (approx. every 3 days); gross pathology and reproductive parameters (PND22-24). F*or F1 generation rats*: malformations (PND0), viability (2x daily), clinical observations (daily during lactation), body weights (birth and approx. every 3 to 4 days), physical (pinna unfolding from PND1, tooth eruption from PND7 and eye opening from PND12 until all pups in the litter showed development), reflexological (righting reflex from PND2, the negative geotaxis test from PND8 and the auricular startle response from PND12 until all pups in the litter have a positive response and behavioral development). Shortly before weaning (PND21), 1 male and 1 female rat were randomly selected from each litter, where possible, to provide the F1 adult generation. All other pups were euthanized on days PND22, 23 or 24 and were given a gross pathological examination. No tissues were retained. *F1 generation adults*: mortality and signs of ill health or reaction to treatment (2x daily), body weight (approx. every 3 days), visual function (pupillary closure and visual placing responses on PND21), physical development (vaginal opening was assessed from PND26 until development for ♀ and preputial separation was assessed from PND35 until development for ♂), behavioral performance (motor activity at PND 35, 60), auditory startle habituation at PND55, 'E' water maze between PND60 and 70), mating procedures (14 day cohabitation beginning at PND85), gross pathology (pregnant ♀ GD6, non-pregnant ♀ GD13, ♂ 3 wks after mating).
Deviation from study protocol:	There were no remarkable deviations reported.

Observations and Results

FO In-life Observations

F_0 Dams

Survival: 4 HD♀ were euthanized on PND0 to 1: dystocia and ↓ in the number of live pups, dams had additional fetuses in utero, littering >7 hours, clinical signs of skin pallor, pale eyeballs. 1 control and 1 HD♀ cannibalized all pups in the litter following parturition.

Clinical signs: Dose-dependent ↑ in the incidence of salivation seen primarily postdose (1, 6, 15, 17 observations in control, LD, MD, and HD♀ during gestation, respectively), as early as GD9. Higher incidence of thin fur cover, red staining and firm abdomen/distension in PKC412-treated dams. Liquid red vaginal discharge in HD♀.

Body weight: HD♀: ↓1% BW GD20, ↓18% (p≤0.05) BW gain on GD6 to 9 and GD18 to 20 (20%↓, p≤0.01). ↑5.6% BW in HD♀ during lactation (p≤0.05).

Food consumption: All PKC412 treated groups: ~↑20% GD15 to 18 (p≤0.001), ~↑7 to 8% on Lactation Days 10 to 14.

Necropsy observation: Dose-related ↑dark/area/foci in the thymus; ↓number of live pups at HD. Also at HD: dark, enlarged lymph node; dark area, cyst of ovary; and pale foci of the lung. All doses: pale liver.

FO Delivery and Litter Observations

Table 63 Summary of Delivery Parameters in Rats – PPND Study

F0 generation female:	**0 mg/kg/day**	**5 mg/kg/day**	**15 mg/kg/day**	**30 mg/kg/day**	**HC Data (b) (4) 1996 to 2009**
Pregnant rate	24/24=100%	24/24=100%	23/24=96%	24/24=100%	
Delivered litter	23[a]	24	23	19[a]	
Duration of gestation (days)	21.7	21.7	21.5	21.8	21.3-22
Gestation index	24/24=100%	24/24=100%	23/23=100%	24/24=100%	88.9-100
Mean no. implantations	13.8	14	14.4	12.9	
Mean no. pups/litter	13	13.1	13.4	11.5	
Mean no. live born pups/litter	12.6	13	13.1	10.8*	12-15.8
Dams with stillborn pups (pups affected)	3 (11)	2 (2*)	2 (7)	7 (13)	
Malformed pups	0	0	1	1	
F1 generation litters:	**0 mg/kg/day**	**5 mg/kg/day**	**15 mg/kg/day**	**30 mg/kg/day**	
Viability index PND0-4	99.7	99.3	97.9	96.7	91.1-100
Viability index PND4-12	98.7	99.7	99.2	99.5	
Lactation index	98.2	99.4	100	99.4	94.3-100
Pup sex ratios (% males)	46.94	52.29	45.97	54.56	41.4-60.6
Pup weight (PND0) (PND21)	6.73 51.68	6.98 52.84	6.36 49.86	5.74*** 48.74	5.8-7.0 45.4-64.0

a Two dams (1 control and 1 30 mg/kg) were euthanized due to cannibalization of pups following parturition. An additional 4 dams at 30 mg/kg were euthanized due to signs of dystocia; *p≤0.05, ***p≤0.001. HC=historical control; (b) (4)

F1 Pups Clinical and Necropsy Observations

Table 64 Summary of F1 Generation Clinical Signs and Gross Observations as Pups – PPND Study

Clinical signs:	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	30 mg/kg/day
Live litters	23	24	23	19
No. of ♂/ ♀ per group	146/156	165/148	139/163	129/115
Breathing labored/respiratory decreased	1/0	0/0	0/1	2/0
Domed skull	1/1	0/0	0/0	2/0
Eyeball shrunken/sunken	0/0	0/0	0/0	0/2
Forelimb right severed	0/0	0/0	0/0	0/1
Swollen soft abdominal	0/0	0/0	0/0	2/2
Swollen soft cranium, soft ventral cervical, skin bruise cranium	0/0	0/0	0/0	1/0
Skin Bruise Dorsal Thoracic	0/0	1/0	1/2	0/3
Skin pallor	0/2	0/0	3/2	4/5
Physical development				
Mean time for pinna unfolding (days)	2.29	2.18	2.49	2.42
Mean time for eye opening (days)	13.79	13.74	13.73	13.11***
Reflexological development				
Mean time of development of auricular startle (days)	12.16	12.2	12.25	12.38
♂	12.21	12.21	12.27	12.37
♀	12.11	12.19	12.23	12.4**
Necropsy:♂/♀	**0 mg/kg/day**	**5 mg/kg/day**	**15 mg/kg/day**	**30 mg/kg/day**
Testis malpositioned near kidney - anomaly	0/NA	0/NA	0/NA	1/NA
Tail shortened - malformation	0/0	0/0	0/1	1/0
Brain: dilatation ventricle	1/0	0/0	0/0	3/0
Cranial cavity: pale fluid clear	0/0	0/0	0/0	1/0
Kidney: area dark	0/0	0/0	0/0	3/1
Lung: discoloration dark	0/0	0/0	0/0	1/0
: spongy	0/0	0/0	0/0	0/1
: uncollapsed	0/0	0/0	1/0	0/1
LN mandibular: area dark	0/0	1/0	1/0	1/0
Eye: area dark or small	0/0	0/0	0/0	0/1

*p≤0.05, **p≤0.01, ***p≤0.001; NA=organ not present in sex

F1 In-Life Observations

Mortality, clinical signs, sexual maturation, necropsy observations were not affected.

- Mean activity counts were consistently lower, but not statistically significant for F1 generation PKC412-treated adult males on PND35 (20%↓ and 7%↓ in ♂ and ♀, respectively), which resolved by PND60.
- Mean 'E' water maze times (sec) were consistently higher (up to 40%↑), but not statistically significant, with generally more errors, for F1 generation 30 mg/kg/day PKC412-treated adult males on PND63 to 69.

Table 65 Summary of F1 Generation Rat Adult Data – PPND Study

Terminal Body Weight:

Body weights:	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	30 mg/kg/day
Terminal body weight				
♂ PND78 (g)	501.4	↓3.7	↓1.4	↓9.7**
♀ PND78 (g)	293.1	↓0.7	↓2	↓7.7*
♀ GD13	381.2	↓1.3	↓1.6	↓6.9

*p≤0.05, **p≤0.01, ***p≤0.001

Mating/Fertility:

Mating/Fertility:	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	30 mg/kg/day
Mean no. days prior to mating	2.9	2.8	3	2.9
# in cohabitation (♂)	23	24	23	18
Mating index (%)	91.3	95.8	95.7	88.9
Fertility index (%)	91.3	91.7	95.7	88.9
Conception rate (%)	100	95.7	100	100
Pregnant	21/21	22/23	22/22	16/16
# in cohabitation (♀)	23	24	23	18
Pregnant (%)	100	95.7	100	100

C-sectioning and litter observations:

C-section data/litter observations:	0 mg/kg/day	5 mg/kg/day	15 mg/kg/day	30 mg/kg/day
Rat examined	21	22	22	16
Rats C-sectioned on GD13	21	22	22	16
Corpora lutea	18.3	18.9	17.1	16.7
Implantation Sites	16.6	17	16	14.9*
Mean % pre-implantation loss	8.93	9.24	6.75	10.83
Live conceptuses/ litter	15.4	16.1	15.2	14.3*
Resorptions	1.2	1	0.8	0.6
Mean % post-implantation loss	6.94	5.79	5.2	3.87

*p≤0.05, but were within the historical control data ranges.

Study title: PKC412: An Oral (Gavage) Toxicity Study in the Juvenile Albino Rat

Study no.:	870600
Study report location:	4.2.3.5.4.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 28, 2009
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	PKC412; Batch No. 0424020; Purity: 95%

Key Study Findings

- Mainly at 15 mg/kg/day, ↓BW and absolute organ weights with reversible microscopic findings in the lungs, pancreas, heart, kidney, and possibly male reproductive organs.
- No effects on fertility on maternal reproductive performance, but not expected since doses/exposures low.
- Overall, comparable toxicities to other rat studies.

Methods

Doses:	0, 2, 5, and 15 mg/kg/day
Frequency of dosing:	Once daily from PND7-70
Dose volume:	3 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	(b) (4)
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	20/sex/group for each reproduction and recovery subsets; 12/sex/group for the pathology subset
Satellite groups:	TK PND7 only: 10/sex and 20/sex for controls and PKC412-treated animals, respectively
Study design:	Sprague Dawley rat pups were treated with either vehicle or test article from PND7 to 70 at the specified doses. On PND21, pups were weaned and housed 2 to 3/sex/cage until PND28, then individually thereafter. At PND105, half the main study animals were subjected to mating procedures whereby 1 female was placed with 1 male (sibling matings were not performed) in the same dosage group for a maximum of 21 days.

Deviation from study protocol: None considered by the Study Director to affect the integrity or interpretation of data from the study

Observations and Times

Mortality:	Twice daily
Clinical signs:	Twice daily; F0 detailed: PND7,14, and 21; F1 detailed: predose and 2-3 hr postdose for wk 1, then twice weekly
Body weights:	PND7, 14, and 21; then, twice weekly (F1 generation only); also, GD 0, 3, 6,9, and 13 (mated F1♀)
Food Consumption:	F1 generation: Twice weekly beginning on PND28; also, GD 0 to 3, 3 to 6, 6 to 9 and 9 to 13 (mated F1♀)
Hematology, Clinical Chemistry:	At necropsy*
Urinalysis:	At necropsy*
Physical Development:	Eye opening (PND14 to 17), vaginal opening (beginning PND26), preputial separation (beginning PND35)
Sensory Development:	Visual function (pupillary closure response) (PND21)
Reproductive Performance:	Mating, GD13 ovarian and uterine examination
Behavioral Performance:	Motor activity (PND91±5) in enclosures (San Diego Instruments) for 1 hour w/ 6, 10 min intervals; 'E' water maze (PND98±5), auditory startle (PND28±3) measured using a San Diego Instruments Inc. SR-Lab Startle Response System
Gross Pathology:	At necropsy*
Organ Weights:	At necropsy*
Histopathology	At necropsy*
TK:	PND7: 4 pups/sex/treated group/timepoint and 2 pup/sex/control group/timepoint at 0.5, 1, 3, 7 and 24 hrs postdose via the vena cava (satellite TK); Last week of dosing from recovery animals (3 animals/sex/timepoint) at 0.5, 1, 3, 7 and 24 hours postdose via the jugular vein.

* Necropsy was conducted on all tissues for the pathology subset (PND71) and animals with unscheduled death and, for the recovery subset, target tissues (kidneys [♂ only], mesenteric lymph node, and lungs), and tissues showing gross abnormalities. Note: following weaning, F0 dams were euthanized without further examination.

Results
Mortality:

Table 66 Summary of Mortalities in Juvenile Toxicology Study in Rats

Dose of PCK412 (mg/kg/day)	Animal #	Found Dead or Euthanized	Day	Potential Cause of Death/Clinical Signs and Macroscopic Findings
Control	152/7F	Found dead	PND10	Gavage injury/abnormal breathing sounds, empty stomach, perforated esophagus
	1931F (recovery subset)	Found dead	PND21	Gavage error/decreased activity, abnormal breathing, red staining of the fur of the lower jaw
15	459/2M	Found dead	PND10	Unknown/decreased activity, pallor skin and abnormal breathing sounds, cold to touch
	456/5F	Found dead	PND10	Unknown/decreased activity, pallor skin and abnormal breathing sounds, labored breathing, thinness and a stomach suspected to be empty
	4920F (reproductive sunset)	Euthanized	PND95	Urinary tract disease/decreased activity, coldness to touch, severe dehydration, decreased muscle tone, labored breathing, piloerection and lying on the side; masses in the urinary bladder, left ureter and both kidneys which were consistent with the marked to severe inflammation with abscesses seen in the urethra, ureter, urinary bladder and kidneys. This animal also had mild cortical hypertrophy of the adrenal gland, minimal myeloid hypercellularity of the bone marrow, mild subacute heart inflammation, minimal to mild hepatocellular vacuolation with chronic inflammation, minimal to moderate lymphoid atrophy/necrosis of the spleen and thymus.

Clinical Signs:
Fur staining/skin brown (hindlimb, tail, or anus) occurred at higher incidence in HD♂+♀ compared to controls.

Reference ID: 4051584

Body Weight:

Table 67 Summary of Body Weights in Juvenile Toxicology Study in Rats

(Excerpted from the Toxicology Tabulated Summary)

Daily dose (mg/kg/day)	0 (Control; Group 1)		2 (Group 2)		5 (Group 3)		15 (Group 4)	
	Males	Females	Males	Females	Males	Females	Males	Females
No. of animals pre-weaning	52	52	52	52	52	52	52	52
No. of animals post weaning	52	51	52	52	52	52	51	51
Body weight (%[a])								
Day 21 *post partum*	63.53 g	61.95 g	-0.3	-4.3	1.8	1.4	-2.1	-1.5
Day 70 *post partum*								
Reproductive subset	511.0 g	305.7 g	-0.9	-9.8**	-0.2	-3.2	-4.8	-2.9
Recovery subset	528.5 g	294.8 g	-3.5	-8.6*	-1.8	-2.7	-6.7	-8.9*
Pathology subset	524.8 g	293.1 g	0.3	-2.5	-3.5	-3.4	-7.8	0.2
Day 112 *post partum* (Recovery subset)	719.2 g	372.1 g	-5.9	-11.2**	-3.5	-2.5	-9.0**	-11.0**
Day 147/108[b] *post partum* (Recovery subset)	747.1 g	384.0 g	-0.3	-9.5*	0.1	-4.7	-2.1	-5.8
Body weight gains (total per interval) (%[a])								
Day 21 to 70 *post partum* (Recovery subset)	463.7 g	231.2 g	-3.6	-8.9	-2.2	-2.9	-7.4	-9.9*
Day 70 to 112 *post partum* (Recovery subset)	190.7 g	77.3 g	-12.5*	-21.0*	-8.2	-1.7	-15.3**	-19.3*

* p<0.05, ** p<0.01, *** p<0.001 (Dunett)

a For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

b Males/females

Food Consumption:
No drug related effect on food consumption was observed.

Hematology and Clinical Chemistry (F1 adults):

- Absolute lymphocytes↓: dose-related MD and HD♀ (-18% and -24%, respectively); HD♂ (-19% compared to controls)
- ALT↑: dose-related MD and HD♂ and HD♀ (ranging from 1.30X to 2.67X individual values compared to control group mean value)
- Glucose↑: All doses ♂; +26% to +37% during treatment; +25% to +41% during recovery; no dose-response

Urinalysis:
Lower specific gravity females at all doses (~2%; p≤0.01)

Physical Development:
HD♀: ↓ in mean days for eye opening (14.1 days vs. 14.3 for controls; p≤0.01)

Sexual maturation:
Statistically significant increase in mean day of vaginal opening at all doses (≤2.5%↑; p≤0.01)

Neurobehavioral testing:
Acoustic startle habituation
While not statistically significant, LD and HD F1 males tended to have lower voltage to startle (mean 21%↓, startle at start) compared to controls.

Motor activity, 'E' water maze:
Unremarkable (differences between treated and controls <10%)

Visual Functioning
Normal (visual placing, pupillary closure)

Sperm analysis:
There was a trend in reduced spermatozoa count and percent motility in LD and HD♂, none of which reached statistical significance.

Reproductive capacity:
There was an unusually low number of pairs mating and a decrease in the conception rate of those that did mate; ↓ in mating index, fertility index and the conception rates in all groups.

Gross Pathology

Table 68 Incidence of PKC412-Related Macroscopic Findings in Juvenile Rats

(Excerpted from the study report)

	Males				Females			
Treatment groups	1	2	3	4	1	2	3	4
Pathology Subset								
Animals examined	12	12	12	12	12	12	12	12
Lungs								
Area dark	4	3	3	10	0	0	1	4

	Males				Females			
Treatment groups	1	2	3	4	1	2	3	4
Recovery Subset								
Animals examined	20	20	20	19	18	20	20	19
Lungs								
Area dark	0	0	1	3	0	0	0	1

- These lung changes correlated with hemorrhages or mixed cell infiltration noted microscopically.
- An increased incidence of pelvic dilatation in the right kidney was observed for males at 2, 5 and 15 mg/kg/day in the pathology subset and at 5 and 15 mg/kg/day in the reproduction and at 15 mg/kg/day in the recovery subsets. This correlated microscopically with compression.
- Small, soft testis or epididymis was observed in HD♂ and correlated with testicular seminiferous epithelium degeneration/atrophy and epididymis oligo/aspermia.
- A higher incidence of dark foci of the thymus was observed in HD animals.

Organ Weights

Mainly at 15 mg/kg/day, decreases in absolute organ weights ≥ 10% persisted during recovery in males in the spleen, thymus, adrenals and liver; and, in both sexes (≥ 7% in females) in the heart and kidneys.

Histopathology

Adequate Battery Yes

Peer Review Yes, signed

Histological Findings

Table 69 Microscopic Findings in Juvenile Rats

(Excerpted from the study report)

Pathology Subset:

Tissue/Finding	Sex	Males				Females			
	Dose (mg/kg/day)	0	2	5	15	0	2	5	15
Lung	Number examined	12	12	12	12	12	12	12	12
Infiltration: mixed cell									
	Total number affected	5	2	4	8	1	0	0	5
	Minimal	5	1	3	3	1	0	0	5
	Slight	0	1	1	3	0	0	0	0
	Moderate	0	0	0	2	0	0	0	0
Hemorrhage									
	Total number affected	2	4	2	7	0	0	1	2
	Minimal	2	4	2	5	0	0	1	1
	Slight	0	0	0	2	0	0	0	1
Mesenteric lymph node	Number examined	12	12	12	12	12	12	12	12
Erythrocytosis/erythrophagocytosis									
	Total number affected	3	2	3	10	1	0	1	7
	Minimal	3	2	2	10	1	0	1	6
	Slight	0	0	1	0	0	0	0	1

Recovery Subset:

Tissue/Finding	Sex	Males				Females			
	Dose (mg/kg/day)	0	2	5	15	0	2	5	15
Lung	Number examined	10	10	10	10	10	11	10	10
Infiltration: mixed cell									
	Total number affected	1	1	2	4	2	0	1	1
	Minimal	1	1	1	3	2	0	1	1
	Slight	0	0	1	1	0	0	0	0

- There were unilateral seminiferous epithelium degeneration/atrophy in the testes and unilateral oligo/aspermia of the epididymis of 2/12 and 1/12 rats at 15 mg/kg/day (minimal to marked) in the pathology subset males. These findings were also present in 3/19 HD♂ in the recovery subset. These findings were noted by the Applicant as being unrelated to PKC412 since they were unilateral and similar findings are noted in the historical control database of the test facility. This appears reasonable since there was evidence of marked unilateral testicular atrophy in control male rats in the 6/12 month rat study. However, since there was also the presence of sperm granulomas which may affect fluid flow in a unilateral fashion to contribute to atrophy,[21] the toxicological relevance and the irreversibility of these findings are uncertain based on the available data.
- Pelvic dilatation in the right kidney was observed with an increased severity in the pathology subset males at 2, 5 and 15 mg/kg/day and in the recovery subset

[21] La DK et al., Efferent Duct Toxicity with Secondary Testicular Changes in Rats Following Administration of a Novel Leukotriene A4 Hydrolase Inhibitor, 2012, Toxicologic Pathology, 40:705-714.

males at 15 mg/kg/day. Marked cortical atrophy, unilateral was observed in the pathology subset; moderate severity, similar to controls was observed during recovery.

- 1MD♂, 2HD♀: minimal to mild heart inflammation: subacute, multifocal, with hemorrhages and cardiomyofiber degeneration (one of the females was preterminal with urinary disease).
- 1MD♂, 2HD♂, 1HD♀: minimal to mild acinar cell atrophy in the pancreas was also observed in the pathology subset; not present in recovery.

Toxicokinetics:
Exposure increased dose proportionally between 2 and15 mg/kg/day. In both genders the exposure to PKC412 after single administrations (PND7) was 5 to 8-fold higher than after multiple administrations (last week of dosing) over the dose groups investigated.

Table 70 TK Parameters in Juvenile Rats

(Excerpted from the study report)

Gender	Study Day	Dose	C_{max}	T_{max}	$AUC_{0\text{-}24\ h}$	C_{max}/Dose	$AUC_{0\text{-}24\ h}$/Dose	C_o
Male	Day 7 post partum*	2	205	3.00	1560	103	782	0.00
		5	499	3.00	4350	99.8	870	0.00
		15	1140	7.00	15600	76.0	1040	0.00
	Last week of dosing**	2	35.6	1.00	275***	17.8	138	11.1
		5	50.6	1.00	526	10.1	105	25.0
		15	215	3.00	2000	14.3	133	51.3
Female	Day 7 post partum*	2	390	1.00	1740	195	871	0.00
		5	344	3.00	3310	68.8	661	0.00
		15	924	7.00	13500	61.6	903	0.00
	Last week of dosing**	2	36.9	1.00	289***	18.5	145	3.17
		5	77.1	1.00	695	15.4	139	28.9
		15	290	1.00	2680	19.3	179	25.7

* Toxicokinetic subset animal

** Main study subset animal

*** Concentration at 24 hours was below the lower limit of quantification. In this instance, the program estimated the AUC based on the estimated concentration at 24 hours and not the concentration at the actual observation time.

Units: Dose (mg/kg/day); C_{max} (ng/mL); T_{max} (Hours); $AUC_{0\text{-}24h}$ (ng*Hours/mL); C_{max}/Dose (ng/mL/mg/kg/day); $AUC_{0\text{-}24\ h}$/Dose (ng*Hours/mL/mg/kg/day); Co (ng/mL)

10 Special Toxicology Studies

- Non-GLP Ames tests in two bacterial strains and in silico modeling did not reveal any genotoxic potential for CGP62221 and CGP52421. No structural alerts for mutagenicity for PKC412, CGP52421, or CGP62221 were detected using Derek Nexus, but there was a structural alert for thyroid toxicity (Study No. PCS-LR1519360). However, both predictions were out of domain due to the 1,3,6-oxadiazepane ring system which is not present in the training set of the in silico tools. Toxtree calculations assigned PKC412 and both metabolites to class III (high toxicity) based on the presence of a heterocyclic ring systems with complex substituents.
- After treatment with impurity [redacted] (b) (4) no notable increases in the numbers of revertant colonies above the negative control values were observed

(concentrations up to 1000 and 500 µg/plate using the plate incorporation and preincubation methods, respectively). Upper concentrations were the highest in which colony counting could still occur in the presence of precipitate (Study No. PCS-R0770319, GLP).

- Results and methods from the chromosomal aberration test with impurity (b) (4) are below.

Study title: (b) (4) (Related Substance of PKC412): Chromosome Aberration Test with Cultured Human Peripheral Blood Lymphocytes

Study no.:	PCS-R0770320
Study report location:	4.2.3.7.7.
Conducting laboratory and location:	Novartis, Basel, SWISS
Date of study initiation:	July 10, 2007
GLP compliance:	Yes
QA statement:	Yes, signed
Drug, lot #, and % purity:	(b) (4) (Related substance of PKC412); Batch No. K33208450 / K36449950; Purity: 89.7%

Key Study Findings

- (b) (4) was not clastogenic under the conditions tested, but was aneugenic in the presence and absence of metabolic activation.

Methods

Cell line:	Human Peripheral Blood Lymphocytes
Concentrations in definitive study:	See table below
Basis of concentration selection:	Highest dose based on solubility, each lower dose was 60% of the higher dose
Negative control:	DMSO
Positive control:	-S9: Ethyl methansulfonate (EMS) (5 or 9.7 µM) +S9: Cyclophosphamide (CP) (55 µM)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	3 hr with metabolic activation, 3 or 21 hr without metabolic activation (3 hr treatments had a 17 hr recovery)

Study Validity

The study is valid. Only concentrations with a mitotic index (MI) 47.3% were evaluated.

Results

Table 71 Summary of Chromosomal Aberrations with (b) (4) Treatment in Peripheral Human Lymphocytes

	Total No. Cells Examined				MI	Total No. of Alterations						% Cells w/ Aberrations		
							ct		cs			Structural		
Conc. µg/mL	Tot.	P	H	E		Gap	Del	Exc	Del	Exc	Mab	inc gaps	exc gaps	num
3h +S9 (A)														
0	203	2	1	0	100	0	2	0	0	0	0	0.5	0.5	1.5
5	324	127	4	1	87.5	0	1	0	0	0	0	0.5	0.5	40.7
8.5	363	144	7	13	70.3	1	2	0	0	0	0	1	0.5	45.2
14.5	315	151	2	18	56.2	1	2	0	0	0	0	2.1	1.4	54.3
CP	51	0	1	0	68.8	1	6	0	0	0	0	14	12	2
3h +S9 (B)														
0	201	0	2	0	100	0	0	0	0	0	0	0	0	0.5
3.7	256	50	1	1	101	1	1	0	0	0	0	1	0.5	20.3
7	248	86	2	0	79	0	1	0	0	0	0	0.6	0.6	35.5
25.9	231	78	3	17	47.6	0	1	0	0	0	0	0.8	0.8	42.4
CP	50	0	0	0	97.1	2	6	0	0	0	0	14	12	0
3h -S9 (B)														
0	202	1	1	0	100	2	0	0	0	0	0	1	0	1
1.9	208	3	4	1	95.4	8	7	0	0	0	0	0	0	3.8
7	217	15	3	0	80.9	0	0	0	0	0	0	0	0	8.3
13.5	246	61	8	0	47.3	0	0	0	0	0	0	0	0	28.8
EMS	51	0	0	0	33.6	2	10	4	0	0	0	21.6	19.6	0
20h -S9 (C)														
0	202	0	2	0	100	0	0	0	0	0	0	0	0	1
1.2	202	0	2	0	102.2	2	2	0	0	0	0	2	1	1
2	207	5	2	0	100	5	6	0	0	0	0	4.5	3	3.4
3.4	295	90	4	6	95.1	0	1	0	0	0	0	0.5	0.5	33.9
EMS	51	1	0	0	23.9	3	25	3	0	0	0	40	40	2

Abbreviations: Gaps chromatid/isochromatid gap; Del (ct) chromatid/chromosome deletions (cd, iso); Exc(ct) all chromatid exchanges (dicentric chromosome, tricentric chromosome, ring chromosome, acentric ring, interstitial deletion); Del (cs) is not used (undetectable without special staining techniques); Exc(cs) all chromosome exchanges (dicentric chromosome, tricentric chromosome, ring chromosome, acentric ring, interstitial deletion); MAb Multiple aberrant cell (cell with more than 5 structural chromosomal aberrations excluding gaps); P polyploid metaphase; H hyperdiploidy; E endoreduplicated metaphase; Num. % of total P, H and E; Cells with chromosome counts between 47 and 68 were classified as hyperdiploids and cells with chromosome counts ≥ 69 were classified as polyploids.

- The proposed limits of impurity (b) (4) in the midostaurin formulations are qualified based on results from the following study.

Study title: PKC412: A 4-week Oral (Gavage) Impurity ([redacted] Qualification Study in the Rat

Study no.:	PCS-R0770470
Study report location:	4.2.3.7.7.
Conducting laboratory and location:	[redacted]
Date of study initiation:	February 6, 2008
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	[redacted] (related substance of PKC412); Batch 07/4; Purity: 99.8% PKC412-NXA.003 (also known as PKC412); Batch 0424020; Purity: 100.5%

Key Study Findings

- Overall, the toxicity profile was comparable in PKC412 formulations with or without [redacted]
- [redacted] is qualified up to the highest level used in the study of [redacted]%.

Methods

Doses:	See study design table below
Frequency of dosing:	Daily for 4 weeks
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	[redacted]
Species/Strain:	Sprague-Dawley Crl:CD(SD)
Number/Sex/Group:	10/sex/group
Age:	9 wks
Weight:	Males: 319 to 382 g, Females: 211 to 256 g
Satellite groups:	None; main study animals were sampled
Unique study design:	No
Deviation from study protocol:	Due to severe mortality and adverse clinical signs noted at 60 mg/kg/day during wks 1 and 2, dosing for Groups 3 and 4 at 60 mg/kg was suspended for 6 days from D9/10 to D16. All males in Groups 3 and 4 were terminated on D17. All females received a lowered dose at 30 mg/kg starting on D16 to the end of dosing period.

Study Design (excerpted from the study report)

Group number identification	Dose level (mg/kg/day)	Dose conc. (mg/mL)$	Animal number Main study Male	Animal number Main study Female
1/ Vehicle control	0	0	1001 to 1010	1501 to 1510
2/ PKC412 low dose + degradation product (b) (4)	10	1	2001 to 2010	2501 to 2510
3/ PKC412 high dose + degradation product (b) (4)	60/30	6/3	3001 to 3010	3501 to 3510
4/ PKC412 high dose	60/30	6/3	4001 to 4010	4501 to 4510

$ Dose concentrations were not corrected for purity.

Observations and Times

Mortality:	Twice daily
Clinical signs:	Pretest, weekly
Body weights:	Pretest, weekly
Food consumption:	Pretest, weekly
Opthalmoscopy:	Pretest, D23 and 24
Hematology, Clinical Chemistry, Urinalysis	D28, (Groups 1 and 2 male heme and cc data analyzed on D28 and Groups 3 and 4 on D17)
Gross Pathology, Organ Weights, and Histopathology:	At necropsy* (organ wt data not available for Groups 3 and 4 males)
TK:	Days 1 and 28: 0.5, 1, 3, 7 and 24 hrs postdose (2 rats/sex/time point)

* Necropsy was conducted on D29. For Group 2, only tissues examined were those showing treatment-related findings including: bone marrow (sternum and femur), epididymis, testes, prostate, mammary gland, spleen, thymus, stomach (non-glandular), colon, and all gross lesions

Summary of Findings:

Overall the addition of the impurity to the formulation does not significantly influence toxicity. The incidence of animals found dead was similar with or without (w/wo) (b) (4) ↑liver weight was observed in formulations w/wo (b) (4) No urinalysis was conducted for Groups 3 and 4, but Group 2 seems to follow a similar trend of ↓ specific gravity as other toxicology studies. Electrolyte imbalances were also observed as in other studies with no apparent difference based on the presence of absence of (b) (4) in the formulation.

There were some differences in toxicity between groups treated w/wo impurity, like decreased creatine kinase in Group 3 only (perhaps unique to impurity) and decreased thymus weights were more pronounced in formulations with (b) (4) compared to PKC412 alone. Also, within the study, there was a slightly higher incidence of inflammation in the stomach and prostate in males with (b) (4) compared to PKC412 alone.

Groups 3 males and Group 4 females had higher exposures on Day 1 than Group 4 males and Group 3 females, respectively (differences <2-fold). AUC and Cmax values were higher in D28 for females with the impurity (Group 3) versus those with PKC412 only (Group 4).

11 Integrated Summary and Safety Evaluation

Midostaurin is a synthetic derivative of staurosporine and a multi-kinase inhibitor indicated for the treatment of patients with advanced systemic mastocytosis (ASM) and FLT3 mutation positive acute myeloid leukemia (AML). Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of midostaurin for this indication.

Pharmacology

Midostaurin and its predominant metabolites CGP62221 and CGP52421 (epimers display similar biological activities) inhibited many kinases more than 80% at concentrations of 1 µM when screened against a panel of 220 kinases in an in vitro enzymatic assay. Using different methodology, midostaurin inhibitory activity was profiled against ~30 kinases. Some of these kinases (and others) had IC50 values within 30-fold of the most potent target, FLT3 D835Y (IC50=3.6 to 6 nM). The binding affinity for 36 kinases was below 100 nM out of a panel of more than 180 purified kinases or kinase domains, with highest affinity for FLT3 (wild-type and mutants, Kd=6 to 15 nM) and the KIT D816V mutant (Kd=7.7 nM). Only kinases with IC50s <100 nM in the kinase panel that also and demonstrated inhibitory activity in other in vitro cellular assays were included as targets of midostaurin in labeling.

Table 72 Kinase Selectivity of PKC412, CGP62221, and CGP52421

	PKC412	CGP62221	CGP52421	Label Inclusion (Y,N)
Kinase	IC50 (nM)	IC50 (nM)	IC50 (nM)	
Free Cmax 50 mg BID	1.17	1.68	1.99	
FLT3 D835Y	3.64, 6	2.29	10.2	Y
SYK	8.38, 95, 140	3.11	110	N
NTRK1 (TRKA)	11.4	14.2	--	N
PHKG1	11.9	--	--	N
NTRK3 (TRKC)	15.2	8.35	--	N
TBK1	15.5	--	93.2	N
PKN1 (PRK1)	15.6, 47	--	81	N
AURKA (Aurora A)	18.2	15	844	N
RPS6KA2 (RSK3)	18.8	24	--	N
FLT3	19.8	11.3	59.5	Y
MAP3K9 (MLK1)	22.6	39	47.4	N
CSF1R (FMS)	25.9	--	27.9	N
BRSK1 (SAD1)	31.8	--	--	N
RPS6KA1 (RSK1)	33.6	10.9	--	N
PDGFRA (PDGFRα)	34.2	--	223	Y
PDGFRB (PDGFRβ)	35.2	--	--	Y
RPS6KA3 (RSK2)	43.8	18.5	--	N
NTRK2 (TRKB)	50.8	49.1	--	N

	PKC412	CGP62221	CGP52421	Label Inclusion (Y,N)
Kinase	IC50 (nM)	IC50 (nM)	IC50 (nM)	
Free Cmax 50 mg BID	1.17	1.68	1.99	
STK4 (MST1)	50.9, 36	--	--	N
PRKCA (PKC α)	203, 22, 280	152	827	Y
PRKCB1 (PKC βI)	263, 30	--	631	Y
PRKCB2 (PKC β II)	119, 31	--	--	Y
PKC-γ	24	--	--	Y
PPK	38	--	--	N
				(b) (4)
KIT	600, 330	--	--	N
PKC-η	160	--	--	Y
FGFR2	91	NE	NE	N
JAK2	96	--	--	N
JAK3	47	NE	NE	N
PDPK1	58	NE	NE	N
PKN2	48	NE	NE	N
FER	--	9.72	--	N
ROS1	--	22.9	--	N

Targets with IC50 values <100 nM from Study Nos. RD-2008-01357, PKF-98-02545, PSP-NVP-BVN113, and Fabbro et al, 2002[7] are included in the above table. "--", IC50 values were not determined; Y = target included in Section 12.1 of the label because of high potency and target inhibition was characterized in multiple in vitro assays; N, not included in the label because did not meet aforementioned criteria; NE = metabolites were not evaluated for some targets in this study.

Midostaurin inhibited mitogen-induced phosphorylation of PDGFR (IC50=80 nM), KIT, PKC, and VEGFR2 (KDR) (IC50=0.3 to 1µM), but not insulin receptor or EGFR. Midostaurin also inhibited PDGF-induced mRNA expression of c-fos (IC50=100 nM) and may also inhibit non-specific mitogen-induced MAP-kinase and p70-S6 kinase activity. Competitive binding studies suggest midostaurin binds to the catalytic domain of PKC isozymes (IC50=11 to 50 nM), but such studies were not submitted for other midostaurin targets. Midostaurin inhibited FLT3 and KIT phosphorylation, as well as downstream targets STAT3, STAT5, and ERK (IC50=10 to 100 nM) in mutant expressing BaF3 cell lines. Midostaurin and its predominant metabolites inhibit IgE-mediated histamine release in human blood basophils (IC50=~10 nM).

Midostaurin demonstrated more potent antiproliferative activity in FLT3-ITD and c-KIT D816V mutant BaF3 cell lines (EC50=39 to 47 nM) compared to FLT3 wild-type expressing BaF3 cell lines. CGP62221 demonstrated comparable potency (EC50=28 to 71 nM); however, CGP52421 was on average ~10-fold less potent than midostaurin. Midostaurin demonstrated antiproliferative activity in various human AML cell lines and was most potent for cell lines with FIP1L-PDGFR and FLT3-ITD mutations (IC50=8 to 48 nM) compared to those expressing FLT3 wild-type. Studies suggest midostaurin can synergize with various cytotoxic agents to reduce proliferation in FLT3-ITD expressing

AML cell lines. Midostaurin also inhibited proliferation in a mast cell line and primary mast cells from a patient with smoldering systemic mastocytosis harboring the KIT D816V mutation (IC50=50 nM). Studies in FLT3-ITD and D816V/V560G KIT mutant expressing AML and mast cell lines indicate midostaurin exerts its effects by promoting apoptosis, specifically in AML cells via upregulation of mRNA expression of apoptosis regulators Bim and Puma.

Regarding midostaurin's in vivo antitumor activity, statistically significant smaller tumors were obtained in nude mice inoculated with a human AML cell line when mice were treated for 12 days orally at doses of 50 and 150 mg/kg (150 to 450 mg/m2) compared to vehicle treated (tumor regression 58 to 100%, respectively).

Off-target activity was assessed for midostaurin and its metabolites for GPCRs, transporters, ion channels, nuclear receptors, and enzymes for as many as 40 targets, including those implicated in suicidality.[20] The lowest IC50 was 2.3 µM for inhibition of COX-1. At the clinical dose, the free Cmax <2 nM for midostaurin and its metabolites, therefore the potential for off-target activity against COX-1 and other potential targets, including phosphodiesterase PDE4D (IC50=3.2 to 6.9 µM), adenosine transporter ENT1 (IC50=8.3 µM), and 5-HT2B antagonism (IC50=18 µM), is low.

Both in vivo and in vitro safety pharmacology studies were conducted to assess the effects of midostaurin on neurological and cardiovascular function (designated studies to assess respiratory function were not conducted). The IC50s for the *in vitro* hERG potassium current were estimated to have a low potential to inhibit hERG in vivo for midostaurin (>12 µM), CGP52421 (38.5% inhibition at 1.5 µM), and CGP62221 (11.3% inhibition at 1.21 µM). In a cardio-renal safety evaluation study in Tif:RAIf (SPF) male rats, decreased mean arterial pressure and heart rate were observed following single IV doses ≥ 8 mg/kg (48 mg/m2)(with no effects at an oral dose of 300 mg/kg, or 1800 mg/m2). Infusion of 2.5 mg/kg/min (15 mg/m2/min) in rats induced severe hypotension and respiratory arrest and was lethal after 14 to 21 minutes of infusion, i.e., after total doses of 35 to 53 mg/kg. Increases in sodium and chloride excretion were observed in female rats administered midostaurin ≥ 100 mg/kg (600 mg/m2) orally as a single dose. In an ex vivo mesenteric artery perfusion assay in male rats, the IC50s for noradrenaline- and KCl-induced vasoconstriction were 0.67 and 0.41 µM, respectively; clinically relevant concentrations. In a neurotransmitter release and uptake study, 10 µM midostaurin induced basal release of noradrenaline, serotonin, and dopamine 20, 26, and 81%, respectively and reduced GABA release by 19%. Effects on neurotransmitter uptake were >100 µM midostaurin and likely are not therapeutically relevant. In an in vivo CNS evaluation study in Tif:MAGf (SPF) male mice, after a single oral dose of midostaurin, ataxia (≥ 10 mg/kg, 30 mg/m2) 4 hours postdose and reduced length to stay on rotarod and increased body temperature (300 mg/kg, 900 mg/m2) 1 hour postdose were observed.

Pharmacokinetics/ADME/Toxicokinetics

Absorption

Orally administered midostaurin ((b) (4) formulation) is absorbed slower in the rat, dog, and rabbit compared to humans (4 to 8 hours versus 1.7 hours). The extent of absorption was estimated to be high in rats and humans (>90%) and moderate in dogs and rabbits (40 to 64%). Bioavailability with the (b) (4) formulation was low in rats (9.3%) and rabbits (1.8%), and moderate in dogs (48.5%), indicating high to moderate first pass metabolism. Clearance was moderate in rats, rabbits, and dogs (0.98, 0.24 and 0.90 L/h/kg, respectively) and less than 35% of hepatic blood flow. Volume of distribution at steady state suggests midostaurin was well distributed to tissues/organs. Concentration versus time profiles were biphasic with a long apparent terminal half-life of 9.6 to 20 hours.

Distribution

Males

In vitro protein binding was high for midostaurin and its predominant metabolites (>98 to 99%). Intravenous (1 mg/kg; 6 mg/m2) and oral (3, 10 or 22 mg/kg (18, 60, or 132 mg/m2) single dose administration of radiolabeled midostaurin to rats reached maximal concentration at 5 minutes and 1 hour postdose, respectively. Concentrations began to taper 2 hours (IV) and 24 hours (oral) postdose. At 168 hours postdose, the liver and kidney displayed >10-fold higher concentrations than blood for both routes. Following 3 mg/kg repeated oral dosing, highest concentrations were found in the liver and small intestine, followed by the stomach, kidney, aorta, thyroid, white fat, spleen, adrenals, brown fat, skin, pancreas, and lung. Whole body radiography (WBA) on IV administered rats showed midostaurin was taken up by the pituitary gland and crossed the blood brain barrier, with the highest [^{14}C] concentrations in the frontal cortex. No melanin binding was observed.

Females

After a single radiolabeled oral dose of 30 mg/kg (180 mg/m2) midostaurin ((b) (4) to pregnant Wistar/Han (albino) rats on GD12 or GD17, WBA revealed the liver, kidney, spleen, and heart had the highest radioactive concentration at 3 or 6 hours GD12 postdose (24 hours GD17 postdose, suggesting delayed absorption). Absorption was delayed and the amount of midostaurin that distributed to the fetus was lower on GD17 versus GD12. Very little drug appeared to enter the fetal brain. A similar study in rats using the (b) (4) formulation did not demonstrate extensive transfer to the fetus on GD13. Similarly, there was not extensive transfer to the fetus in rabbits treated with a single oral radiolabeled dose of midostaurin ((b) (4) formulation) to pregnant New Zealand white rabbits on GD17. The highest concentrations in rabbit dams were in the bile, liver, white fat, kidney, and lung. Uptake of radioactivity into the mammary glands suggests possible excretion of midostaurin in milk, which was confirmed in an excretion study in lactating rats where midostaurin and metabolites were transferred to milk within 1 hour postdose with a milk/plasma ratio ~5.

Metabolism

The major circulating components were midostaurin are CGP52421 (epimers 1 and 2, mono-hydroxylation metabolites) and CGP62221 (O-demethylation metabolite) in animals and humans (>10% of the total AUC). The apparent half-life of CGP52421 and

CGP62221 in human was long (495 and 33.4 hours, respectively). CGP52421 was present in the rat, rabbit, dog and human; however, while CGP62221 accounted for 28% of total AUC in humans, it was detected at lower levels in the rabbit and dog (1.9 and 4.6%, respectively). CGP62221 was not present in the rat. CYP3A4 was mainly involved in both hydroxylation and demethylation pathways of midostaurin, with a very minor contribution of CYP1A1.

Excretion
Hepatobiliary route was a route of excretion, with the majority of midostaurin and its metabolites being detected in the feces and 4% or less in the urine. Unchanged drug accounted for 0.91 to 18.7% in feces across species (highest in the rabbit). In lactating Wistar/Han rats treated on PND8 or 9, unchanged midostaurin was present at higher relative concentrations in rat milk (51%) compared to than observed in plasma (38%).

Based on similarities in pharmacokinetics for midostaurin, CGP52421, and CGP62221 between humans and animals, the animal models for pharmacology are generally predictive of the time course of pharmacologic activity of the drug, and can be relied upon for predicting activity of alternative regimens in the clinical setting. The only exception is that CGP62221 is not present in the rat and is at low levels in the rabbit, and additionally targets ROS1 kinase, which may under predict potential reproductive and developmental toxicity.

General Toxicology
Single dose toxicity studies were conducted in mice, rats and dogs and the repeat dose studies were performed on rats and dogs for up to 12 months duration with daily oral dosing. In one acute toxicity study, female rats were more sensitive than the males to the lethal toxicity of midostaurin (Study No. 926033), but this result was not reproduced in another single dose study with similar dosing regimen in rats, but different batch (Study No. 936216). Single doses of 1400 mg/kg (4200 mg/m2) midostaurin were well tolerated in mice and the highest non-severely toxic dose in dogs was 120 mg/kg (2400 mg/m2). A summary of significant findings from the single dose studies are listed in Table 74 (excerpted from the 30-Day Safety Review by Dr. Hua Zheng with minor modifications by this reviewer).

In a 3-month repeat dose daily oral general toxicology study in Tif:RAIf (SPF) rats, there were no mortalities, but anemia and toxicities to the heart, lungs, GI tract, thyroid, lymph node, and male reproductive organs were observed, mainly at 30 mg/kg/day (180 mg/m2). Toxicities to the liver and thymus were observed at doses ≥ 20 mg/kg/day (120 mg/m2) in rats, with the NOAEL determined to be the low dose of 10 mg/kg/day (60 mg/m2). In a 26-week repeat dose daily oral general toxicology study in Sprague Dawley rats, 5/30 (4 males and 1 female) high dose (100 mg/kg/day, 600 mg/m2/day) rats died between days 19 and 21. Severe atrophy of the thymus was noted as a probable cause of death in some animals. The NOAEL was less than the low dose of 30 mg/kg/day (180 mg/m2/day). Other hematopoietic organs as well as the GI tract, male reproductive organs, liver, uterus, ovaries, kidneys, urinary bladder, glands (pancreas, adrenal, submaxillary, lacrimal and salivary), and heart displayed toxicities.

Reference ID: 4051584

The GI, liver, heart, and hematological toxicities were more adverse in the 26-week than 3-month study, but higher doses were also used in the 26-week study as well as a different, more clinically relevant formulation ((b) (4) In a 6/12 month, repeat dose, daily oral general toxicology study in Tif:RAIf (SPF) rats, interstitial pneumonitis emerged at the 30 mg/kg/day (180 mg/m2/day) dose. Hematopoietic and liver toxicities were most prominent. Given that AUC exposures in rats used in the general repeat dose toxicology studies are much lower than those observed in patients at clinical doses of 50 and 100 mg BID, many of the aforementioned toxicities may be clinically relevant.

In a 3-month repeat dose daily oral general toxicology study in Beagle dogs, there were no mortalities, but toxicities to the heart, lungs, GI tract, liver, urinary bladder, and glands were observed mainly at 30 mg/kg/day (600 mg/m2/day). Toxicity to male reproductive organs, hematological toxicity, and kidney toxicity were observed at all doses (≥ 3 mg/kg/day, 60 mg/m2/day). In a 6/12 month repeat dose daily oral general toxicology study in Beagle dogs, there were no mortalities. Toxicities were comparable to the 3-month study except the hematopoietic toxicity was more pronounced, there were inflammatory foci in brain, and a high incidence of pituitary cysts. The majority of these findings were present at doses ≥ 3 mg/kg/day (60 mg/m2/day). Toxicities in dogs were very comparable to those observed in rats. Overall, hematopoietic and renal toxicities displayed better reversibility in dogs than rats.

Genetic Toxicology

Midostaurin was not mutagenic in vitro in the bacterial reverse mutation assay (Ames test) or in Chinese hamster V97 cells. Midostaurin increased the frequency of polyploidy cells in an in vitro chromosomal aberrations assay in Chinese hamster ovary cells, but was not clastogenic in an in vivo rat bone marrow micronucleus assay when tested to the maximum tolerated dose (MTD) of 200 mg/kg (1200 mg/m2). This dose is approximately 20-fold the recommended human dose, based on body surface area.

In silico modeling predicted metabolites CGP52421 and CGP62221 as having a similar toxicity profile as midostaurin. There is also no evidence to suggest the metabolites are mutagenic (non-GLP tests in TA98 and TA100 strains +/- S9). The impurity (b) (4) was not mutagenic in vitro in the bacterial reverse mutation assay (Ames test) or clastogenic in human peripheral blood lymphocytes under the conditions tested. However, (b) (4) was aneugenic (increasing the number of polyploidy, hyperdiploid, and endoreduplicated metaphase cells) in the presence and absence of metabolic activation.

Carcinogenicity studies have not been conducted and are not necessary for NDA approval for the proposed indication.

Reproductive and Developmental Toxicology

Reproductive toxicology studies consisted of one fertility and early embryonic development study in rats, embryofetal developmental (EFD) studies in rats and rabbits, a pre- and postnatal development study in rats, and a juvenile toxicology study in rats.

Fertility in Rats
In the oral repeat dose fertility and early embryonic development study, treated male and female rats (0 (vehicle), 10, 30, and 60 mg/kg/day; 0, 60, 180, 360 mg/m2/day of midostaurin) were mated. Females were dosed until gestational day 6. There were 6/24 mortalities in males at the high dose with clinical signs of weight loss, stained forelimbs and mouth, stool changes, unthriftiness, salivation after dosing, emaciation, lethargy, and distended abdomen/stomach. At doses ≥ 30 mg/kg/day (180 mg/m2/day), males exhibited reduced body weights and at 60 mg/kg/day (360 mg/m2/day), females displayed reduced body weight gain and lower gravid uterine weights. This correlated with increased resorptions (including total resorption of litter), decreased pregnancy rate, and decreased number of implants and live embryos. In males, testicular degeneration and atrophy was observed at doses ≥ 10 mg/kg/day (60 mg/m2/day) and reduced sperm count and motility and a decrease in reproductive organ weights were observed at 60 mg/kg/day (360 mg/m2/day).

In a 3-month toxicology study in dogs, there was inhibition of spermatogenesis at doses ≥ 3 mg/kg/day (AUC(0-24hr)=0.858 µmol.hr/mL on Day 92). Toxicokinetic evaluations were not performed in the rat fertility study, therefore, relevant comparison of exposures in animals and humans for midostaurin labeling will be based on the steady state AUC from the 6/12 month rat study with the (b) (4) formulation for the 10 mg/kg/day dose (1.8 µmol.hr/L) and based on AUCs from the 26-week rat toxicity study with the (b) (4) for the 30 and 60 mg/kg/day doses (9.05 and 14.04 µmol.hr/L, respectively on Day 30). The human AUC(0-24hr) used for animal:human comparisons is 166 µmol•hr/mL. Thus, the doses of 10, 30, and 60 mg/kg/day of midostaurin in rats resulted in exposures (AUC) of approximately 0.01, 0.05, and 0.08 times, respectively, and 0.005 times in male dogs at 3 mg/kg/day, the human exposure at the recommended dose.

Embryo-fetal Development in Rats and Rabbits
In the rodent embryo-fetal developmental (EFD) oral repeat dose study, pregnant rats were administered oral doses of midostaurin during the period of organogenesis at doses of 0 (vehicle), 3, 10, and 30 mg/kg/day (0, 18, 60, 180 mg/m2/day). At all doses, midostaurin increased the number of resorptions (mainly late) in the absence of maternal toxicity. Reduced fetal weight was observed only at the high dose of 30 mg/kg/day (180 mg/m2), but side effects of reduced fetal growth (dilated brain ventricles and reduced skeletal ossification) were observed at all doses. This could be related to sporadic low fetal weight of individual fetuses versus a test article-related effect. Reduced skeletal ossifications, severe renal pelvic cavitation, and widened anterior fontanelle were also observed in rat pups at the highest dose of 30 mg/kg/day.

In the nonrodent EFD oral repeat dose study, pregnant rabbits were administered oral doses of midostaurin during the period of organogenesis at doses of 0 (vehicle), 2, 10, and 20 mg/kg/day (0, 24, 120, 240 mg/m2/day). There was a similar incidence in mortality in treated dams and controls. Midostaurin was maternally toxic (dose-related ↓BW gain and food consumption, ↑incidence of spontaneous abortions) at all doses (≥ 2

mg/kg/day). Fetotoxicity (↓fetal weight, with reduced gall bladder size and reduced skeletal ossifications) was observed mainly at doses ≥ 10 mg/kg/day (120 mg/m2/day).

Pre-and postnatal Development in Rats
In the oral repeat dose pre- and postnatal development (PPND) study, pregnant Sprague Dawley rats were administered the midostaurin (b) (4) formulation daily from gestational day 6 through postnatal days 21, 22, or 23 at doses of 0 (vehicle), 5, 15, and 30 mg/kg/day (0, 30, 90, 180 mg/m2/day). Midostaurin was maternally toxic (↓BW gain) at 30 mg/kg/day (180 mg/m2/day). Adverse effects upon maternal performance at 30 mg/kg/day included dystocia and decreased live litter size. In the F1 generation there were reductions in body weight that did not resolve by the end of the study, accelerated eye opening in pups and delayed auricular startle ontogeny. Additionally, learning behavior (as measured by time to complete the maze and number of errors in the 'E' water maze) may have been affected in males at 30 mg/kg/day, but there was no statistical significance due to the high degree of animal variability. When F1 generation males were mated with F1 generation females (roughly 2 months after receiving the final dose of midostaurin/weaning), there were some effects on fertility parameters. There was a slight reduction in male fertility rate, and statistically significant reductions in the number of implantation sites and live fetuses/litter in F1 females at 30 mg/kg/day (180 mg/m2/day).

The reproductive and developmental toxicology studies suggest that administration of midostaurin may pose a risk for embryo-fetal toxicity. No toxicokinetics were conducted as part of the reproductive toxicology studies, so results from pharmacokinetic studies using similar doses will be relied upon to make animal:human AUC comparisons for labeling purposes. Relevant comparison of exposures in rats and humans for midostaurin labeling will be based on: (1) steady state AUC(0-24hr) values from pharmacokinetic data from the rat 6/12 month study (Study No. DMPK-R1995093) with comparable doses to those demonstrated to have embryo-fetal toxicity (0.423, 2.347, and 9.283 µmol.hr/L at doses of 3, 10, and 30 mg/kg/day, respectively); and, (2) the human AUC(0-24hr) of 166 µmol.hr/mL. Of note, in the 26-week rat general toxicology study using the (b) (4) formulation, similar to the clinical formulation, the AUC(0-24hr) on Day 30 at 30 mg/kg/day was 9.760 µmol.hr/L in females. Since PK data is not available with the (b) (4) formulation at the lower doses used in the rat EFD study and AUC is comparable between formulations in female rats at 30 mg/kg/day, animal AUC exposures for animal:human comparisons based on the (b) (4) formulation appear relevant. Thus, the doses of 3, 10, and 30 mg/kg/day of midostaurin in rats resulted in exposures (AUC) of approximately 0.003, 0.014, and 0.05 times, respectively, the human exposure at the recommended dose.

For relevant rabbit:human comparisons, exposures (AUC(0-24hr)) in rabbits will be based on pharmacokinetic data obtained on gestational day 20 using the (b) (4) formulation from the EFD dose range finding study (Study No. DMPK-R936242) from doses comparable to those with positive findings in the pivotal rabbit EFD study (1.581 and 2.422 µmol.hr/L at 10 and 30 mg/kg/day, respectively). There is no pharmacokinetic data for the lowest rabbit dose with positive findings of 2 mg/kg/day.

Since there were comparable findings (↓BW gain in dams, ↓fetal birth weight) between the dose finding and pivotal studies, animal AUC exposures for animal:human comparisons based on the dose finding study appear relevant. Thus, doses of 2, 10, and 30 mg/kg/day of midostaurin in rabbits resulted in exposures (AUC) lower than human exposure, 0.009, and 0.015 times, respectively, at the recommended dose.

Juvenile Toxicology in Rats

A juvenile toxicology study was also conducted in Sprague Dawley rats administered the midostaurin (b) (4) formulation at doses of 0 (vehicle), 2, 5, and 15 mg/kg/day (0, 12, 30, 90 mg/m2/day) from postnatal day 7 to 70, with a 6-week recovery. There were mortalities that were not treatment related, but there was one unusual mortality in a high dose female with urinary tract infection/disease. Since the mechanism of action of midostaurin includes neutrophil and lymphocyte suppression, it cannot be ruled out that midostaurin treatment did not contribute to the mortality. Decreased body weight, mainly at 15 mg/kg/day (90 mg/m2/day) with statistically significant reductions in body weight gain even at the low dose, that may have contributed to reduced weights of various organs (heart, kidney, liver, spleen, thymus, and adrenals) were observed. Similar to the PPND study, there was accelerated eye opening at 15 mg/kg/day (90 mg/m2/day) in females. The maximum voltage to startle juvenile rats in an auditory startle habituation study was lower in males at 15 mg/kg/day (90 mg/m2/day)(although there was high individual animal variability in the study). There was an approximate 15%↓ in sperm count at 2 and 15 mg/kg/day (12 and 90 mg/m2/day, respectively) in males (a similar percentage to that observed in the fertility study), but no apparent effects on sperm motility and fertility. However, this interpretation is somewhat confounded by the unusually low fertility rate present in all groups after mating, including controls. At doses ≥ 2 mg/kg/day (12 mg/m2/day), there was a delay in sexual maturation in females that did not appear to affect fertility or the number of live embryos. Of note, most effects on fertility and maternal performance in rats in the reproductive and development toxicology studies were at doses and AUC exposures greater than the highest dose tested of 15 mg/kg/day in the juvenile study. Organ toxicities were comparable to those seen in other general toxicology studies except there was irreversible glucose elevations that lacked a dose response and some uncertainty of the toxicological significance of marked seminiferous epithelium degeneration/atrophy in the testis and oligo/aspermia in the epididymis (males) in recovery animals. Since the juvenile toxicology study contributed no new significant and solid information regarding the toxicity of midostaurin, data from this study was not included in the label.

Other Toxicity
See Summary in Appendix

12 Appendix/Attachments

Table 73 Binding Affinity of Midostaurin to Purified Kinases[22]

(Excerpted from the Pharmacology Written Summary)

Enzyme	midostaurin (PKC412) Kd /nM
FLT3 (N841I)	6
FLT3 (D835H)	6.8
KIT (D816V)	7.7
PKN1	9.3
FLT3	11
FLT3 (ITD)	11
JAK3 (JH1domain-catalytic)	12
FLT3 (D835Y)	15
MLK1	15
PKN2	15
MLK3	17
CAMK2A	20
MARK3	21
CAMK2D	36
GCN2 (Kin.Dom.2,S808G)	39
MST1	40
ARK5	41
SRPK1	42
AAK1	48
AURKB	62
SNARK	63
PLK4	66
CAMKK2	73
PRKG2	74
TNK1	83
SYK	88
JAK2 (JH1domain-catalytic)	94

Dissociation constants were determined by competition binding assays using immobilized midostaurin and purified kinase isoforms. FLT3 and KIT kinases are shown in bold

Table 74 Summary of Significant Findings in Single Dose Studies with Midostaurin

Species (Study No.)	Route & Duration	Dose mg/kg (N/dose)	Critical Doses	mg/kg	mg/m2	Significant findings
Mouse (936215)	Oral	1400 (1♀), 2000 (5/sex)	~LD_{10} NOAEL	2000 1280	6000 3840	**6,000 mg/m2**: 4/10 died, ataxia, stiff and staggered gait, piloerection, dyspnea; no gross pathology changes **3840 mg/m2:** asymptomatic
Mouse (946016)	Oral	1400 (1 ♀), 2000 (5/sex)	~LD_{10} NOAEL	2000 1400	6000 4200	**6,000 mg/m2**: 2/10 died, abnormal gait, ↓ activity, and cool body, no gross pathology changes **4200 mg/m2:** asymptomatic
Rat (Tif:RAIf) (926033)	Oral	1280 (5/sex)	Lethal (~LD_{50})	1280	7680	**7680 mg/m2**: 4/5 died, 2 on D3 and 1 on D6 and D8, respectively; signs of emaciation and depression, more obvious in females; pulmonary edema and gastric hemorrhage in animals that died. Note: ~LD_{50} was lower than 7680 mg/m2 in female rats. Batch number was 1056/1.
Rat (Tif:RAIf) (936216)	Oral	1280 (5/sex)	STD_{10}	< 1280	< 7680	**7680 mg/m2:** No mortality or clinical signs were observed Note: The results from this study were dramatically different from that of Study No. 926033. Different batch number (1058/6) for PKC412 was used in this study.

[22] Karaman MW et al, 2008, A Quantitative Analysis of Kinase Inhibitor Selectivity, Nat Biotechnol, 26(1):127-32.

Species (Study No.)	Route & Duration	Dose mg/kg (N/dose)	Critical Doses	mg/kg	mg/m2	Significant findings
Dog (926034)	Oral (capsules)	120 (1 ♀)	HNSTD	120	2400	**2400 mg/m2:** salivation, diarrhea, emesis and loose feces

Table 75 Summary of Significant Findings in Repeated Dose Studies with Midostaurin

Species (Study No.)	Route & Duration	Dose mg/kg (N/dose)	Critical Doses	mg/kg	mg/m2	Significant findings
Rat (Tif:RAIf) (926037)	Oral 3-mo. (13-wks)/ 1-mo. recovery	10, 20, 30 (10/sex/group, 5/sex/group for recovery)	STD10 NOAEL	30 10	180 60	**180 mg/m2:** no death, salivation, liver toxicity (↑ ALT, AST, ↑ relative liver weight), anemia (↓ RBC parameters), irreversible ↓ thyroid weights, hematological toxicity (LN mineralization, congestion, small thymus), GI toxicity (hyperplasia), heart toxicity (focal necrosis), lung toxicity (perivascular edema and ↑ lung weights), testis toxicity (tubular atrophy), epididymis toxicity (aspermia) **60 mg/m2:** asymptomatic
Rat (Sprague Dawley) (956016)	Oral 26-wks/ 1-mo. recovery	30, 60, 100 (25/sex/group, 10/sex/group for recovery (control, HD))	STD10 NOAEL	60-100 < 30	360-600 < 180	**600 mg/m2:** 5/30 (4♂ and 1♀) died between D19-D21, BW↓, GI toxicity (hyperplasia, epithelial vacuolation, luminal dilation, edema), liver toxicity (multifocal necrosis), pancreas toxicity (degranulation, vacuolation, edema, acinar atrophy), hematological toxicity (hypocellularity, hemorrhage, lymphoid depletion, erythrophagocytosis), thymic atrophy, uterine distension/dilation (possible abnormalities of estrous cycle), urinary bladder dilation, submaxillary gland toxicity (plasmacytosis), reproductive organ toxicity (degeneration, vacuolation, debris, ♂), heart (myocarditis, myocardial degeneration, ♀), ovaries (hyperplasia, vacuolation), adrenal toxicity (diffuse cortical hypertrophy), kidney toxicity (↓ specific gravity, hypoalbuminemia), lacrimal and salivary gland toxicity (acinar necrosis) **360 mg/m2:** 1/15 (♂) died, similar to HD except no pancreas, heart, urinary bladder, or ♂ reproductive organ toxicity **180 mg/m2:** abdominal distension, GI toxicity, liver toxicity (necrosis), renal toxicity (↓ specific gravity, hypoalbuminemia) and hematological, and ovary (follicular distension) toxicity
Rat (Tif:RAIf) (936281)	Oral 6/12 mo. (26/52-wks), 1-mo. recovery	3, 10, 30 (10/sex/group-26 wks, 20/sex/group-52 wks)	STD10 NOAEL	30 3	180 18	**180 mg/m2:** many deaths due to dosing errors; salivation, liver toxicity, hematological toxicity (↓ RBC parameters (slow to recover)) and mild marrow toxicity (reductions in cellularity), interstitial pneumonitis, bile duct proliferation **60 mg/m2:** no death; salivation, reversible liver toxicity After 6 mo, more exposure to CGP5242 epimer 1 than parent; highest [CGP62221]=8 nmol/L (BLQ) (Study No. DMPK-R1997520)
Dog Beagle (926041)	Oral 3-mo. (13-week), 1-mo. recovery	3, 10, 30 (3/sex/group, 3/sex/group for recovery (HD))	HNSTD	30	600	**600 mg/m2:** No mortality; emesis, cardiotoxicity (↓ in HR at wk 13 and during recovery, prolongation in P-Q interval, clotted blood:heart inflammation), hematologic toxicity (↓ RBC parameters (slow to recover) and WBC (including segmented neutrophils), lymphoid hyperplasia and depletion, erythrophagocytosis of the mesenteric LN, ↓thymus weight); lungs (bronchopneumonia), GI tract toxicity (hemorrhage, congestion), liver (multicellular necrosis, ↑ liver weight), urinary bladder (congestion), gland toxicity (thyroid and lacrimal atrophy, adrenal hypertrophy), reproductive organ toxicity (aspermia, ↓organ weights, ♂), kidney toxicity (pyelitis, hypoalbumemia) **60 mg/m2:** Hematological toxicity (↓thymus weight, ↓ RBC parameters (slow to recover) erythrophagocytosis of the

						mesenteric LN), kidney toxicity (pyelitis), male reproductive organ toxicity (aspermia, ↓organ weights)
Dog Beagle (946003)	Oral 6/12 mo. (26/52-week), 1-mo. recovery	1, 3, 10 4/sex/group (12 mo); 2/sex/group (6 mo); 2/sex/group (recovery)	HNSTD NOAEL	10 1	200 20	**200 mg/m2:** No mortality, cardiotoxicity (prolonged PQ interval), kidney toxicity (↓ total protein, hypoalbuminemia, ↓ urea, electrolyte imbalances), hematological toxicity (anemia, ↓ segmented neutrophils, ↓ lymphocytes, thymus atrophy, spleen congestion and hyperplasia, bone marrow hypercellularity, erythrophagocytosis of the mesenteric LN), liver toxicity (↑ AST, ↑liver weight, tension lipidosis), lung toxicity (bronchopneumonia), adrenal toxicity (vacuolation), pituitary cysts, reduced spermatogenesis, and inflammatory foci in the brain **60 mg/m2:** Similar toxicities to HD, except no liver toxicity, no LN erythrophagocytosis, no pituitary or adrenal findings, no male reproductive toxicity **30 mg/m2:** ↓ urea, electrolyte imbalances, lung toxicity (bronchopneumonia), ↓ thymus weights/atrophy, bone marrow hypercellularity (findings were generally reversible)

Table 76 Tabulated Summary of Reproductive and Developmental Toxicology Studies

Fertility – Males and Females	
Study #	PCS-964123
Title	An Oral Study for Effects on Fertility and Early Embryonic Development in Rats
Methods	♂: dosed 70 days prior to mating, during the 2 wk mating period and for 11 to 15 days thereafter; ♀: dosed 14 days prior to mating, during mating and until presumed GD6.
Formulation	(b) (4) ((b) (4) in purified water)
Key Study Findings	• HD♂: reduced sperm count and motility and a decrease in reproductive organ weights. Reversibility not determined. This was also a lethal dose (6/24 found dead or euthanized). • HD♀: increased resorptions (including total resorption of litter), decreased pregnancy rate, and decreased number of implants and live embryos. • All doses ♂: testicular degeneration and atrophy
Species	Rat/ Sprague-Dawley (Crl:COBS CD[SD]BR)
Doses	0, 10, 30, or 60 mg/kg/day (0, 60, 180, 360 mg/m2/day)
AUC*	0, 1.8, 9.05, 14.04 µmol.hr/L
Animal:Human Safety Margins	0, 0.01, 0.05, 0.08
Mortality/ Clinical Signs	• Six males at 60 mg/kg/day were found dead or euthanized between D29 and D60 during the study. • Clinical signs: weight loss, stained forelimbs and mouth, stool changes, unthriftiness, salivation after dosing, emaciation, lethargy, distended abdomen/stomach.
BW/FC	MD and HD♂: body weight loss HD♀: reduced body weight gain and gravid uterine

	weights
Embryonic Fetal Development	
Rodent	
Study #	PCS-936241
Title	CGP41251 Segment II (Teratology) Study in Rats by Oral Administration
Methods	Females dosed daily from GD 6-17 with a uterine examination on GD20
Formulation	(b) (4) ((b) (4) in purified water)
Key Study Findings	• All effects on embryos and fetuses occurred in the absence of maternal toxicity. • Late embryofetal death (↑ late resorptions); reduced fetal weight, dilated brain ventricles, severe renal pelvic cavitation, reduced skeletal ossifications, and widened anterior fontanelle in fetuses were observed mainly at 30 mg/kg/day.
Species	Rat/Hsd/Ola: Sprague-Dawley
Doses	0, 3, 10, and 30 mg/kg/day (0, 18, 60, 180 mg/m2/day)
AUC**	0, 0.423, 2.347, 9.283 µmol.hr/L
Animal:Human Safety Margins	0, 0.003, 0.014, 0.05
Mortality/ Clinical Signs	None in dams
BW/FC	Unremarkable in dams
Nonrodent	
Study #	PCS-936243
Title	CGP41251 Segment II (Teratology) Study in Rabbits by Oral Administration
Methods	Females dosed daily from GD7 to 20 with a uterine examination on GD29
Formulation	(b) (4) ((b) (4) in purified water)
Key Study Findings	• Midostaurin was maternally toxic (↓BW gain and food consumption, ↑spontaneous abortions) at all doses (≥ 2 mg/kg/day). • Fetotoxicity (↓fetal weight, with reduced gall bladder size and reduced skeletal ossifications) was observed at doses ≥ 10 mg/kg/day
Species	New Zealand White Rabbits
Doses	0, 2, 10, and 20 mg/kg/day (0, 24, 120, 240 mg/m2/day)
AUC[†]	0, not measured, 1.581, 2.422 µmol.hr/L
Animal:Human Safety Margins	0, presumed less than human AUC, 0.009, and 0.015
Mortality/ Clinical Signs	• 2/20 control, 2/20 LD, and 2/20 HD dams were found dead or euthanized between GD15 and GD29. Clinical signs: weight loss, reduced food and water intake, distended gall bladder, dosing errors • 1/20 LD, 2/20 MD, and 4/20 HD dams aborted with body weight loss and ↓food consumption and distended gall

	bladder • MD and HD dams: hypoactivity, body weight loss, slightly pale kidneys, distended vulva with gelatinous fluid, clear fluid in the pericardium, and hairloss with reddened areas (fur balls in stomach and a distended and gaseous intestines at HD only)
BW/FC	MD and HD: body weight loss All doses: ↓body weight gain and ↓food consumption
Pre- and Postnatal Development	
Study #	PCS-770270
Title	PKC412: An Oral (Gavage) Pre and Postnatal Study in the Rat
Methods	Dams dosed from GD6 to PND 21, 22, or 23
Formulation	(b) (4)
Key Study Findings	• Midostaurin was maternally toxic with adverse effects upon maternal performance at 30 mg/kg/day. • Midostaurin was fetotoxic with effects of reduced body weight (irreversible), accelerated complete eye opening and delayed auricular startle ontogeny, and impaired fertility in male (↓fertility index) and female offspring (↓number of implantation sites and live fetuses/litter).
Species	Sprague-Dawley (Crl:CD[SD])
Doses	0, 5, 15, and 30 mg/kg/day (0, 30, 90, 180 mg/m2/day)
AUC	0, 5 and 15 mg/kg AUCs not measured, 30 mg/kg/day = 9.05 µmol.hr/L based on 26-week rat toxicity study
Animal:Human Safety Margins	0, presumed to be less than human AUC, 0.05
Mortality/ Clinical Signs	Dams: • 4 HD♀ were euthanized on PND0 to 1: dystocia and ↓ in the number of live pups, dams had additional fetuses in utero, littering >7 hours, clinical signs of skin pallor, pale eyeballs • 1 control and 1 HD♀ cannibalized all pups in the litter following parturition. • Salivation, thin fur cover, red staining and firm abdomen/distension at all doses. Liquid red vaginal discharge and pale foci in lungs in HD♀. Pups: • A low incidence of pups had labored breathing, domed skull, shrunken eyeball, swollen soft abdomen, soft cranium/bruise, skin pallor. • At necropsy a low incidence of pups had malpositioned testis, short tail, dilated brain ventricles, fluid in cranial cavity, dark kidney, spongy, uncollapsed, dark lung,

	dark LN, small eye. • HD♂: Longer water maze times with more errors, but large standard deviation
BW/FC	F0 dams HD: ↓body weight gain F1 offspring HD: ↓body weight
Juvenile Toxicology	
Study #	870600
Title	An Oral (Gavage) Toxicity Study in the Juvenile Albino Rat
Methods	Dosed from PND7 to 70
Formulation	(b) (4)
Key Study Findings	• Mainly at 15 mg/kg/day, decreases in BW and absolute organ weights with reversible microscopic findings in the lungs, pancreas, heart, kidney, and possibly male reproductive organs. • No effects on fertility on maternal reproductive performance, but not expected since doses/exposures low. • Overall, comparable toxicities to other rat studies.
Species	Sprague Dawley rat (Crl:CD[SD])
Doses	0, 2, 5, and 15 mg/kg/day (0, 12, 30, and 90 mg/m2/day)
AUC	PND7: 1.65, 3.83, 14.55 µmol.hr/L ~PND70: 2.82 (estimated, BLQ), 0.611, and 2.34 µmol.hr/L
Mortality/ Clinical Signs	• 2 control and 3 HD animals had premature death. Clinical signs for 4/5 suggest gavage error (1♂, 3♀), 1♀ death at PND95 was due to severe urinary tract disease. • HD♂+♀:fur staining/skin brown
BW/FC	↓ BW without major effects on food consumption

* The 10 mg/kg/day steady state mean (♂+♀) AUC was based on a (b) (4) formulation from the 6/12 month rat study (Study No. DMPK-R1995083) and the mean (♂+♀) AUCs for the 30 and 60 mg/kg/day doses were based on the (b) (4) formulation from the 26-week rat toxicity study (Study No. 956016); **Steady state female AUC data for all doses was based on the (b) (4) formulation from the 6/12 month rat study (Study No. DMPK-R1995083); † Day 20 AUC(0-24hr) values for the 10 and 20 mg/kg/day doses were taken from the rabbit EFD dose finding study (Study No. PCS-R936242).

Table 77 TK Parameters from the 6/12 Month Rat Study to Support Labeling

(Excerpted from Study No. DMPK-R1995083)

		Day 1			Steady-state		
Rat Sex	Dose [mg/kg]	C_{max} [nmol/L]	t_{max}* [h]	AUC(0-24h) [h·nmol/L]	C_{max} [nmol/L]	t_{max}* [h]	AUC(0-24h) [h·nmol/L]
M	3	85	2	374	73	1	385
M	10	240	2	2628	147	2	1250
M	30	397	1	3251	385	3	4011
F	3	83	2	483	116	1	423
F	10	229	1	1912	246	2	2347
F	30	409	1	5831	778	6	9283

* median

List of Studies Not Reviewed (from Section 3.2):

Primary Pharmacology

Study#	Title	Module
PKF-98-02525	CGP41251 Inhibition of Anti-IgE Induced Histamine, LTC4/D4 and PGE2 Release from Human Basophils (Biology Report 42/92)	4.2.1.1.
RD-2015-00401	Anti-Proliferative Activity of Separated Epimers of CGP52421	4.2.1.1.

Pharmacokinetics

Study #	Title	Module
Analytical Methods and Validation Reports		
DMPK R0300287-01	[13C6]CGP62221 Synthesis and Release Analysis	4.2.2.1.
DMPK R0300287-02	[13C6]PKC412 Synthesis and Release Analysis	4.2.2.1.
DMPK R0300287-03	[13C6]CGP52421 Synthesis and Release Analysis	4.2.2.1.
DMPK R0800179	[14C2]PKC412 Synthesis and Release Analysis	4.2.2.1.
DMPK R0900478	Quantitative Determination of PKC412 (Midostaurin) in Rat Plasma by LC-MS/MS. Method Description and Validation.	4.2.2.1.
DMPK R0900478-01	Quantitative Determination of PKC412 (Midostaurin) in Rat Plasma by LC-MS/MS. Method Description and Validation Amendment No. 1.	4.2.2.1.
DMPK R1200031	[14C]PKC412 Synthesis and Release Analysis	4.2.2.1.
DMPK R1300460	[14C]CGP52421 Synthesis and Release Analysis	4.2.2.1.
DMPK R1300461	[3H]CGP62221 Synthesis and Release Analysis	4.2.2.1.
DMPK-R1996-107	Validation of an Analytical HPLC Method for the Determination of CGP41251 in Animal Plasma	4.2.2.1.
Absorption		
DMPK-R0900185	Absorption, Metabolism and Excretion of [14C]PKC412 following Oral (3 mg/kg) and Intravenous (0.5 mg/kg) Dosing in Dogs	4.2.2.2.
Metabolism		
DMPK-R0201774	In vitro Metabolism of [14C]PKC412 in Rat, Dog Monkey, Human Liver Slices and Human Hepatocytes	4.2.2.4.
DMPK-R1500239	Exposure of PKC412 and its Metabolites at Steady State; Summary of Data from Preclinical Species	4.2.2.4.
DMPK-1997461	Semiquantitative Determination of CGP41251 and Three Metabolites in Plasma of Cynomolgus Monkeys of a 3 Months Intravenous Toxicity Study	4.2.2.4.
DMPK-1997462	Semiquantitative Determination of CGP41251 and Three Metabolites in Plasma of Rabbits (Chinchilla) after 1 and 14 Days of Peroral Treatment with CGP41251	4.2.2.4.

Single Dose Toxicology

Study#	Title	Module
PCS-R946041	CGP41251: Acute Intravenous Toxicity Study in Dogs	4.2.3.1.
PCS-R946081	CGP41251: Acute Intravenous Toxicity Study in Mice	4.2.3.1.
PCS-946040	CGP41251: Acute Intravenous Toxicity Study in Rats	4.2.3.1.

Repeat Dose Toxicology

Study#	Title	Module
DMPK-R1996148	Plasma Concentrations of CGP41251 in a Pilot 14-Day Intravenous Toxicity Study in Dogs	4.2.3.2.

Reference ID: 4051584

PCS-R946053	Pilot 14-Day Intravenous Toxicity Study in Dogs	4.2.3.2.
DMPK-R1994021	Plasma Concentrations of CGP41251 on Days 1 and 91 of a 3-Month Oral Toxicity Study in Dogs	4.2.3.2.
PCS-R926039	Pilot 14-Day Oral Range-Finding Study in Dogs	4.2.3.2.
PCS-R936198	3-Month Oral Toxicity Study in Male Dogs	4.2.3.2.
PCS-R1996074	Plasma Concentrations of CGP41251 in a 10-day Oral Dose Range Finding Study in Dogs	4.2.3.2.
PCS-R916023	Pilot 10-Day Oral Toxicity Study in Dogs	4.2.3.2.
PCS-R981021	4-Week Oral Dose Range-Finding Study in Mice	4.2.3.2.
DMPK-R1995084	Plasma Concentrations of CGP41251 in an Intravenous Dose Range Finding Study in Cynomolgus Monkeys	4.2.3.2.
DMPK-R956014	PKC412-3 Month Intravenous Toxicity Study in Cynomolgus Monkeys Toxicokinetics of PKC412 in Monkey Plasma	4.2.3.2.
PCS-R956013	Intravenous Dose Range Finding Study in Cynomolgus Monkeys	4.2.3.2.
PCS-956014	3 Month Intravenous Toxicity Study in Cynomolgus Monkeys	4.2.3.2.
PCS-R966090	Pilot 2-Week Oral toxicity Study in Rabbits	4.2.3.2.
DMPK-R956015	13 Weeks Intravenous Toxicity Study in Rats Toxicokinetics of PKC412 in Rat Plasma	4.2.3.2.
PCS-R1996146	Plasma Concentrations of CGP41251 in a Pilot 14-Day Intravenous Toxicity Study in Rats	4.2.3.2.
PCS-946052	Pilot 14-Day Intravenous Toxicity Study in Rats	4.2.3.2.
PCS-R956015	13-Week Intravenous Toxicity Study in Rats	4.2.3.2.
PCS-R906263	10-Day Oral Dose Range Finding Study in Rats	4.2.3.2.
PCS-R926036	14-Day Oral Toxicity Study in Rats	4.2.3.2.

Reproductive Toxicology

Study#	Title	Module
DMPK-R936242	Plasma Concentrations of CGP41251 in a Segment II Dose Range Finding Study in Rabbits	4.2.3.5.2.
PCS-R936240	Segment II Dose Range Finding Study in Rats by Oral Administration	4.2.3.5.2.
PCS-R936242	Segment II (Teratology) Dose Range Finding Study in Rabbits by Oral Administration	4.2.3.5.2.
PCS-R0870599	PKC412: An Oral (Gavage) Range-Finding Toxicity Study in the Juvenile Albino Rat	4.2.3.5.4.

Local tolerance:

Study#	Title	Module
PCS-R0317010-01	Assessment of Contact Allergenic Potential with the Murine Local Lymph Node Assay (LLNA tier I)	4.2.3.6.
PCS-R0317017	Assessment of Contact Allergenic Potential with the Murine Local Lymph Node Assay (LLNA tier II)	4.2.3.6.

Other Toxicity Studies:

Study#	Title	Module
	Antigenicity	
PCS-R3041	Antigenicity Study of the CGP41251 Formulation in Guinea Pigs	4.2.3.7.1.
	Metabolites	
PCS-LR001647	CGP52421: Ames Test (non-GLP, no raw data)	4.2.3.7.5.
PCS-LR001648	GGP62221: Ames Test (non-GLP, no raw data)	4.2.3.7.5.

Reference ID: 4051584

Other		
PCS-0310044	PKC412 (Daunorubicin, Ara-C): 5-Week Combination Toxicity Study in Rats	4.2.3.7.7.
PCS-R0310045	PKC412 (Daunorubicin, Ara-C): 5-Day Dose-Range Finding Combination Study in Rats	4.2.3.7.7.
PCS-0970338	Local Intravenous Tolerability Study in Rabbits	4.2.3.7.7.
PCS-1570160	In Vitro Assessment of the Ocular Irritation Potential of PKC412 Using the Bovine Corneal Opacity and Permeability Assay	4.2.3.7.7.
PCS-R1588802	PKC412 (b) (4) t: 4-Week Local Ocular Irritation and Systemic Toxicity Study of PKC412 (b) (4) by Instillation into the Conjunctival Sac of Albino Rabbits	4.2.3.7.7.
PCS-R15889102	PKC412 (b) (4): 4-Week Local Ocular Irritation and Systemic Toxicity Study of PKC412 (b) (4) by Instillation into the Conjunctival Sac of Beagle Dogs	4.2.3.7.7.
PCS-R16025-02	PKC412 (b) (4): 3-Week Ocular Irritation and Histopathology Study of PKC412 (b) (4) by Single Subconjunctival and Retrobulbar Injection into the Eye of Albino Rabbits	4.2.3.7.7.
936267	Skin Phototoxicity Study in Hairless Mice	4.2.3.7.7.
PCS-R946042	Local Intravenous Irritation Study in Rabbits	4.2.3.7.7.
PCS-R956134	5-Day Local Intravenous Tolerability Study in Rabbits	4.2.3.7.7.

Summary of Other Toxicity Studies:

A 4 week oral toxicity study in Sprague Dawley rats was conducted to qualify the degradation product (b) (4) in a (b) (4) formulation.

Local tolerance and many other toxicity studies including studies on antigenicity, combination studies, local intravenous tolerability studies, ocular toxicity studies, and phototoxicity studies were not fully reviewed but are summarized below.

Local tolerance studies in female mice identified midostaurin as a weak lymph node activator in its ability to cause hyperplasia and increase lymph node weights and a weak skin irritant, but not a skin sensitizer. In an antigenicity study in male Guinea pigs, no antibodies against midostaurin were detected and there were no signs of anaphylaxis due to an antigen-antibody reaction. In a skin phototoxicity study in male and female hairless mice, mild skin reactions were noted after UV-A irradiation at the 300 mg/kg (900 mg/m2) dose level. Treatment with oral midostaurin in combination with daunorubicin (IV) and Ara-C (IV) resulted in slightly more pronounced effects on body weight, food consumption, and hematology parameters in rats when compared to treatment with midostaurin alone. Local tolerability IV studies in rabbits indicate a mild potential for inflammation, thrombosis, or hemorrhage at the injection site.

Midostaurin was categorized as a non-irritant at concentrations ≤ 2 mg/mL in an ocular irritation assay in bovine cornea, although the highest concentration of midostaurin tested, 200 mg/mL, could not be categorized. In vivo results of ocular irritation studies in Beagle dogs indicated that midostaurin (b) (4) was irritating in concentrations as low as 50 µg /eye. Additionally, a number of in-vivo ocular irritation studies in rabbits and dogs were conducted (b) (4)

(b) (4). The low levels of midostaurin residues ((b) (4) µg/capsule) on capsules would not be expected to pose a high risk of ocular irritation to handlers of midostaurin during clinical use, since positive findings were observed with a different formulation than intended clinically.

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/s/

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02/03/2017

CHRISTOPHER M SHETH
02/04/2017