CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202429Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Vemurafenib (Zelboraf)

Date: July 27, 2011
To: File for NDA 202429
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology/Toxicology
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review and labeling provided by Drs. McGuinn and Aziz and the memorandum provided by Dr. Helms. I concur with their conclusions that Zelboraf may be approved for the proposed indication and that no additional nonclinical studies are needed.

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/s/

JOHN K LEIGHTON
07/28/2011

MEMORANDUM

Date: July 27, 2011
From: Whitney S. Helms, Ph.D.
Acting Pharmacology Team Leader
Division of Drug Oncology Products
To: File for NDA #202,429
Vemurafenib (ZELBORAF)
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies examining the pharmacology and toxicology of vemurafenib provided to support NDA 202,429 for the treatement of patients with V^{600E} mutation positive metastatic melanoma were reviewed in detail by W. David McGuinn, Ph.D., D.A.B.T., and Robeena M. Aziz, MPH, Ph.D. The submission included studies of orally administrated vemurafenib in rats, dogs, mice, and rabbits that investigated the drug's pharmacology, pharmacokinetics, safety pharmacology, general toxicology, genetic toxicity (*in vivo* and *in vitro*), and reproductive toxicity. The reviewed studies consist primarily of original research conducted by the sponsor but also include some studies previously submitted for publication in peer reviewed journals. The pivotal studies reviewed were performed with the microprecipitated bulk powder that was introduced during the course of development due to solubility issues with an earlier formulation.

The pharmacology studies submitted to this NDA demonostrate that vemurafenib is a kinase inhibitor. Like other approved kinase inhibitors vemurafenib targets a number of unique enzymes at clinically relevant concentrations, but it has highest activity against activating mutations of the BRAF protein, particularly mutations at the V600 amino acid site, predominantly V600E. Vemurafenib was able to inhibit the growth of tumors derived from various cell types expressing the $BFAF^{V600E}$ mutation in a series of xenograft experiments conducted in athymic mice. Treatment of these mice with the drug also led to decreased phosphorylation of MEK and ERK in the tumors, further demonstrating the pharmacologic activity of vemurafenib. Though vemurafenib does not selectively target $BRAF^{V600E}$, this mutation was highlighted in the mechanism of action section of the label and the inhibition of wild type BRAF was de-emphasized. Several factors influenced this decision. While the concentrations at which there was clear *in vitro* inhibition of kinases other than $BRAF^{V600E}$, including wild type BRAF, were achieved clinically, there is data suggesting that the downstream signaling effects of vemurafenib mediated inhibition of the wild type protein may differ from the effects of inhibition of the mutant. The bulk of data to support this finding were not submitted as study reports to the NDA and were not reviewed in detail. For this reason, and to enhance clarity regarding patient safety, the review team agreed that the mechanism of action statement in the vemurafenib label should focus on the *in vitro* and *in vivo* effects of the drug on the $BRAF^{V600E}$ mutation, which were the most thoroughly characterized effects of the drug. If in the future, use of vemurafenib in other patient populations is explored, further pharmacology studies may need to be submitted in order to understand the activity of the drug in these populations.

Clinically, treatment with vemurafenib has been associated with the development of cutaneous squamous cell carcinomas. The sponsor has made an effort to investigate the pharmacologic basis for the development of these secondary tumors. Experiments in which nude mice implanted with SCC cells were administered vemurafenib showed a dose-dependent increase in tumor growth. No clear mechanism was determined for the increases in tumor growth, though other experiments suggested that compensatory upregulation of CRAF expression and MEK phosphorylation may play a role.

The major target organ for toxicity in both rats and dogs appeared to be the liver. In both species there was evidence of a dose-dependent increase in cholesterol accompanied, in the dog, by significant increases in liver enzymes. Liver function abnormalities have also been reported in the clinical trials. In dogs significant elevations in eosinophils, neutrophils, and monocytes were observed at the high dose in both sexes during the dosing period. Moribund dogs also had clinical observations of reddened skin. These findings are suggestive of the clinically observed immune-mediated toxicity. In addition one high dose male dog developed a wart-like growth on the front left paw which was not fully examined. This growth may have been similar to the cutaneous skin cell carcinomas observed clinically. Finally, in cardiovascular safety pharmacology studies both *in vivo* and *in vitro* there were indications of a potential cardiovascular risk. In the long term dog study prolongation of QTc was observed that was similar to what has been observed clinically. Genotoxicity tests were negative both *in vivo* and *in vitro*. Carcinogenicity studies were not required to support the marketing application and, with positive findings of carcinogenicity in humans, carcinogenicity studies are neither planned nor expected as a post-marketing requirement.

The reproductive toxicity studies for vemurafenib were negative for embryo-fetal toxicity in either rats or rabbits. The high doses used in the pivotal studies resulted in exposures approximately equal to the exposures seen clinically; higher doses to fully explore toxicity were not explored as maximum feasible doses were reached. At these doses there was evidence of mild maternal toxicity in both rats and rabbits as evidenced by decreased maternal body weight and food consumption. Based on these studies the sponsor requested a Pregnancy Category (b) (4) designation. Despite the lack of clear findings in the embryo-fetal development studies, the review team felt that Pregnancy Category (b) (4) was inappropriate for several reasons. The sponsor did not perform fertility or post-natal development studies. These studies are not required for a drug with a proposed use of treating patients with advanced cancer, however, the designation of Pregnancy Category (b) (4) is predicated on the assumption that a full battery of non-clinical reproductive toxicity tests were performed. The embryo-fetal development tests that were performed did not exceed the clinical exposure and, as such may not fully predict toxicity. Finally, alterations in the BRAF signaling cascade have been associated with serious embryo-fetal toxicity including embryolethality, severe growth retardation, and post-natal death. In addition, congenital mutations of *BRAF* in humans have been associated with serious genetic disorders. With these data in mind, based on its mechanism of action, Pregnancy Category D is recommended for vemurafenib.

A single impurity was identified during the course of the review as being above the level for qualification. The specification for this impurity is (b) (4)% and it was qualified in the 13-week dog study. Other impurities discussed with the chemistry review team did not exceed the 0.15%

level described in the ICH Q3A(R2) guidance and, therefore, did not require qualification in animal studies.

Recommendations: I concur with the conclusion of Drs. McGuinn and Aziz that the pharmacology and toxicology data support the approval of NDA 202,429 for ZELBORAF. There are no outstanding nonclinical issues related to the approval of ZELBORAF for the proposed indication in the $BRAF^{V600E}$ positive patient population.

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/s/

WHITNEY S HELMS
07/27/2011

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

PHARMACOLOGY and TOXICOLOGY NDA REVIEW and EVALUATION

Application number	202429
Supporting document	000
Applicant's letter date	February 14, 2011
Product	ZELBORAF (Vemurafenib)
Indication	Unresectable Stage IIIc or Stage IV BRAF Mutation Positive Melanoma by the cobas® 4800 BRAF V600 Mutation Test
Applicant	Hoffmann-La Roche Inc.
Review Division	Division of Drug Oncology Products (HFD-150)
Reviewers	W. David McGuinn, Jr., M.S., Ph.D., D.A.B.T.
	Robeena M. Aziz, M.P.H, Ph.D.
Acting Supervisor/Team Leader	Whitney S. Helms, Ph.D.
Division Director	Robert L. Justice, M.S., M.D.
Project Manager	Theresa Ferrara

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1 Executive Summary

1.1 Introduction

Vemurafenib is a small organic molecule that the sponsor selected for its ability to inhibit the enzymatic activity of a mutant variant of the cytosolic serine/threonine-protein kinase, BRaf. BRaf is normally inactive until it is phosphorylated by upstream kinases; this phosphorylation activates BRaf such that it phosphorylates other downstream kinases that stimulate cell growth and division. One mutated form of BRaf is $BRaf^{V600E}$. This mutant occurs frequently in malignant melanoma and results in a constitutively active protein that is able to constantly send downstream signals and promote cell division. The sponsor postulates that inhibition of this kinase activity will delay disease progression and increase survival of patients with tumors expressing the mutated protein. For the marketing application the sponsor has submitted pharmacology and toxicology studies to support the use of ZELBORAF in patients with unresectable or metastatic melanoma with the $BRaf^{V600E}$ mutation.

1.2 Brief Discussion of Nonclinical Findings

In vitro studies show that vemurafenib binds to the active site of BRaf and numerous mutants of that enzyme at nanomolar concentrations. It is not specific for BRaf as it inhibits numerous other kinases at sub-micromolar concentrations as well. In *in vitro* whole cell assays vemurafenib inhibited the growth of cells expressing the V600E mutation at sub-micromolar concentrations. In addition, vemurafenib shows *in vivo* activity in xenograft studies in mice bearing various tumors with the V600E mutation. Despite the sub-nanomolar concentrations required for *in vitro* kinase inhibition, the concentrations that must be achieved to attain the *in vivo* activity are relatively high with C_{max} usually being greater than 100 µM. The discrepancy is explained by the fact that in humans vemurafenib is 99.5% bound to serum proteins.

Vemurafenib caused no signs of neurotoxicity in the standard Functional Observational Battery. Tests with isolated canine cardiac Purkinje fibers were inconclusive but showed a decrease in V_{max} of about 50% suggesting the possibility of inhibition of conduction. This correlated with an increase in AV block and QT prolongation seen in dogs. QT prolongation occurs clinically.

Rats clear vemurafenib much more rapidly than humans. Studies using the maximum feasible dose cause little toxicity despite achieving exposures comparable to or higher than those that cause toxicity clinically.

Dogs show signs of toxicity at exposures significantly less than those seen clinically. Doses of 450 mg/kg/day BID (18000 mg/m^2/day) caused unacceptable toxicity and mortality by Day 8. Prior to Day 8 the dogs showed clinical signs of excessive salivation, vomiting, dehydration, soft or malformed feces and hypoactivity. Hypoactivity correlates with the fatigue seen clinically. Vomiting also occurs clinically. These two toxicities were dose limiting in Phase I clinical studies. In a 13-week study, a lower dose of 300 mg/kg/day caused three deaths by Day 31. No dogs died after that time, an observation that correlated with a steady decrease in exposure. This decrease was probably because of induction of metabolism. Dogs that survived had increases in ALT, GGT, amylase and ALP suggesting damage to the liver and pancreas. Hepatic toxicity occurs clinically. Cholesterol and triglycerides were increased. Cholesterol biosynthesis

is controlled by a kinase that might be directly affected by the vemurafenib. Treatment also caused a dose-dependent neutrophilia with profound eosinophilia in a pattern that resembled that of an allergic response. There were numerous organ weight changes not accounted for by weight loss, the most troubling being a small decrease in relative heart weight. Thymus weight increased in the mid dose males but decreased in females, the pattern was just the opposite in HD animals suggesting some unusual derangement of thymus function. Adrenal and liver weight decreased. Changes seen microscopically included perivascular infiltrates in the liver, atrophy in the thymus. Most of these toxicities showed signs of resolution after a drug free recovery period.

Vemurafenib did not cause fetal damage in reproductive toxicity studies done during organogenesis at doses that caused only minimal or no toxicity to the dams. Nevertheless, in BRaf knockout mice the placenta fails to form properly. In addition, mutations in *BRAF* have been associated with congenital disorders in humans. Therefore, vemurafenib is labeled Pregnancy Category D.

Vemurafenib did not cause an increase in reverse mutations in the Ames assay. Neither did it cause chromosomal aberrations in an *in vitro* test using fresh human peripheral blood lymphocytes. *In vivo*, vemurafenib did not cause chromosomal damage in the rat bone marrow erythrocytes micronucleus test. Nevertheless, clinically vemurafenib causes the development and growth of cutaneous squamous cell carcinomas and there is some indication that it may promote the development of new primary melanomas. Nonclinical carcinogenicity were not submitted or required for approval in a patient population with advanced cancer. Pharmacology studies investigating mechanisms for the cutaneous squamous cell cancer promotion seen clinically were performed. Several potential mechanisms for tumor promotion were suggested by these studies but a clear mechanism of action was not determined.

Vemurafenib absorbs UV-A and UV-B light. It was phototoxic to mouse fibroblasts *in vitro* but the concentrations reached in an *in vivo* study in rats were too low to demonstrate this toxicity which is seen clinically.

1.3 Recommendations

1.3.1 Approvability

There are no non-clinical findings of unacceptable toxicity that would prevent the approval of vemurafenib for this indication.

1.3.2 Additional Non Clinical Recommendations

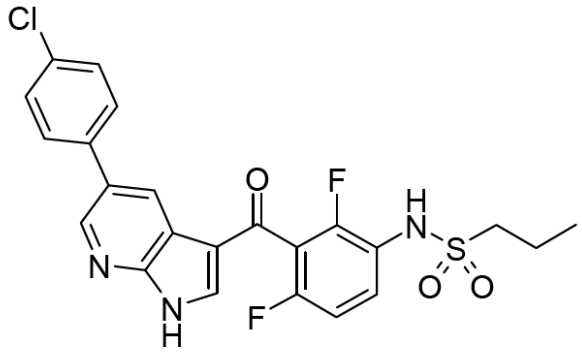
None

1.3.3 Labeling

Labeling will be addressed in a separate document.

2 Drug Information

2.1 Drug

CAS Registry Number	918504-65-1
Generic Name	Vemurafenib
Code Name	R05185426, PLX4032, RG7204
Chemical Name	propane-1-sulfonic acid {3-[5-(4-chlorophenyl)-1H pyrrolo [2,3-b]pyridine-3-carbonyl]-2,4-difluoro-phenyl}-amide
Molecular Formula	$C_{23}H_{18}ClF_2N_3O_3S$
Molecular Weight	489.93 g/mole
Structure	
Pharmacological Class	Kinase inhibitor
Relevant IND	IND 73620 originally submitted by Plexxikon Inc.

2.3 Drug Formulation

Film coated tablet, 240 mg
(Excerpted from the submission)

Table 1: Composition of RO5185426 Film-Coated Tablets 240 mg

Components[1]	Quality	Function	Actual Weight (mg/tablet)
Tablet core			
RO5185426-000	in house monograph	Drug substance	240.000
Hypromellose acetate succinate	NF		(b) (4)
(b) (4) (Colloidal silicon dioxide)	Ph. Eur., NF		
Croscarmellose sodium	Ph. Eur., NF		
(b) (4) (Hydroxypropyl cellulose)	Ph. Eur., NF		
Magnesium stearate	Ph. Eur., NF		
Mass of tablet core			
Film-coating mixture[2]			
Poly(vinyl alcohol)	Ph. Eur., USP		
Titanium dioxide	Ph. Eur., USP		
(b) (4)			
(b) (4) (Polyethylene glycol 3350)	Ph. Eur., NF		
Talc	Ph. Eur., USP		
Iron oxide red (b) (4)	2008/128/EC, NF, 21 CFR		
Mass of film-coating mixture			
		Total tablet mass	*870.000*

Chemical properties:

RO5185426-000 is a white to off white, crystalline, non-hygroscopic powder (b) (4)

The compound is achiral.

2.4 Comments on Novel Excipients

In initial clinical studies, the low aqueous solubility and evidently poor bioavailability resulted in lower and more variable exposure after oral administration than expected. The actual oral bioavailability has not been determined, (b) (4)

[1] Previously disclosed in US Patent 6,350,786 issued to Roche on February 26, 2002.

(b) (4)

In many of the studies reviewed below the investigators identified a lot number but not a recognizable batch number that we could use to identify the compound used in the experiment in such a way as to coordinate the information with CMC. CMC requested a comprehensive identification of the Batches used in the non-clinical experiments. The sponsor supplied the following two tables. The formulation used in the studies listed in Table 2 was the MBP formulation; that used in the studies listed in Table 3 was the initial crystalline formulation.

Table 2: Toxicity studies performed with amorphous MBP

RO5185426-006, RO5185426-007, and RO5185426-004

Study No.	RDR	Type of study	DP Lot No. (MBP with (b) (4)	MBP Lot No.	API Lot No.
10263	1026332	Micronucleus test (MNT)	91389	38668-208-A	X537RWFP-07-001M
			91390	38668-208-A	X537RWFP-07-001M
			91391	38668-208-A	X537RWFP-07-001M
10165	1025760	26-Week repeat-dose toxicity, rat	97617	BS0711SA12-M	X537RWFP-07-001M
10164	1025759	13-Week repeat-dose toxicity qd dosing, dog	97617	BS0711SA12-M	X537RWFP-07-001M
11260	1032862	13-Week repeat-dose toxicity bid dosing, dog	120785	BS0906SH10	BS0805SA04
			120787	BS0906SH10	BS0805SA04
11025	1033163	39-Week repeat-dose toxicity, dog	120785	BS0906SH10	BS0805SA04
			120785	BS0906SH10	BS0805SA04
			120787	BS0906SH10	BS0805SA04
			120788	BS0906SH11	BS0805SA04
			120789	BS0906SH11 BS0906SH12	BS0805SA04 BS0805SA05
			120790	BS0906SH12	BS0805SA04 BS0805SA05

Table 3: Toxicity studies performed with crystalline API RO5185426-000

Study No.	RDR	Type of study	API Lot No.
(b) (4) 578004	1040820	4-Week repeat-dose toxicity, rat	02PLE04B-01-79
578003	1040819	4-Week repeat-dose toxicity, dog	02PLE04B-01-79
578008	1040807	Other toxicity studies, hERG	02PLE04B-01-79
578011	1040810	Other toxicity studies, Purkinje Fiber	02PLE04B-01-79
AB29FU.503 (b) (4)	1040822	Genotoxicity, Ames	02PLE04B-01-79
AB29FU.341	1040821	Genotoxicity, Chromosome Aberration Test	02PLE04B-01-79

2.5 Comments on Impurities or Degradants of Concern

The CMC reviewer, Dr. Anne Marie Russell, requested our comments on the qualification of four impurities. The sponsor specified three of those impurities ((b) (4)) within the ICH limit of 0.15% or less so no qualification is needed. The fourth ((b) (4)) is qualified at a concentration of (b) (4) % or less by the 13 week toxicology study in dogs (study 1032862 reviewed below). In this study, dogs in the low dose group received 5.1 mg/m^2/day of the impurity; the human exposure would be 3.2 mg/m^2/day at the specification limit.

2.6 Proposed Clinical Population and Dosing Regimen

Indication	Unresectable Stage IIIc or Stage IV BRAF Mutation Positive Melanoma by the cobas® 4800 BRAF V600 Mutation Test
Route:	Oral
Clinical Dose:	960 mg (533 mg/m^2/dose, 1066 mg/m^2/d)
Clinical Schedule:	Four pills taken twice daily

Plexxikon discovered vemurafenib by screening compounds for their ability to inhibit the kinase activity of a particular mutated form of the serine/threonine-protein kinase BRAF. When the gene, *BRAF,* which encodes the protein BRAF has a mutation resulting in a valine to glutamic acid substitution at residue 600, the resulting protein is designated BRAF^{V600E}. Other amino acid residues can substitute for valine, such as arginine (V600R), aspartic acid (V600D) and lysine (V600K). These mutated forms of BRAF are constitutively active; that is BRAF phosphorylates the kinase MEK which in turn phosphorylates the kinase ERK which subsequently stimulates cell growth and division. BRAF mutations, predominantly V600E, occur in approximately 8% of all solid tumors, including 50% of metastatic melanomas, 30% to 70% of thyroid carcinomas, 30% of ovarian carcinomas, and 10% of colorectal carcinomas.[2,3,4,5,6] The sponsor

[2] Davies H, Bignell GR, Cox C, *et al.* Mutations of the BRAF gene in human cancer. *Nature* 2002;**417**:949–954
[3] Fransen K, Klintenas M, Osterstrom A, *et al.* Mutation analysis of the BRAF, ARAF and RAF-1 genes in human colorectal adenocarcinomas. *Carcinogenesis* 2004: **25**:527–533.
[4] Garnett MJ and Marais R. Guilty as charged: B-RAF is a human oncogene. *Cancer Cell* 2004; **6**:313–319.
[5] Libra M, Malaponte G, Navolanic PM, et al. Analysis of BRAF mutation in primary and metastatic melanoma. *Cell Cycle* 2005; **4**:1382–1384.

proposes that inhibition of $BRAF^{V600E}$ by vemurafenib will slow the progression of cancer and lead to an increase in overall survival.

Melanoma was chosen for initial clinical investigation because of the high incidence of the V600E mutation in this disease. (b) (4) Because the company considered the compound inhibition specific for this mutated form of BRAF, they only recruited patients with this mutation into the clinical trial. They tested for the mutation using a diagnostic test, the cobas® 4800 BRAF V600 Mutation Test. This assay determines the presence of mutations at valine 600 by real-time polymerase chain reaction (PCR). Experience in patients who have cancers that do not express this mutation is limited. The product label will mandate the use of this test to select patients for treatment with vemurafenib.

2.7 Regulatory Background

Plexxikon Inc. submitted the original IND for PLX4032 on October 2, 2006. The company entered a partnership with Roche that year to facilitate the clinical development of the drug.

In 2009, Roche sought scientific advice from Health Authorities in the EU (MPA, DMA, BfArM) and the US on development plans to support registration for vemurafenib. The clinical overview document states that the sponsor reached consensus with these agencies on the following:

- The design of the proposed Phase 3 trial was considered acceptable, with a strong recommendation that OS should be the primary endpoint and the trial design should be open-label.
- The maximum tolerated dose of 960 mg b.i.d. was considered appropriate for further clinical development based on preliminary safety data from 20 patients treated for 28 days.
- The Risk Management Plan (RMP) for squamous cell carcinoma (SCC) was expanded to include head and neck exams, and periodic chest CT scans, and was extended post treatment until death, withdrawal, loss to follow-up, or withdrawal of consent.
- The clinical pharmacology program was considered appropriate to support registration, including plans to conduct a rigorous QT sub-study in the Phase 2 trial and to conduct a new study characterizing the pharmacokinetics of vemurafenib across the therapeutic dose range of 240 mg b.i.d. to 960 mg b.i.d. using the proposed commercial formulation of 240 mg film-coated tablets (study NP25163).
- The nonclinical program was considered acceptable to support registration, although insufficient exposure multiples in toxicology studies relative to clinical exposures at the maximum tolerated dose and the lack of an identified organ of toxicity were noted. Following these comments, the Sponsor conducted a 13- week repeat dose toxicology and toxicokinetic study in dog with twice daily dosing that identified the liver as the target organ of toxicity and the dog as the most sensitive species.

[6] McCubrey JA, Steelman LS, Chappell WH, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochim Biophys Act*a 2007;**1773**:1263–1284.

3 Studies Submitted

3.1 Studies Reviewed

See Table of Contents
Dr. Aziz reviewed studies below that are marked with RMA in the title. Dr. McGuinn reviewed all other studies.

3.2 Studies Not Reviewed

1) An *in vitro* dog, rat and human hemotoxicity study of R05185426 & R05212054 (Study 1036231)
2) PLX4032, a selective B-Raf inhibitor has potent anti-tumor activity in B- colorectal xenografts (Study 1026800)
3) A Four-day Oral Tissue Distribution Study in Male Crl:LE Rats (Study 1025573)
4) 13-Week Oral Gavage Toxicity and Toxicokinetic Study with RO5185426-007 (b-Raf Inhibitor) in Dogs with a 4-Week Recovery Phase. (Study 1025759)
5) Single Dose Oral and IV Tolerability Study of PLX4032 in the Male Sprague-Dawley Rat. (Study 1040813)
6) Single Dose Oral Tolerability Study of PLX4032 in the Male Beagle Dog (Study 1041530)
7) A Four-day Oral Tissue Distribution Study in Male Crl:LE Rats (Study 1025573)
8) In Vitro Plasma Protein Binding, Blood to Plasma Ratios and Partitioning to Red Blood Cells in Human and Various Animal Species (Study 1031038).
9) *In Vitro* Metabolic Profiles of RO5185426 in Liver Microsomes and Hepatocytes (Study 1033024)
10) In vivo Metabolic Profiles of RO5185426 in Rat and Dog Following PO Administration (Study 1037920)
11) In vivo Metabolic Profiles of RO5185426 in Dog Plasma for the Dog Toxicity (Study 1039931)
12) Stability and Identification of Metabolites of PLX4032 and PLX4720 in Human, Monkey, Dog, Rat and Mouse Hepatocytes (Study 1040856)
13) Fluorescent Human Serum Protein Binding: Effects of PLX4032 (Study 1040870)
14) A Crossover Oral (Capsule and Tablet) Dose Pharmacokinetic Study in Male Beagle Dogs (Study 1041443)
15) In vivo Metabolic Profiles of RO5185426 in Human Plasma for the Phase II Study NP22657 (Study 1041546)
16) *In vivo* Metabolic Profiles of RO5185426 in Rat Following PO and IV Administration From Excretion Balance Study (Study 1041579)

3.3 Previous Reviews Referenced

On October 26, 2006, Dr. Haleh Saber reviewed the following studies submitted by Plexxikon, Inc. to IND 73,620 on October 2, 2006.

1) A 28-day oral toxicity study in Sprague-Dawley rats with a 14 day recovery. Study (b) (4) 578004
2) A 28-day oral (gavage) toxicity study of PLX4032 with a 14-day recovery period in Beagle dogs. Study (b) (4) 578003

On July 7, 2007, Dr. Haleh Saber reviewed the following studies submitted by Plexxikon, Inc. to IND 73,620 on October 2, 2006.

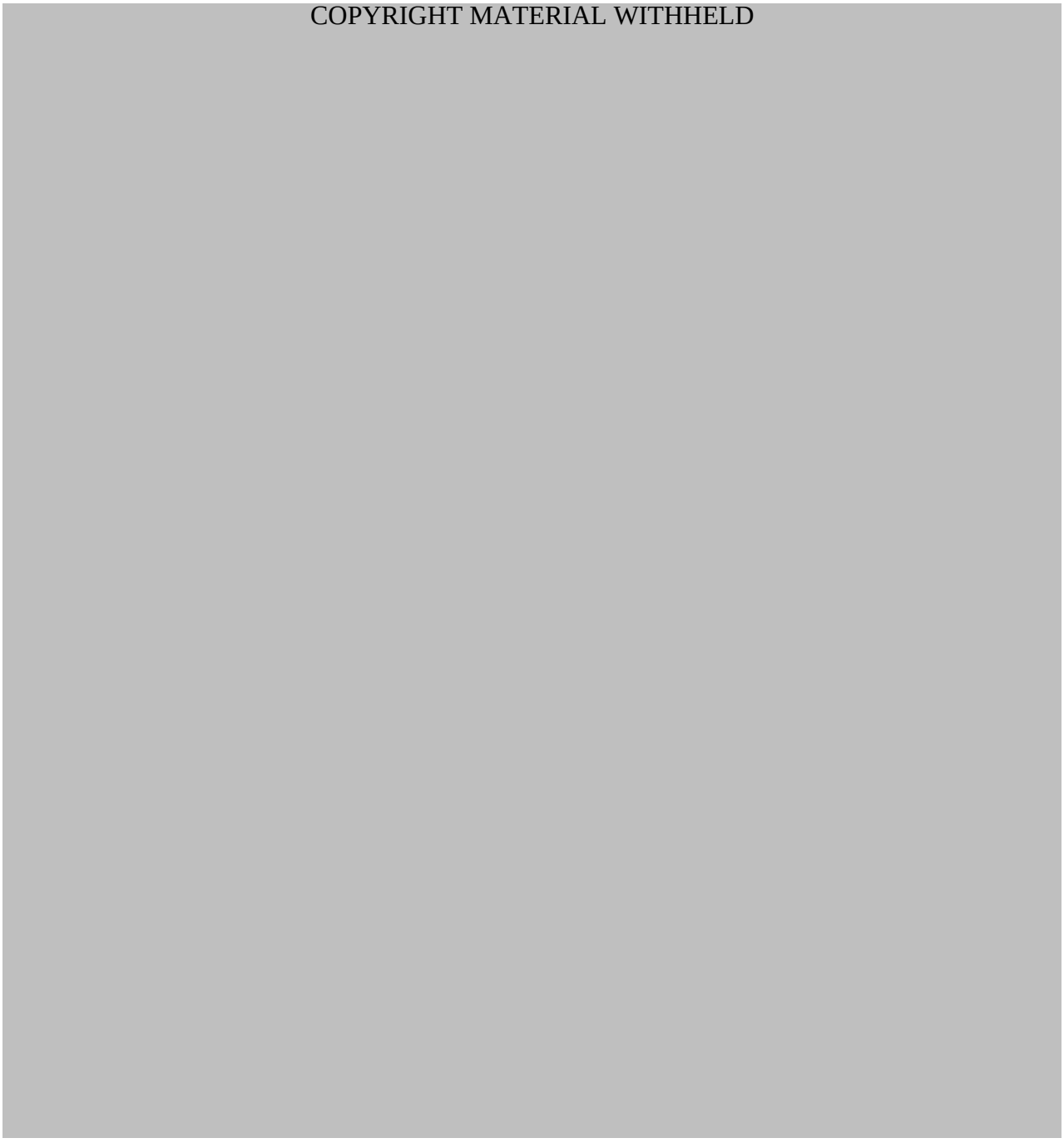
1) Bacterial Reverse Mutation Assay: Study # AB29FU.503 (b) (4)
2) *In Vitro* Mammalian Chromosome Aberration Test: Study # AB29FU.341 (b) (4)

4 Pharmacology

4.1 Primary Pharmacology

1) Pharmacodynamic Characterization of the Efficacy Signals Due to Selective BRAF inhibition with PLX4032 in Malignant Melanoma. WD Tap *et al.* *Neoplasia*, August 2010, 12(8):637-649.

Tap *et al.* treated a panel of 35 melanoma cell lines with varying concentrations of vemurafenib *in vitro* to determine growth inhibition over an incubation period of 7 days. They used PCR and sequencing to determine the mutational status of the cell lines. The following chart from the article shows the results of these assays.



The authors color coded the response by arbitrarily assigning cutoffs for “clear sensitivity” (pink, IC_{50} < 1 μM), “intermediate sensitivity” (orange, IC_{50} < 3 μM) and resistant” (gray, IC_{50} > 3 μM). Nevertheless, all but four of the tested cell lines had IC_{50} values at least 10 fold lower than the concentrations achieved clinically. Sensitivity to inhibition appeared to be associated with mutations of at V600 regardless of zygosity or amino acid substitution. The only discernable correlation between higher IC_{50} values and vemurafenib sensitivity is whether or not cells have wild-type or mutated BRAF, and that correlation is not absolute. The data is inadequate to provide any information about

the influence of RAS mutational status because all the cells with relatively high IC_{50} values and mutated RAS also have wild-type BRAF.

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2) Proliferation of Human Cell Lines: Effects of PLX4032

Major Findings

Vemurafenib inhibited the growth of four human cancer cell lines expressing the product of the $BRAF^{V600E}$ mutation with IC_{50} values ranging from 0.02 to 0.55 µM. In cell lines that did not have the $BRAF^{V600E}$ mutation, IC_{50} values for growth inhibition ranged from 6.5 to 12.9 µM. The IC_{50} value for growth inhibition of normal (non-cancerous) human kidney cells was 19 µM.

Study Number	1041086, PLX4032-002, DRN-102-078
File name	1041086
Laboratory	Plexxicon Inc., Berkeley, CA
Study Date	August 2006
GLP	No
Audited	No
Drug	RO5185426, purity and lot not specified
Methods	
Cells	A375 cells HEK293T cells WM2664 cells COLO205 cells COLO829 cells SW620 cells H460 cells H1299 cells
Concentrations	At least five concentrations per cell line
Solvent	DMSO
Treatment time	72 hours
Assay	Fluorescent assay [Cell Titer Glow (Promega)] to detect ATP concentration, which correlates with cell viability

Results

The following graph from the report shows the decrease in growth of A375 Cells as a function of increasing concentration of the compound. Similar plots determined the IC_{50} values for the other cell lines.

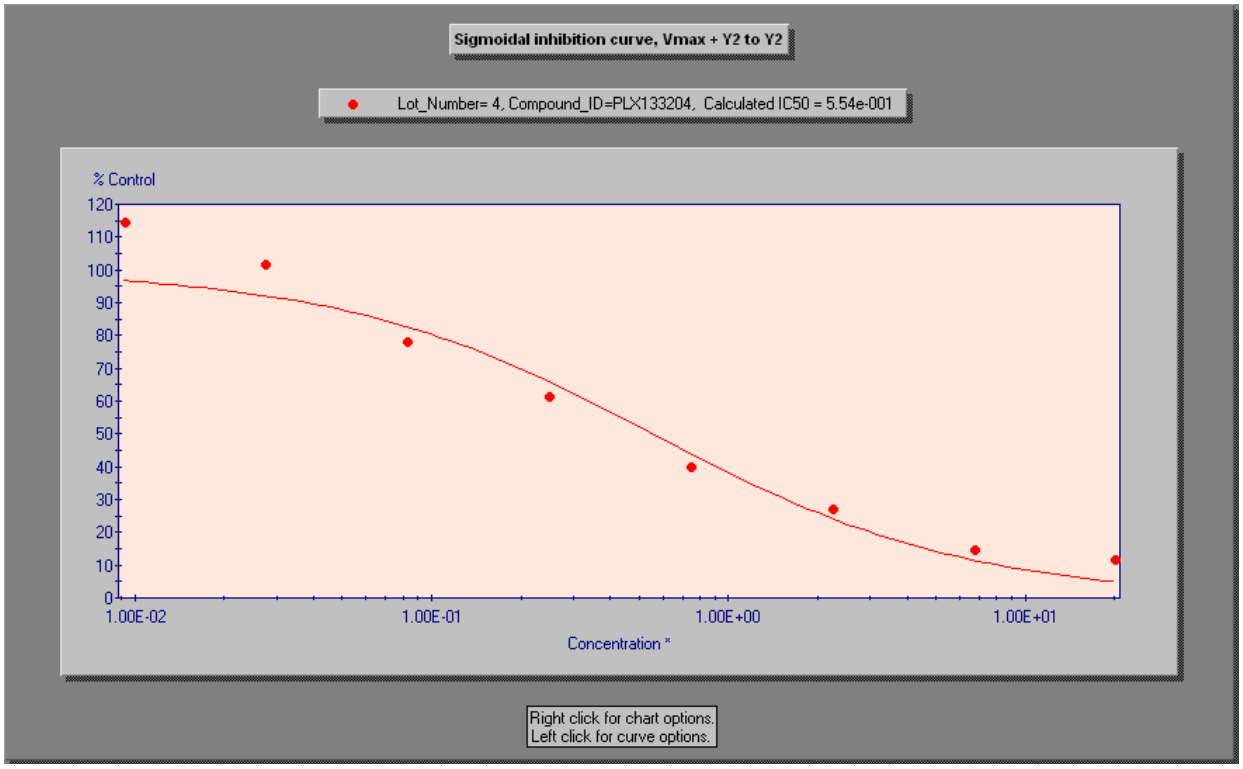

Table 4 from the study report shows the IC_{50} values for growth inhibition for each of the cell lines used in this study. The table also indicates the tissue type from which each of the oncogene-containing cells were derived.

Table 4: Inhibition of Proliferation: IC_{50} Summary of PLX4032 in a variety of cell lines

Tumor	Melanoma			Colorectal		NSCLC	
Oncogene	B-Raf^{V600E}			B-Raf^{V600E}	K-Ras	K-Ras	
Cell Line	A375	WM2664*	COLO829	COLO205	SW620	H1299	H460
IC_{50} (μM)	0.55	0.42	0.081	0.042	6.5	12.9	8.9

* WM2664 cells harbor the V600D mutation instead of V600E

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3) BRaf Kinase Activity: Effects of PLX4032

Major findings

Vemurafenib inhibited the activity of the isolated catalytic region of BRaf wild type protein with an IC_{50} of 140 nm. It inhibited the activity of the isolated catalytic region of BRaf^{V600E} with an IC_{50} of 50 nM and that of the isolated catalytic region of another mutant oncogene CRaf-1 with an IC_{50} of 16 nM. Thus, vemurafenib inhibits the phosphorylation of all these enzymes at concentrations more than 100 fold lower than those achieved clinically.

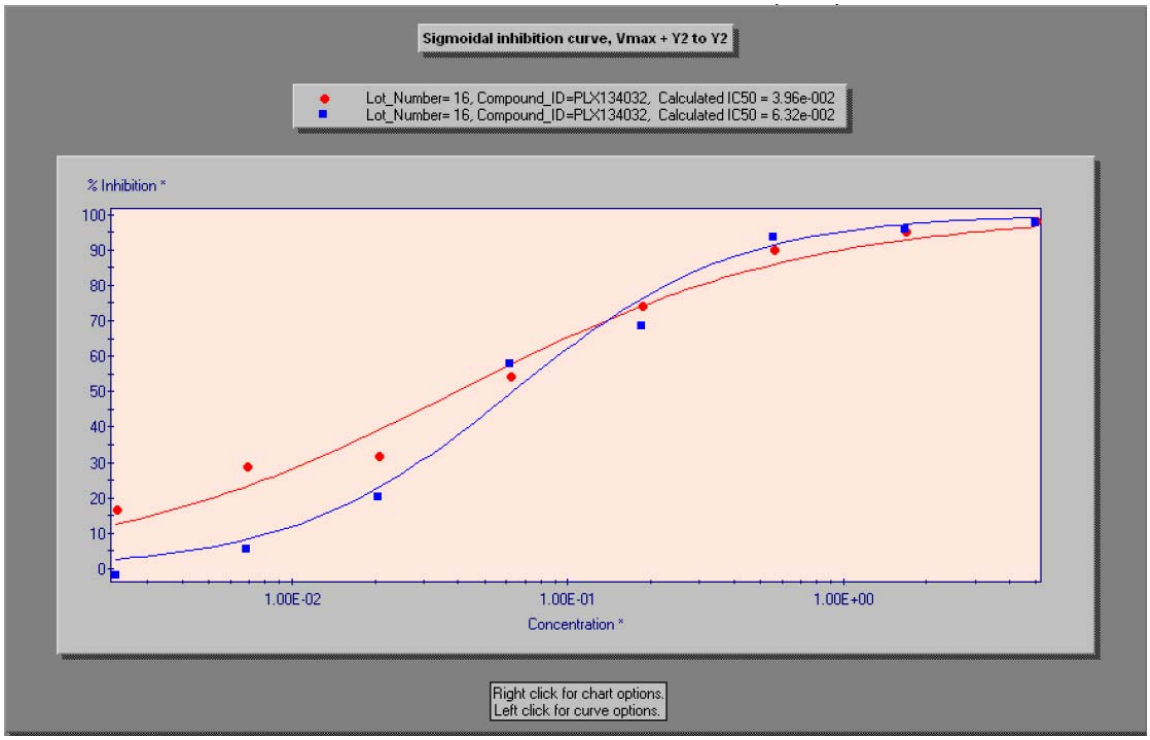
Study Number	1041081, PLX4032-003, DRN-102-076
File name	1041081
Laboratory	Plexxikon Inc., Berkeley, CA
Study Date	August 2006
GLP	No
Audited	No
Drug	RO5185426, purity not specified, lot 16 (not specified in the body of the report but shown on the graphs of the data)
Methods	
Enzymes	
$BRaf^{V600E}$	(b) (4)
$BRaf^{WT}$	
CRaf	
Biotinylated-Mek1	
Vehicle	DMSO
Assay	AlphaScreen™ (PerkinElmer)

In this study, the investigators transfected insect cells to express the catalytically active regions of $BRaf^{V600E}$, wild type BRaf, or a CRaf mutant protein. They isolated these proteins and determined their catalytic activity in the absence of inhibitor and in the presence of increasing concentrations (not specified except graphically) of vemurafenib using the AlphaScreen™ assay. When BRaf is catalytically active, it phosphorylates a biotinylated Mek1 substrate on serine residues. Biotinylated Mek1 is immobilized by the AlphaScreen™ Streptavidin donor beads and, upon serine phosphorylation by BRaf and binding to anti-phospho-Mek1 antibody, binds to AlphaScreen™ Protein A Acceptor beads. The assay then demonstrates kinase activity by the intensity of a fluorescence signal at 580 nm after 680 nm illumination.

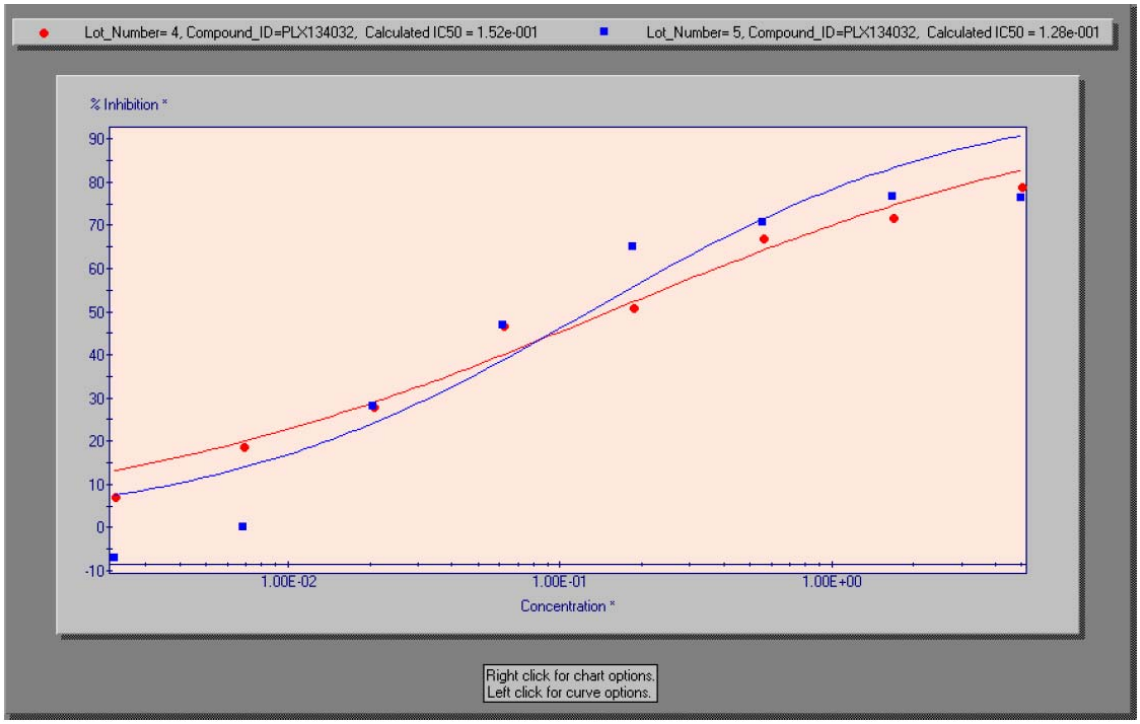
Results

Unfortunately, the investigators did not do thorough enzyme kinetics in this study but rather only determined IC_{50} values within an unspecified software program. They did not include the original data but only graphical presentations of the data. They assayed each enzyme twice across the range of concentrations. The following graphs from the study report show the percent of inhibition as a function of increasing vemurafenib concentration.

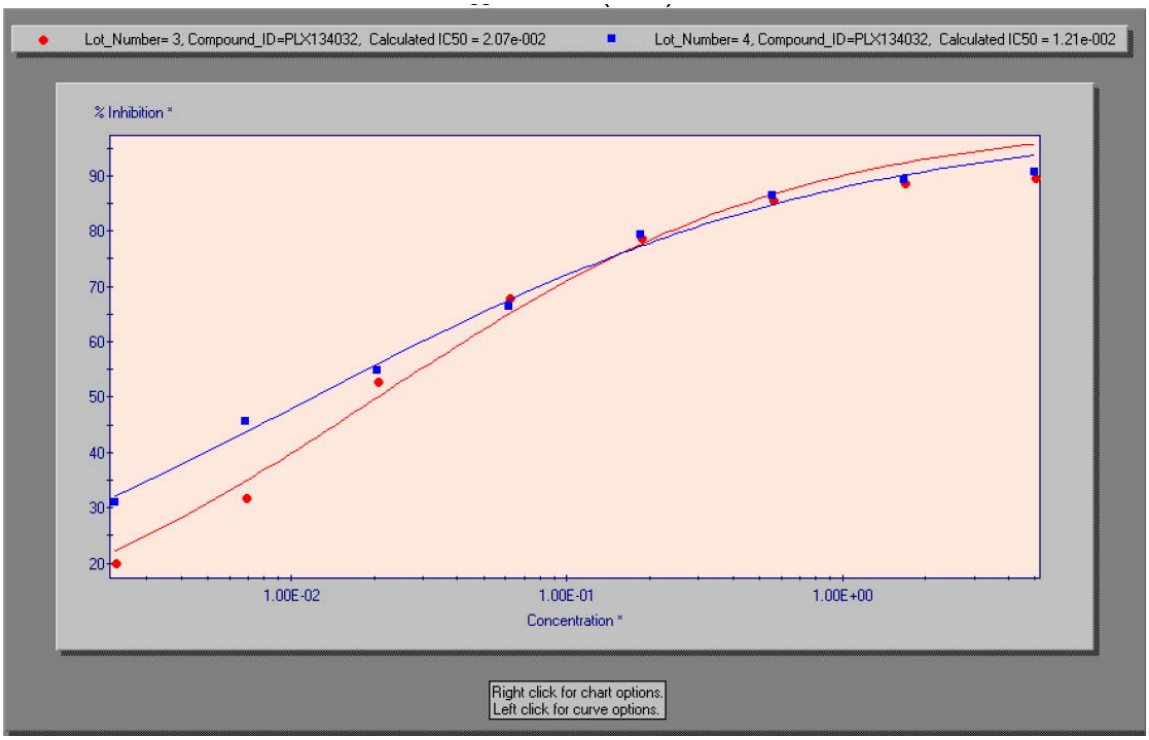
PLX4032 Inhibition of $BRaf^{V600E}$ IC_{50} = 50 nM (n=2 per concentration)



PLX4032 inhibition of BRaf wild type IC_{50} = 140 nM (n=2 per concentration)



PLX4032 inhibition of CRaf-1 IC_{50} =16 nM (n=2 per concentration)



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4) Immunoassays of ERK Phosphorylation in Human Cell Lines

Study Number	1041082, DRN-102-080
File name	1041082
Laboratory	Plexxikon Inc., Berkeley, CA
Study Date	August 2006
GLP	No
Audited	No
Drug	RO5185426, purity and lot 16 (not specified in the body of the report but shown on the graphs of the data)
Methods	
Cells	See table below
Assay	Immuno-blot and immuno-stain assays to detect phosphorylation of ERK in lysed cells

Major findings

Vemurafenib inhibited downstream ERK phosphorylation in melanoma and colorectal cell lines containing the $BRaf^{V600E}$ mutation at nM concentrations. In cells expressing $BRaf^{WT}$, IC_{50} values were at least three orders of magnitude higher but still within the range of clinically achievable concentrations. The presence of human serum in the cell growth medium greatly increases the IC_{50} values due to the binding of vemurafenib to soluble proteins. Although the table indicates N-Ras or K-Ras for the two BRafWT cell lines the text does not explain the meaning of this designation.

Table 1. Inhibition of ERK Phosphorylation: IC_{50} of PLX4032 in a variety of cell lines

Tumor	Melanoma			Colorectal	
Oncogene	B-Raf^{V600E}		N-Ras	B-Raf^{V600E}	K-Ras
Cell Line	A375	COLO829	SK-MEL2	COLO205	SW620
IC_{50} (nM)	11	16	> 40,000	32	> 40,000

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5) Kinase Selectivity Profiling: Effects of PLX4032

Major findings

At clinically relevant concentrations, vemurafenib inhibition is not specific for BRaf^{V600E} kinase. It inhibited the phosphorylation activity of at least 10 other wildtype kinases at IC_{50} values less than 20 µM. The report does not provide the IC_{50} values for those kinases with values higher than 20 µM.

Study Number	1041080, DRN-102-077
File name	1041080
Laboratory	(b) (4)
Study Date	August 2006
GLP	No
Audited	No
Drug	RO5185426, purity and lot not specified
Methods	

The sponsor contracted investigators at (b) (4) to determine the ability of vemurafenib to inhibit the activity of 58 kinases representing most branches of the protein kinase family. (b) (4) uses their proprietary (b) (4) assay, which measures phosphorylation flourometrically.

Compounds were tested at 20, 5, 1.25, 0.31 and 0.078 µM in duplicate. ATP concentration was 10 µM for most of the following kinases tested:

ADRBK1 (GRK2); AKT3 (PKB gamma); CAMK2A (CaMKII alpha); CDK2/cyclin A; CHEK1 (CHK1); CHEK2 (CHK2); CSNK1E (CK1 epsilon); DAPK3 (ZIPK); GSK3B (GSK3 beta); IKBKB (IKK beta); IRAK4; MAP4K4 (HGK); MAPK1(ERK2); MAPKAPK2; NEK2; PAK3; PIM1; PLK1; PRKACA (PKA); PRKCQ (PKC theta); PRKD1 (PKC mu); ROCK1; RPS6KB1 (p70S6K); STK22D (TSSK1); STK4 (MST1);STK6 (Aurora A) ABL1; BTK; CSK;FER; FRK (PTK5); HCK; JAK3; PTK2 (FAK); PTK6 (Brk);SRC; SYK ALK; CSF1R (FMS);EGFR (ErbB1); EPHA2; EPHB4; FLT3; IGF1R; KDR (VEGFR2); MERTK (cMER); MET (cMet); PDGFRA (PDGFR alpha); TEK (Tie2)

Some kinases required assay conditions with ATP at 100 µM. These included:

CLK1; MAP2K1 (MEK1); MAP2K6 (MKK6); MAP3K9 (MLK1); MAPK14 (p38 alpha); PDK1;TAOK2 (TAO1) KIT; NTRK1 (TRKA)

The sponsor did not report the results for any kinase with an IC_{50} greater than 20 µM. Table 5 from the study report shows the results for all kinases with an IC_{50} less than 20 µM.

Table 5: IC_{50} Values for Inhibition of PLX4032-Sensitive Kinases

Kinase	IC_{50} (µM)
PTK6 (BRK) 5pt	0.24
FRK (PTK5) 5pt	2.2
SRC 5pt	2.8
CSK 5pt	2.9
KDR (VEGFR2) 5pt	5.3
PTK2 (FAK) 5pt	6.3
STK4 (MST1) 5pt	8.2
CSF1R (FMS) 5pt	17
HCK 5pt	17
PDGFRA (PDGFR alpha) 5pt	18

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6) Kinase Selectivity Assays

Major findings

Vemurafenib inhibited 28 human kinases with IC_{50} values of about 2 µM or less. These included CRAF, ARAF, SRMS, ACK1, MAP4K5 and FGR, all with IC_{50} values of 100 nM or less, and wild-type BRaf (IC_{50} = 39 nM in this assay).

Study Number	1041139, DRN-102-094
File name	1041139
Laboratory	Plexxikon Inc., Berkeley, CA (b) (4)
Study Date	October 2010
GLP	No
Audited	No
Drug	RO5185426, purity and lot not specified
Methods	
Assay	PLX4032 was tested against a broad panel of 274 kinases at a concentration of 1 µM in duplicate. The inhibition screen was carried out against the combined and overlapping panel of kinases both at (b) (4). The assays determined inhibition of the phosphorylation activity of the kinases. IC_{50} values were determined only for kinases that were inhibited by 40% at 1 µM.

Results

Table 6 shows that vemurafenib inhibited numerous kinases at concentrations well below those achieved clinically.

Table 6: IC_{50} Values for Selected Kinases

Assay	IC_{50} nM[1]
B-RAF-V600E	8
C-RAF	16
A-RAF	29
B-RAF	39
SRMS	18
ACK1	19
MAP4K5 (KHS1)	51
FGR	63
BRK	209
LCK	217
NEK11	317
FYN	533
KIT	538
BLK	547
LYNB	599
KDR	723
YES1	799
WNK3	877
STK3 (MST2)	891
LYNA	995
FRK (PTK5)	1320
CSFR1 (FMS)	1500
MNK2 (GRK7)	1717
CSK	1770
STK4 (MST1)	1790
GCK	1850
FLT4	1920
SRC	2011

7) Biochemical Kinase Inhibition of BRAF Proteins with Varying Amino Acid Substitutions for Valine-600: Effects of PLX4032

Major findings

Vemurafenib inhibited the kinase activity of 10 BRaf mutant proteins at IC_{50} values ranging from 7 to 31 nM. This included protein expressed by a $BRAF^{V600K}$, a mutation erroneously detected by the sponsors screening assay. Thus, inhibition is independent of any particular mutation at protein residue 600. Vemurafenib also inhibited the kinase activity of BRaf proteins with mutations at residue 601 and 599. The investigators did not study wild-type BRaf protein in this assay.

Study Number	1042026, DRN-102-095
File name	1042026
Laboratory	Plexxikon Inc., Berkeley, CA
Study Date	December 2010
GLP	No
Audited	No
Drug	RO5185426, Lot 26, Purity ≥ 98%
Methods	
Assay	AlphaScreen™, a fluorometric assay that measures phosphorylation of biotinylated MEK1 by BRaf Kinases
Enzymes	Human BRaf, amino acid 284 through 766 or 432 through *765* with various mutations, is fused at its N-terminus with a 26 residue HIS-tag, expressed and purified from insect cells
Solvent	DMSO

Results

Table 7 shows the results of this experiment. In This table the column reporting "# of Exp" refers to the number of concentration data points used to determine the IC_{50} value.

Table 7: Inhibition of BRAF Mutants

Kinase	IC_{50} (µM)	# of Exp
BRAF_V600E	0.0099	14
BRAF_V600A	0.014	6
BRAF_V600G	0.008	8
BRAF_V600K	0.007	6
BRAF_V600M	0.007	6
BRAF_V600R	0.009	6
BRAF_K601E	0.011	8
BRAF_T599I	0.031	10

8) Biochemical Kinase Inhibition of BRAF Proteins with Varying Amino Acid Substitutions for Valine-600: Effects of PLX4032.

Major findings

Vemurafenib inhibited the kinase activity of 12 BRaf mutant proteins at IC_{50} values ranging from 3 to 110 nM. Again, this included protein expressed by a $BRAF^{V600K}$, a mutation erroneously detected by the sponsors screening assay. Thus, inhibition is independent of the particular mutation at protein residue 600. Vemurafenib also inhibited the kinase activity of BRaf proteins with mutations at residues 586, 595, 464, 469 and 601. The investigators did not study wild-type BRaf protein as a control.

Study Number	1040741, DRN-102-092
File name	1040741
Laboratory	Plexxikon Inc., Berkeley, CA
Study Date	December 2010
GLP	No
Audited	No
Drug	RO5185426, Lot 26, Purity ≥ 98%
Methods	
Assay	AlphaScreen™, a fluorometric assay that measures phosphorylation of biotinylated MEK1 by BRaf Kinases
Enzymes	Human BRaf, amino acid 284 through 766 with various mutations, is fused at its N-terminus with a 26 residue HIS-tag, expressed and purified from insect cells
Solvent	DMSO

Results

Table 8 shows the results of this experiment.

Table 8: BRAF Mutant Inhibition at V600 and Other Amino Acids

Kinase	Source	ATP (µM)	IC_{50} (µM)
BRAF_V600E	Baculo	100	0.009
BRAF_E586K	Baculo	10	0.042
BRAF_F595L	Baculo	10	0.056
BRAF_G464V	Baculo	10	0.003
BRAF_G469A	Baculo	10	0.007
BRAF_K601E	E. coli	10	0.068
BRAF_V600A	Baculo	100	0.028
BRAF_V600E	Baculo	100	0.009
BRAF_V600K	E. coli	10	0.110
BRAF_V600D	E. coli	100	0.007
BRAF_V600M	E. coli	100	0.013
BRAF_V600R	E. coli	10	0.034

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10) RG7204 (PLX4032), a Selective BRaf^{V600E} Demonstrates Potent Anti-tumor Activity in Preclinical Melanoma Models

Major Findings

In vitro, in 10 cell lines expressing the BRAF^{V600E} mutation, vemurafenib inhibited cell growth at IC_{50} values less than 1 μM. Cells expressing a mutation at some site other than V600 or wild type BRAF had IC_{50} values that ranged between about 4 and 20 μM except for three that had mutations at NRAS or KRAS. No lines with NRAS or KRAS mutations had IC_{50} values less than 1 μM. Inhibition of MEK and ERK by vemurafenib was concentration dependent in isolated LOX cells. The inhibition of MEK was consistently greater than ERK inhibition.

In vivo in athymic mice, vemurafenib inhibited the growth of LOX, Colo829 and A375 xenografts implanted subcutaneously. All these cell lines express the BRAF^{V600E} mutant. Treated mice survived longer than controls and some achieved complete tumor cure. In mice bearing LOX xenografts, a dose of 100 mg/kg of vemurafenib caused decreases in the phosphorylation of MEK and ERK in tumor tissue. This inhibition was time dependent and coincided with the elimination of vemurafenib. Again, MEK phosphorylation was more completely inhibited than ERK phosphorylation suggesting ERK phosphorylation by pathways other than RAF-MEK-ERK or a nonlinear effect.

Study Number	1037755
File name	1037755
Laboratory	Hoffmann-La Roche Inc., Nutley, NJ Plexxikon Inc., Berkeley, California
Study Date	February 2010
GLP	No
Audited	No
Drug	RO5185426, Lot and purity not specified, MBP RO5185426, original crystalline form Temozolomide
Methods *In vitro*	
Cells	a panel of 32 human tumor cell lines with known BRAF, KRAS and NRAS mutational status
Assay	Cellular proliferation was evaluated by MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (b) (4) assay Western Blots to determine inhibition of phosphorylation of MEK and ERK
Methods *in vivo*	
Animals	Athymic nude mice (Crl:NU-Foxn1nu) 13−14 weeks old, weighing approximately 23−25 g
Xenografts	LOX, Colo829 and A375 cells All these cell lines are invasive human melanoma lines containing the BRAF^{V600E} mutation All implanted subcutaneously
Dose	12.5, 25, 75

Vehicle aqueous vehicle containing 2% Klucel LF (Hydroxypropylcellulose; Aqualon) and adjusted to pH 4 with dilute HCl

Results

Figure 1 shows that *in vitro*, in 10 cell lines expressing the $BRAF^{V600E}$ mutation, vemurafenib inhibited cell growth at IC_{50} values less than 1 μM. Cells expressing a mutation at some site other than V600 or wild type BRAF had IC_{50} values that ranged between about 4 and 20 μM except for three that had mutations at NRAS or KRAS. No lines with NRAS or KRAS mutations had IC_{50} values less than 1 μM.

Figure 1: *In vitro* Cell Line Inhibition

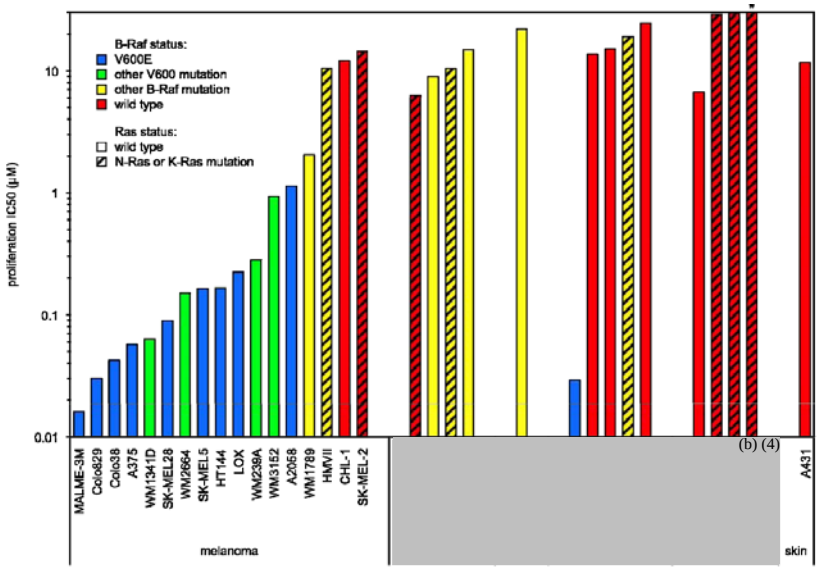


Figure 2 shows that increasing concentrations of vemurafenib cause increasing inhibition of MEK1/2 and ERK phosphorylation in LOX cells. These results suggest that inhibition of the BRaf mutant blocks downstream phosphorylation.

Figure 2: Inhibition of Downstream Phosphorylation

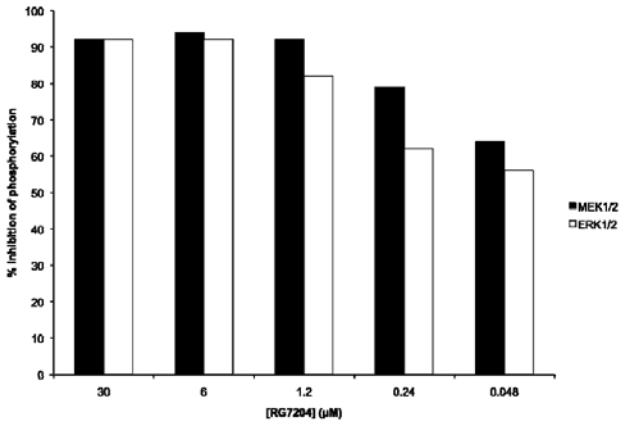
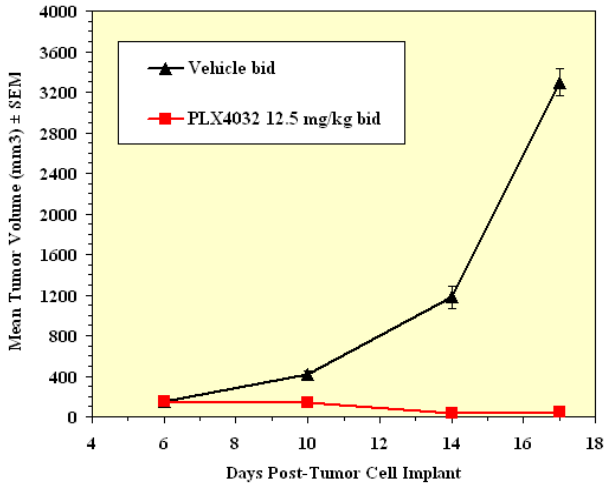


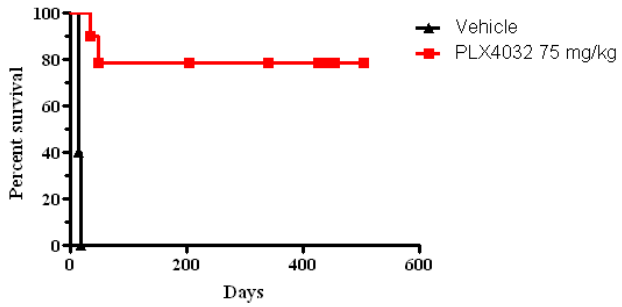
Figure 3 shows that repeated treatment with vemurafenib at doses as low as 12.5 mg/kg (BID) resulted in decreased xenograft growth in mice. The results after treatment with higher doses are similar (not shown).

Figure 3: PLX4032 Inhibition of LOX Tumor Growth



A Kaplan-Meyer plot from the study report shows (Figure 4) that mice treated with 75 mg/kg BID lived longer than untreated mice. Results at lower doses were not as good but showed distinct improvement over control (not shown).

Figure 4: Survival of LOX Tumor Bearing Mice



Some animals achieved complete xenograft cure as shown in Table 9.

Table 9: Tumor Regression in Xenograft Bearing Mice after PLX4032 Administration

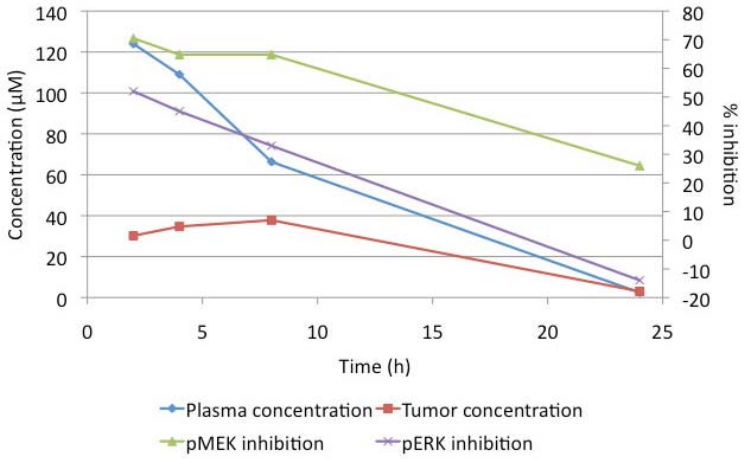
RG7204 dose (mg/kg bid)	N	Partial regression	Complete regression	Cured	Tumor growth inhibition (TGI, %)	TGI P value*	Increased life span (ILS, %)*	ILS P value*
12.5	9	4	5	1	>100	<0.001	147	<0.0001
25	10	0	10	4	>100	<0.001	321	<0.0001
75	10	0	10	8	>100	<0.001	3500	<0.0001

*Relative to vehicle control group.

Similar experiments using Colo829 melanoma xenografts or A375 melanoma xenografts, cell lines that express the $BRaf^{V600E}$ mutation, also demonstrated antitumor activity *in vivo* (not shown).

This series of experiments also demonstrated inhibition of MEK and ERK phosphorylation *in vivo* in mice given 100 mg/kg of vemurafenib after implantation of LOX melanoma xenografts. This inhibition was time dependent and coincided with the concentration of vemurafenib as shown in Figure 5.

Figure 5: Relationship of Inhibition of Phosphorylation to Vemurafenib Concentration



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11) Effects $BRAF^{V600E}$ inhibitors RG7204 (PLX4032, RO5185426) and RG7256 (PLX3603, R05212054) monotherapy or in combination with MEK inhibitor RO5068760 on A431 squamous cell carcinoma xenograft

Major Findings

In this experiment, mice were implanted with human cutaneous squamous cell carcinoma (cuSCC) xenografts and treated with various doses of vemurafenib, R05212054 (another compound selected to inhibit $BRaf^{V600E}$, Sorafenib, or RO5068760 (a compound selected to inhibit MEK). Consistent with the finding that vemurafenib stimulates the development and growth of cutaneous squamous cell carcinomas in clinical trials, treatment with vemurafenib caused a dose dependent acceleration of the growth of the xenographs in these mouse models. Treatment with R05212054 resulted in tumor growth that was about the same as that in the vehicle controls. Treatment with Sorafenib resulted in significantly decreased tumor growth relative to controls. Treatment with MEK inhibitor RO5068760 also resulted in significantly decreased tumor growth. Lastly treatment with both vemurafenib and RO5068760 in combination resulted in almost complete suppression of tumor growth at the highest doses tested. These highest doses tested caused some weight loss or decreased weight gain indicating that they were at or close to the MTD.

Study Number	1037653
File name	1037653

Laboratory Hoffmann-La Roche, Inc., Nutley, NJ, USA
Study Date September 2010
GLP No
Audited No
Drug RO5185426, Lot and purity not specified, MBP formulation
R05212054 BRAF inhibitor
RO5068760 a MEK inhibitor

Methods

Animals Female athymic Crl: NU-Foxnlnu
10-12 weeks of age and weighing 23-25 g

Implants A431 cells (derived from skin epidermis) were grown in DMEM medium supplemented with (b) (4) [redacted]

Doses The following table from the report shows the doses and study design

Table 1 Study Design

Study No.	Tumor Model	Implant Date	Treatment Start Date (days post implant)	Starting Tumor Volume (mm^3)	Treatment End Date (days post implant)	Treatment Groups
1515	A431	5/27/09	6/8/09 (day 13)	~130	6/24/09 (day 29)	1. Vehicle *bid* 2. RG7204 6.25 mg/kg *bid* 3. RG7204 12.5 mg/kg *bid* 4 RG7204 25 mg/kg *bid* 5 RG7204 75 mg/kg *bid*
1549	A431	7/25/09	8/4/09 (day 11)	~130	8/21/09 (day 28)	1. Vehicle *bid* 2. Sorafenib 90 mg/kg qd 3. RG7256 3.125 mg/kg *bid* 4. RG7256 6.25 mg/kg *bid* 5 RG7256 12.5 mg/kg *bid* 6 RG7256 25 mg/kg *bid*
1568	A431	10/16/09	10/26/09 (day 10)	~130	11/9/09 (day 24)	1. Vehicle *bid* 2. RG7204 75 mg/kg *bid* 3. RG7256 25 mg/kg *bid* 4. RG7256 75 mg/kg
1623	A431	1/26/10	2/4/10 (day 9)	~130	2/17/10 (day 22)	1. Vehicle *bid* 2. RG7204 75 mg/kg *bid* 3. RO5068760 75 mg/kg *bid* 4. RO5068760 100 mg/kg *bid* 5. RG7204 75 mg/kg *bid* + RO5068760 75 mg/kg *bid* 6. RG7204 75 mg/kg *bid* + RO5068760 100 mg/kg *bid*
1660	A431	5/4/10	5/13/10 (day 9)	~130	5/25/10 (day 21)	1. Vehicle *bid* 2. RG7204 75 mg/kg *bid* 3. RO5068760 25 mg/kg *bid* 4. RO5068760 50 mg/kg *bid* 5. RG7204 75 mg/kg *bid* + RO5068760 25 mg/kg *bid* 6. RG7204 75 mg/kg *bid* + RO5068760 50 mg/kg *bid*

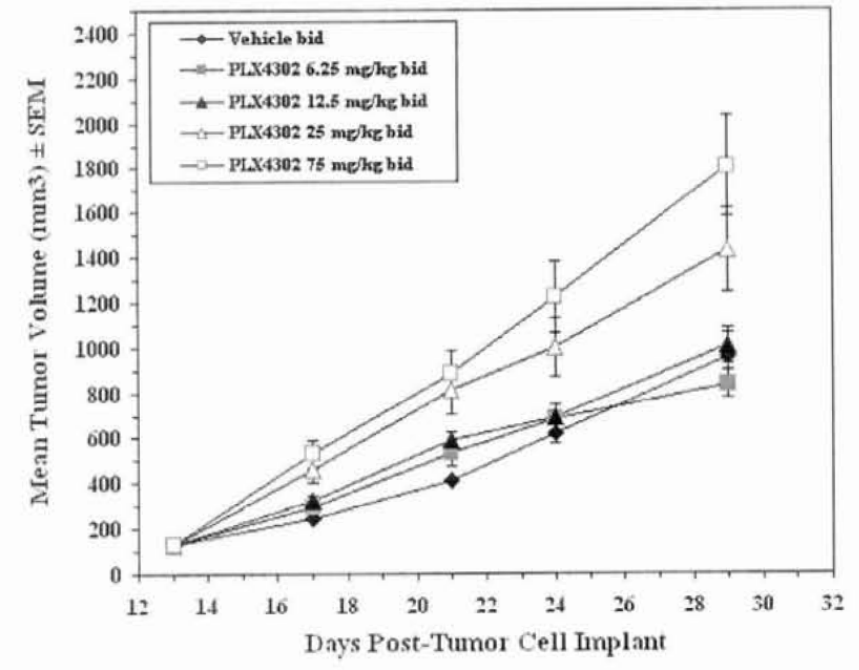
Results

Body weight In these experiments treated mice usually showed a dose-dependent weight loss indicating that the dose was high enough to cause some toxicity and that it approached the MTD.

Experiment EFF1515 with vemurafenib

Figure 6 excerpted from the study report shows that the volume of the implanted cuSCC tumors increased more rapidly with increasing dose of vemurafenib. Kaplan Meier curves demonstrated somewhat poorer survival in mice treated with vemurafenib (not shown).

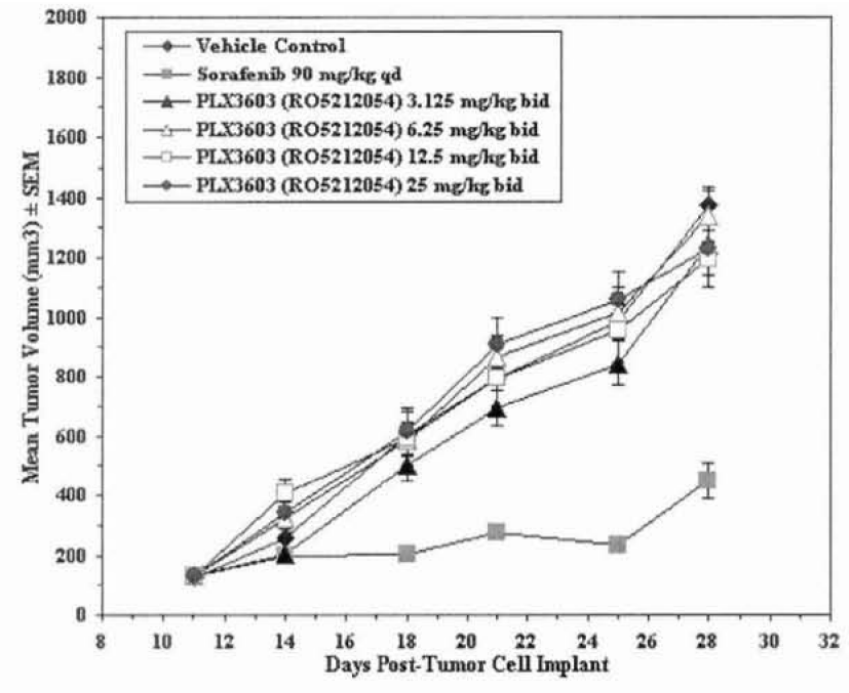
Figure 6: Increased cuSCC Tumor Volume Following Vemurafenib Administration



EFF1549

The following figure (Figure 7) from the study report shows that treatment with PLX3603, another compound selected to inhibit $BRAF^{V600E}$, caused increases in cuSCC tumor growth with increasing dose but these increases were not as great as with vemurafenib. Sorafenib treatment slowed growth considerably.

Figure 7: Comparison of Effects of Vemurafenib, PLX3603, and Sorafenib on cuSCC



EFF1568

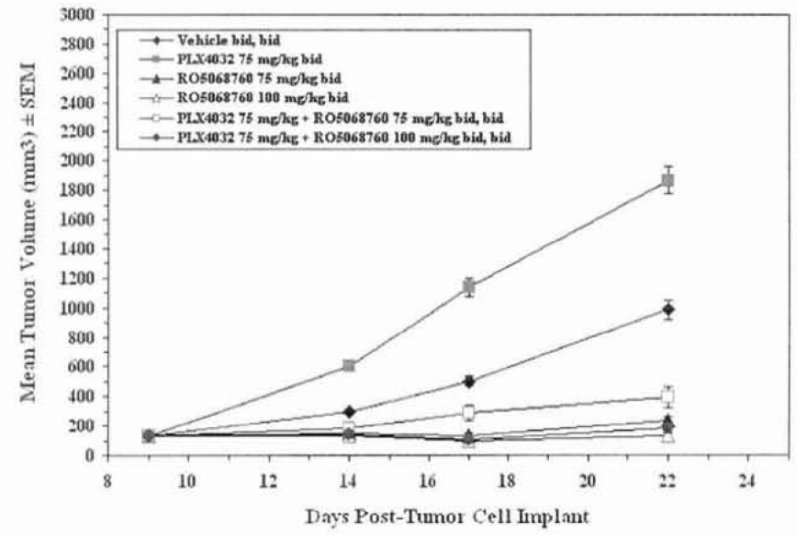
In this experiment, investigators treated groups of mice with either vemurafenib or PLX3603. Here tumor growth in the mice treated with PLX3603 was about the same as in vehicle but tumors in the group treated with vemurafenib grew considerably faster (not shown).

EFF1623

In this experiment, investigators treated groups of mice with vehicle, vemurafenib, RO5068760 (a MEK inhibitor) or both. Again tumor volume in mice treated with vemurafenib increased at a rate grater than in those treated with vehicle. Tumor size in mice treated with both drugs simultaneously remained stable (Figure 8).

(Excerpted from the study report)

Figure 8: Effects of Vemurafenib and MEK Inhibitor on cuSCC



EFF1660

This experiment was similar to EFF1623 but used somewhat lower doses of the MEK inhibitor resulting in similar but somewhat poorer results (not shown).

12) Evaluation of the effects of PLX4032, PLX3606, and PLX5215 in the A431 squamous cell carcinoma model, subcutaneously engrafted in athymic nude mice.

Major Findings

Roche investigators implanted nude mice subcutaneously with human squamous cell carcinoma tumors and then treated with vemurafenib at doses of 75 mg/kg bid for two weeks. An earlier experiment had shown that this dose caused regression of melanoma xenografts in mice. By subjective evaluation, this treatment caused a slight increase in invasive tumor growth and inflammatory cell infiltration. PLX5215 and PLX3603 also caused slight increases in inflammatory cell infiltration. None of the treatments caused an easily discernable change in phosphorylated MAPK or MEK1 stain intensity in immunostained tumor sections.

Study Number	1037110, PLX_Nu_A431_001
File name	1037110
Laboratory	(b) (4) [redacted]

Study Date October 2009
GLP No
Audited No
Drug RO5185426, Lot and purity not specified
PLX5215 and PLX3603 are drug candidates selected to inhibit $BRAF^{V600E}$.

Methods

Animals Athymic nude mice with subcutaneously engrafted A431 human squamous cell carcinoma tumors

Drug and dose The following table from the study report shows the drugs tested used in this experiment, it also shows the doses and the schedule

Group	Animal Number	Compound	Dose		Treatment Schedule
1	101-105	Vehicle	0.2	mg/kg	i.p. 2x/wk.
2	201-205	PLX4032 (RO5185426)	75	mg/kg	bid
3	301-305	PLX3603 (RO5212054)	25	mg/kg	bid
4	401-405	PLX3603 (RO5212054)	75	mg/kg	bid
5	501-505	PLX5215 (RO5309972)	50	mg/kg	bid

Doses PLX4032 – 225 mg/m^2
PLX3603 – 75 and 225 mg/m^2
PLX5215 – 150 mg/m^2

Parameters Rate of proliferation / Rate of apoptosis (per field of view at 200x magnification)
Invasive Growth
Necrosis / cysts estimated in % of total tumor area
Cellular / nuclear polymorphism estimated between (+) and ++++
Capsule formation estimated between (+) and ++++
Intratumoral fibrosis estimated between (+) and ++++
Inflammatory cell infiltration estimated between (+) and ++++
Sections of tumor were stained immunohistochemically for phosphorylated MAPK and MEK1 and subjectively evaluated for the intensity of staining

Results

The following two tables from the study report (Table 10 and Table 11) show the results of this experiment:

Table 10: Effects of RAF Inhibitors on cuSCC

Group	Animal #	Polymorphism	Rate of proliferation	Necrosis / cyst formation (%)	Apoptosis	Invasive growth	Capsule formation	Intratumoral fibrosis	Inflammatory cell infiltration
Group 1: vehicle	101	++ / +++	++ / +++	30	+ / ++	+	++	+	+
	102	++ / +++	++	40	+	+	++	+	++
	103	++ / +++	++ / +++	35	+ / ++	+ / ++	+ / ++	+	+ / ++
	104	++ / +++	++	35	+ / ++	+	++	+	+
	105	++ / +++	+++	40	+ / ++	++	++	+	+
Group 2: PLX4032	201	++ / +++	++ / +++	40	+ / ++	++	++	+	++
	202	++ / +++	++	35	+ / ++	++	++	+	++
	203	++ / +++	++ / +++	35	+ / ++	+	+ / ++	+	++
	204	++ / +++	++ / +++	40	+ / ++	++	++	+	++
	205	++ / +++	++ / +++	40	+ / ++	++	+ / ++	+	+ / ++
Group 3: PLX3603 (25 mg/kg)	301	++ / +++	++	45	+ / ++	+	++	+	++ / +++
	302	+ / + + +	+ +	35	+ / + +	+	+ +	+	+ + / + + +
	303	++ / +++	++ / +++	35	+ / ++	+ / ++	++	+	+ / ++
	304	++ / +++	++	30	+ / ++	+ / ++	+ / ++	+	++ / +++
	305	++ / +++	++ / +++	40	+ / ++	+	++	+	++ / +++
Group 4: PLX3603 (75 mg/kg)	401	++ / +++	++ / +++	40	+ / ++	+	+ / ++	+	++
	402	++ / +++	++ / +++	30	+ / ++	++	+ / ++	+	++ / +++
	403	++ / +++	++	35	+ / ++	++ / +++	++	+	++
	404	++ / +++	++	40	+ / ++	++	+ / ++	+	+ / ++
	405	++ / +++	++	35	+ / ++	+	++	+	+ / ++
Group 5: PLX5215	501	++ / +++	++	30	+ / ++	+	+ / ++	+	++ / +++
	502	++ / +++	++ / +++	40	+ / ++	+	+ / ++	+	++ / +++
	503	++ / +++	++ / +++	45	+ / ++	++	+ / ++	+	++
	504	++ / +++	++ / +++	30	+ / ++	++	++	+	+ / ++
	505	++ / +++	++	40	+ / ++	+ / ++	++	+	+ / ++

Table 11: Effects of Raf Inhibitors on Downstream Phosphorylation in cuSCC

		p-MAPK*			p-MEK1		
Group	Animal #	Staining intensity	Positive cells in %	Staining pattern	Staining intensity	Positive cells in %	Staining pattern
Group 1: vehicle	101	++ / (+++)	60 (30)	c (n)	(+)	60	c
	102	++	60 (30)	c (n)	(+) / +	60	c
	103	++ / (+++)	60 (30)	c (n)	(+) / +	60	c
	104	++ / (+++)	60 (30)	c (n)	(+) / +	60	c
	105	++	60 (30)	c (n)	(+) / +	60	c
Group 2: PLX4032	201	++ / (+++)	60 (30)	c (n)	+	60	c
	202	++ / +++	60 (30)	c (n)	(+) / +	60	c
	203	++ / +++	60 (30)	c (n)	(+) / +	40	c
	204	++ / (+++)	60 (30)	c (n)	(+) / +	60	c
	205	++ / +++	60 (30)	c (n)	(+) / +	60	c
Group 3: PLX3603 (25 mg/kg)	301	++	60 (30)	c (n)	+	60	c
	302	++ / (+++)	60 (30)	c (n)	(+) / +	60	c
	303	++	60 (30)	c (n)	(+) / +	60	c
	304	++ / +++	60 (30)	c (n)	(+) / +	60	c
	305	++	60 (30)	c (n)	+	60	c
Group 4: PLX3603 (75 mg/kg)	401	++	60 (30)	c (n)	(+)	60	c
	402	++ / +++	60 (30)	c (n)	(+) / +	60	c
	403	++ / (+++)	60 (30)	c (n)	+	60	c
	404	++ / (+++)	40 (30)	c (n)	(+) / +	60	c
	405	++	60 (30)	c (n)	(+) / +	60	c
Group 5: PLX5215	501	++ / (+++)	60 (30)	c (n)	(+) / +	60	c
	502	++	60 (30)	c (n)	(+) / +	60	c
	503	++ / (+++)	60 (30)	c (n)	+	60	c
	504	++	60 (30)	c (n)	(+) / +	60	c
	505	++	60 (30)	c (n)	(+) / +	60	c

Legend: (+) very weak; + weak; ++ moderate; +++ strong
c = cytoplasm; n = nuclear; ( ) = minor characteristics

*p-MAPK: Extremely heterogeneous staining concerning intensity and distribution; averagely moderate, but ranging between + and +++. Additional ++ / +++ nuclear staining of about 30 % of the tumor cells.

13) Preclinical investigation of acquired resistance mechanism using cell lines and a xenograft model with acquired resistance to RO5185426

Major Findings

In a series of studies designed to examine mechanisms of resistance to the inhibition of vemurafenib *in vitro*, investigators developed six strains of cells derived from a parental A375 melanoma line by clonal selection under the pressure of increasing concentrations of vemurafenib. They showed that the parental line was sensitive not only to inhibition by vemurafenib but also to two other drugs targeting RAF, and to drugs targeting MEK, CDK, PI3K, or mTOR but not to a drug targeting AKT. The results for the three RAF inhibitors are notable as the values span two orders of magnitude. In the resistant clones, vemurafenib had IC_{50} values 90 to 120 fold greater than those seen in the parental line. The six resistant clones were also relatively resistant to MEK inhibition but became more sensitive to one RAF inhibitor, a CDK inhibitor, a dual inhibitor of PI3K and mTOR, and an AKT inhibitor. The selection mechanism appears to increase expression of CRAF and to some extent BRaf. Addition of vemurafenib suppressed the phosphorylation of ERK in sensitive cells but had little effect on ERK phosphorylation in one resistant line. The selection process also increased the phosphorylation of AKT in this cell line. Further experiments suggested that Pgp may contribute to the resistance mechanism as may CRAF upregulation. All six clones had elevated expression of activated RAS and all six had an uncommon, activating mutation (K117N) in the *KRAS* gene. The parental cell line did not have this mutation. Knocking down KRAS expression with siRNA constructs had no effect on the vemurafenib sensitivity of the parental A375 cells, but caused increased sensitivity in the resistant cells, with 2.3-fold and 5.8-fold decreases in IC_{50} values in resistant cell lines R1 and R6, respectively.

Study Number	1042270
File name	1042270
Laboratory	Hoffmann-La Roche, Inc., Nutley, NJ, USA
Study Date	January 2011
GLP	No
Audited	No
Drug	RO5185426, Lot and purity not specified RO5068760 a MEK inhibitor RO4584820 a CDK inhibitor (abbreviated CDKi) PD0325901 a MEK inhibitor (abbreviated PD) MK-2206 a AKT inhibitor (abbreviated AKTi) NVP-BEZ235 an inhibitor of PI3K and mTOR (abbreviated BEZ) AZ628 a RAF kinase inhibitor RAF265 a RAF kinase inhibitor Sorafenib a RAF kinase inhibitor
Methods in vitro	
Cells	Melanoma cell lines with acquired resistance to vemurafenib were generated by propagating parental A375 cells in increasing concentrations of vemurafenib to achieve chronic selection. Six

cell lines with increased IC_{50} values measured by MTT assay were isolated for further characterization

Methods in vivo

Animals	Female SCID-beige mice
Implants	A375 xenografts (parental and vemurafenib resistant cell lines), 10×10^6 cells implanted subcutaneously
Doses	See below

Results

The following table from the study report shows the IC_{50} for inhibition of growth of the parental cell line for each of the drugs in this study.

	RO5185426	RAF265	AZ628	Sorafenib	CDKi	MEKi	PD	AKTi	BEZ235
IC_{50} (μM)	0.071	0.233	0.022	4.468	0.078	0.033	<0.005	>10	0.007

The table shows that these cells are also relatively sensitive to inhibitors selected for MEK, CDK, PI3K and mTOR but not AKT. The results for the three RAF inhibitors are notable as the values span two orders of magnitude.

The investigators cultured the A375 melanoma cells for three month in the presence of increasing concentration of vemurafenib. At the end of that time they selected six cell lines that had become resistant to the effects of vemurafenib. The IC_{50} values for vemurafenib in these cell lines were 90 to 120 fold greater than that of the parent cell line. The $BRAF^{V600E}$ mutation was conserved in all the selected clonal lines. The following table shows the fold increases in resistance for each of these cell lines to all the drugs used in this study.

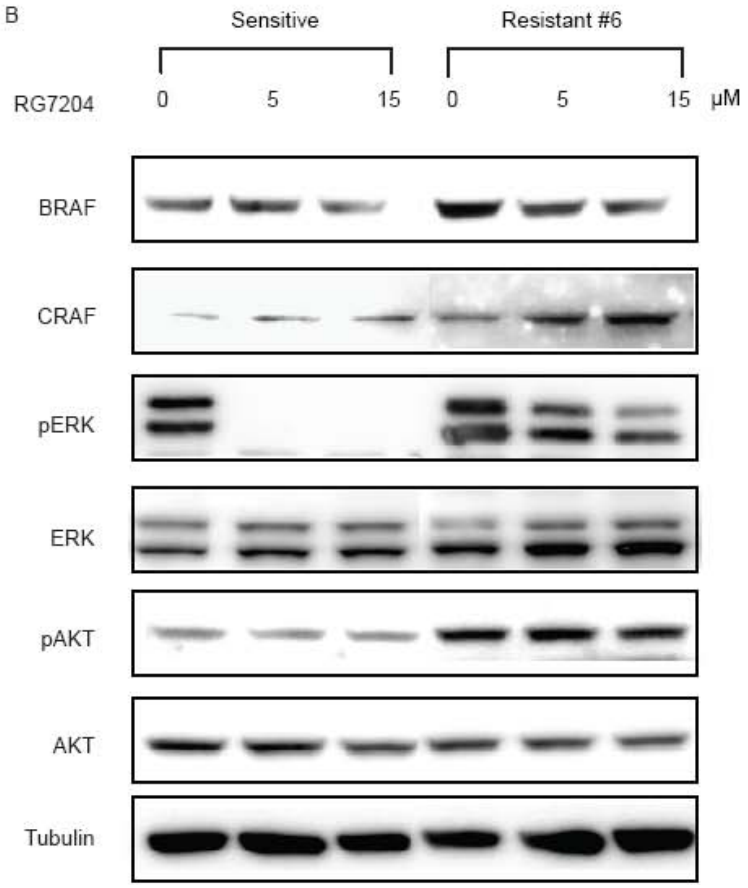
Fold of resistance over the parental cells

Resistant cell lines	RO5185426	RAF265	AZ628	Sorafenib	CDKi	MEKi	PD	AKTi	BEZ
1	91	-7.7	1.1	1.1	-1.7	11.3	21.2	-1.4	-2.3
2	93	-3.3	1.3	0.9	-1.7	10.3	25.0	-2.3	-2.4
3	96	-1.7	2.5	1.1	-1.7	11.4	5.7	-2.6	-2.7
4	86	-1.4	2.1	0.8	-1.4	7.0	3.8	-4.7	-2.8
5	119	-2	2.6	0.8	-1.7	7.8	2.8	-9	-2.7
6	119	-3.3	1.2	0.9	-1.4	7.1	3.9	-33	-2.6

The table shows that the selection process also increased the cells resistance to inhibition at MEK.

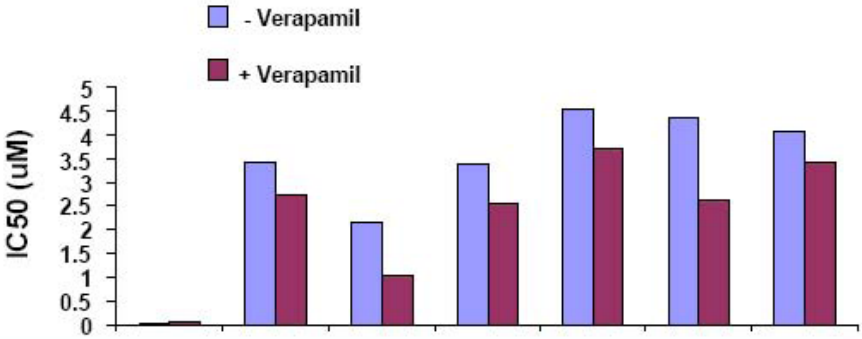
Figure 9 shows Western Blots that semi-quantify the expression of various kinases, some phosphorylated, in parent A375 cells (sensitive) and resistant cell line number six. The selection mechanism appears to increase expression of CRAF and to some extent BRAF. Addition of vemurafenib suppresses the phosphorylation of ERK in sensitive cells but has little effect ERK phosphorylation in the resistant line. The selection process appears to have little effect on the expression of AKT but increases its phosphorylation in this cell line.

Figure 9: Protein Expression in Vemurafenib Sensitive and Resistant A375 Cells



The investigators examined the possibility that increased expression of p-glycoprotein (Pgp) might be a mechanism of resistance to vemurafenib inhibition. They determined the IC_{50} values for each cell line in the presence and absence of verapamil, an inhibitor of Pgp. Figure 10 shows the results of this experiment. Prior treatment with verapamil had no effect on the IC_{50} of vemurafenib in the parental cell line, but in each selected resistant line verapamil marginally decreased the IC_{50} value. This result suggests that increased expression of Pgp may contribute to resistance to vemurafenib. The sponsor did not submit a standard assay to demonstrate whether or not vemurafenib is a substrate for Pgp. A Western Blot to demonstrate expression of Pgp was inconclusive as it failed to detect any expression in any of the selected lines or the parent (not shown).

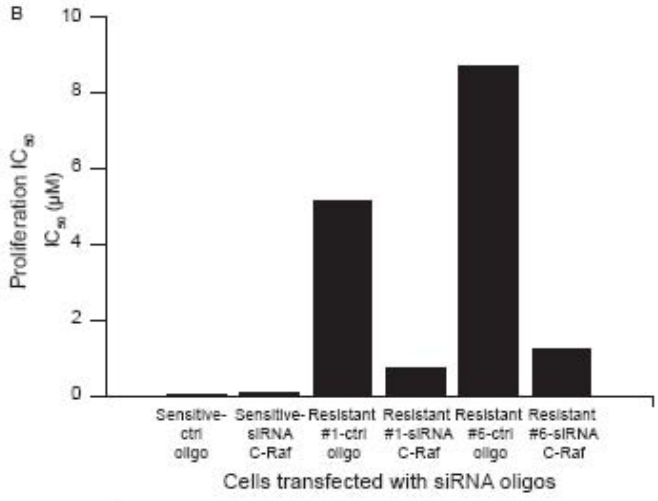
Figure 10: Effects of Pgp Upon Resistance to Vemurafenib



	parental	1	2	3	4	5	6
-verapamil	0.043	3.42	2.17	3.37	4.53	4.38	4.09
+ verapamil	0.045	2.73	1.05	2.55	3.69	2.62	3.43

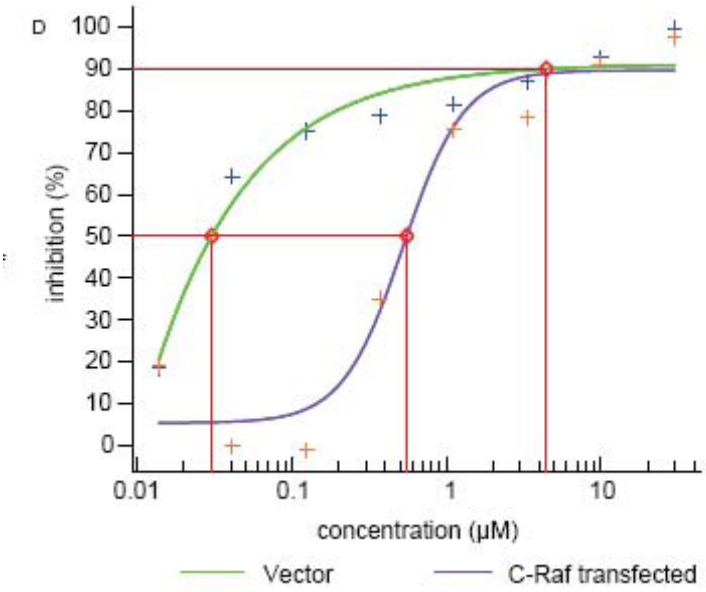
In the next experiment, cells of the parental line and cells of two resistant lines were depleted of CRAF by treatment with an siRNA construct. Figure 11 shows that transfection with the siRNA construct decreased the IC_{50} in the two resistant cell lines. These results imply that upregulation of CRAF is a mechanism of resistance.

Figure 11: Effects of Inhibition of CRAF Expression on Vemurafenib Resistance



The investigators then transfected parental A375 cells with a plasmid encoding CRAF, and demonstrated successful overexpression of the CRAF protein by Western blot analysis (not shown). Overexpression of CRAF protein with this transfected plasmid decreased the sensitivity of the parental A375 cells to vemurafenib inhibition by about 18-fold (IC_{50} shifted from 0.03 to 0.553 μM) (Figure 12).

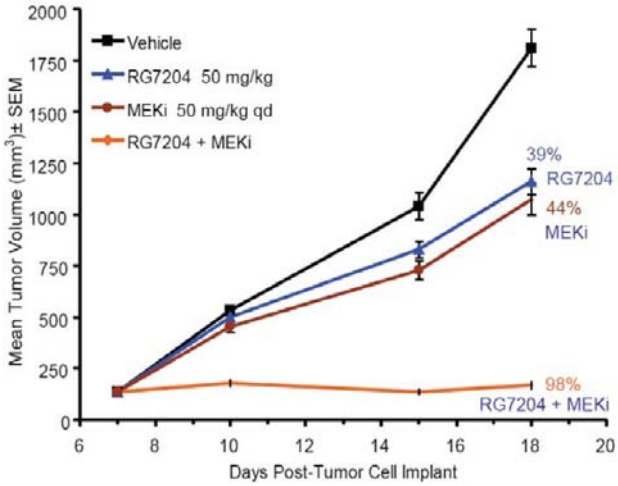
Figure 12: Effects of CRAF Overexpression on Vemurafenib Resistance



Next the investigators determined that all six resistant clones expressed elevated concentrations of activated RAS, again using immunoblotting. This finding led them to sequence the whole exome (targeted exome capture) of the parental and resistant clones, looking particularly at the sequences for N-, H-, and K-RAS. They identified four likely deleterious mutations that were present in all the resistant lines but not in the parental line. One of these was an uncommon, activating mutation (K117N) in the *KRAS* gene. To further evaluate the role of KRAS in the resistance to vemurafenib, they ablated KRAS in each of the cell lines using a KRAS-directed siRNA construct. KRAS down-regulation had no effect on the vemurafenib sensitivity of the parental A375 cells, but caused increased sensitivity in the resistant cells, with 2.3-fold (14.4 decreased to 6.1 µM) and 5.8-fold (11.5 decreased to 2.0 µM) decreases in vemurafenib IC_{50} values in resistant cell lines R1 and R6, respectively.

The investigators postulated that effective growth suppression of the resistant clones would require combination therapy. To test this concept they evaluated the effects of combining vemurafenib and the MEK inhibitor, RO5068760, in vemurafenib-resistant A375R6 cells. Single drug treatment with either vemurafenib or RO5068760 did not effectively inhibit ERK phosphorylation, as expected, since the resistant cells were also cross-resistant to MEK inhibitors (*vide infra*); nevertheless, in combination, BRAF and MEK inhibition significantly diminished the constitutive upregulation of ERK phosphorylation, inhibited cell cycle progression as monitored by Cyclin D1 levels, and induced apoptosis as indicated by cleaved PARP in the resistant cells (not shown). In an *in vivo* tumor implant study, treatment with both drugs was more effective than treatment with either drug alone (Figure 13, excerpted from the study report).

Figure 13: Inhibition of Vemurafenib Resistant Tumor Growth with Vemurafenib + MEKi



4.2 Secondary Pharmacology

1) PLX4032 NovaScreen Assay Report Major findings

This report did not provide enough information for comprehensive review. Nevertheless, at what appears to be a concentration of 10 µM, vemurafenib did not to inhibit any of the 63 major pharmacological active sights tested *in vitro*.

Study Number	1041083, DRN-102-057
File name	1041083
Laboratory	Plexxikon Inc., Berkeley, CA
Study Date	January 2006
GLP	No
Audited	No
Drug	RO5185426, Lot and purity not specified
Methods	Not specified
Assay	Not specified
Enzymes	63 standard pharmacological targets
Solvent	DMSO

4.3 Safety Pharmacology

1) Effects of PLX4032 on Action Potentials in Isolated Canine Cardiac Purkinje Fibers

Major findings

The tests at 0.2 and 2 µM and two of the tests at 20 µM were uninterpretable because the actual amount of vemurafenib in the perfusate was below the limit of quantitation by HPLC. The drug evidently had limited or highly variable solubility under

the conditions of this assay. Two tests at determined concentrations of 7.26 and 7.86 µM demonstrated a clear decrease in V_{max} of about 50% suggesting the potential for conduction delay. Visual inspection of the action potentials suggests a dose dependent delay in repolarization consistent with the QT prolongation seen clinically.

This study is inadequate to characterize the potential for cardiac toxicity associated with vemurafenib but it is strongly suggestive of such toxicity.

Study number	Sponsor – 1040810, Laboratory (b) (4) 578011, DRN-102-060
Filename	1040810
Laboratory	(b) (4)
Date	February 2007
GLP	Yes
Audited	Yes
Drug	PLX4032, Lot 02PLE04B-01-79, purity ≥ 96.8% This drug product is not the microprecipitated powder
Method	
Species	Beagle Dog
N	4 fibers per concentration
Tissue	isolated canine Purkinje fibers
Concentrations	0.2, 2, and 20 µM nominal Actual 7.26 and 7.86
Vehicle	DMSO
Electrical pace	0,5, 1 and 2 Hz
Parameters	Action potential duration
Positive control	100µM dl-sotalol (gave a positive result)

Results

The investigators sent samples of the perfusate from each test in this series to (b) (4) to determine the concentration of vemurafenib by HPLC. In two separate tests the vemurafenib concentration was below the limit of quantitation in all samples. A third test determined that the samples with nominal concentrations of 2 and 0.2 µM were below the limit of quantitation. In the samples from the tests at nominal concentrations of 20 µM, the concentrations in perfusate from two fibers was BLQ but that used to perfuse the other two fibers, A 07 TY 060718 and B 07 TY 060718, were 7.26 µM and 7.86 µM respectively. Despite this finding, the investigators reported the results of the analysis in Table 12.

(Excerpted from Sponsor)

Table 12: Summary of the Effects of PLX4032 on the Action Potential Parameters Measured from Isolated Cancine Purkinje Fibers

2 s BCL					
PLX4032	APD60	APD90	RMP	APA	Vmax
μM	(Δ%)	(Δ%)	(ΔmV)	(ΔmV)	(Δ%)
0.2*	-4.3 ± 3.6	-3.9 ± 3.3	-0.5 ± 0.4	1.4 ± 2.5	9.8 ± 8.5
2*	3.4 ± 2.4	0.3 ± 2.2	-1.0 ± 0.8	2.9 ± 3.0	0.3 ± 15.9
20*	5.7 ± 2.3	2.0 ± 1.8	-1.0 ± 0.5	0.7 ± 2.5	-15.7 ± 18.5

1 s BCL					
PLX4032	APD60	APD90	RMP	APA	Vmax
μM	(Δ%)	(Δ%)	(ΔmV)	(ΔmV)	(Δ%)
0.2*	-0.2 ± 4.4	-0.9 ± 3.8	-0.7 ± 0.5	0.6 ± 1.6	7.8 ± 6.2
2*	3.3 ± 2.4	0.0 ± 2.0	0.9 ± 1.4	-1.2 ± 2.0	-0.9 ± 10.5
20*	5.4 ± 1.6	2.1 ± 0.8	-0.3 ± 0.4	-0.4 ± 1.5	-17.5 ± 18.3

0.5 s BCL					
PLX4032	APD60	APD90	RMP	APA	Vmax
μM	(Δ%)	(Δ%)	(ΔmV)	(ΔmV)	(Δ%)
0.2*	-0.8 ± 2.7	-0.7 ± 2.8	-0.5 ± 0.9	-0.1 ± 1.8	6.1 ± 6.6
2*	3.7 ± 1.1	1.0 ± 1.3	0.1 ± 0.6	0.2 ± 1.9	-4.2 ± 11.1
20*	4.4 ± 1.5	1.8 ± 1.1	0.4 ± 0.7	-1.1 ± 1.5	-19.0 ± 17.8

None of the parameters show any significant change, but those for the nominal concentrations of 0.2 and 2 μM are uninterpretable as there is apparently no measureable drug in the perfusate and those done at 20 μM are obfuscated by the fact that results from the two fibers that were perfused with concentrations of drug BLQ are averaged with those that were measurable. Despite this inappropriate analysis the results demonstrate a decrease in V_{max} that appears to be toxicologically significant.

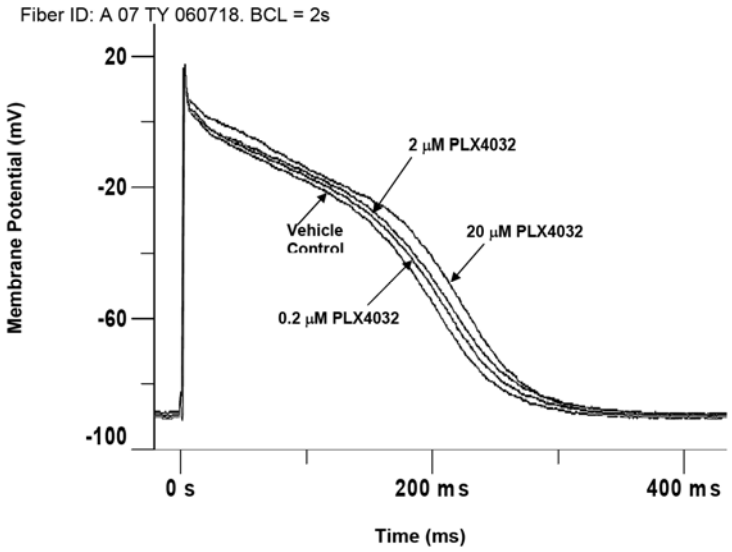
A visual comparison of the individual results for the two fibers actually perfused with measureable concentrations of drug showed that there was no effect on action potential duration 60% (APD60), action potential duration 90% (APD90), resting membrane potential (RMP) and action potential amplitude (APA). Perfusion with vemurafenib did cause a change in the maximum rate of depolarization (V_{max}) at all three stimulation frequencies. Table 13 shows this decrease in V_{max} for the two fibers that were actually perfused with drug. Due to the small sample size statistical analysis is not reliable but the values are sufficiently large as to clearly demonstrate toxicological significance, particularly in light of the fact that vemurafenib causes QT prolongation clinically.

Table 13: Vemurafenib Effects on V_{max}

	[Vemurafenib]	dV/dt max	
2 sec BCL	µM	mV	
A 07 TY 060718	0	244.1	
B 07 TY 060718	0	366.2	
A 07 TY 060718	7.26	131.8	-46%
B 07 TY 060718	7.86	195.3	-47%
	[Vemurafenib]	dV/dt max	
1 sec BCL	µM	mV	
A 07 TY 060718	0	244.1	
B 07 TY 060718	0	376	
A 07 TY 060718	7.26	122.1	-50%
B 07 TY 060718	7.86	200.2	-47%
	[Vemurafenib]	dV/dt max	
0.5 sec BCL	µM	mV	
A 07 TY 060718	0	239.3	
B 07 TY 060718	0	371.1	
A 07 TY 060718	7.26	127	-47%
B 07 TY 060718	7.86	190.4	-49%

Additionally, visual inspection of the graph of the action potentials from the submission (Figure 14) generated with stimulation at 2 Hz show what appears to be a clear dose effect.

Figure 14: Overlapping Action Potential Traces Obtained after Equilibrium with Control and 0.2, 2, and 20 µM PXL4032 (Nominal)



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2) Effects of PLX4032 on Cloned hERG Channels Expressed in Human Embryonic Kidney Cells

Major findings

Vemurafenib inhibited the delayed rectifier cardiac potassium current (I_{Kr}) in human embryonic kidney cells expressing hERG with an IC_{50} of 1.24 µM and a Hill Coefficient of 1.7. Thus, vemurafenib has the potential to cause cardiac toxicity at physiologically relevant concentrations. Under identical conditions, the positive control, terfenadine produced 83.6 ± 0.1% block of the hERG currents at 60 nM.

Study number	Sponsor – 1040807, Laboratory – 060329.XSM, (b) (4)-578008
Filename	1040807
Laboratory	(b) (4)
Date	January 2007
GLP	Yes
Audited	Yes
Drug	PLX4032, Lot 02PLE04B-01-79, purity ≥ 96.8% This drug product is not the microprecipitated powder
Method	
Cells	Human embryonic kidney cells transfected with the human ether-a-go-go gene (hERG)
N	≥ 3
Vehicle	DMSO
Doses	0.3, 1, and 3 µM nominal 0.35, 0.61 and 1.90 µM actual
Positive control	Terfenadine, 60 nM

Results

(Excerpted from the submission)

Table 14: hERG Inhibition vs. PLX4032 Concentrations

PLX4032 Measured Concentration (µM)	Mean % hERG Inhibition	SD	SEM	n
Vehicle	0.8%	0.6%	0.3%	3
0.35	8.7%	1.4%	0.8%	3
0.61	25.5%	1.2%	0.7%	3
1.90	66.8%	4.2%	2.1%	4

The calculated IC_{50} of PLX4032 for hERG currents was estimated to be 1.24 µM with a Hill Coefficient of 1.7. Under identical conditions, the positive control, terfenadine produced 83.6 ± 0.1% (Mean ± SD) block of the hERG currents at 60 nM when tested on two cells (n=2).

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3) Cardiovascular Assessment of Orally Administered PLX4032 in Conscious Radiotelemetry-Implanted Beagle Dogs

Major findings

In this standard study in conscious dogs, single oral doses as high as 1000 mg/kg (20000 mg/m^2) of vemurafenib caused no mortality and no clinical signs. Treatment was associated with a relative decrease in systolic, diastolic and mean arterial blood pressure between 11 and 24 hours (difference about 20 mm in systolic pressure at 18 hours relative to control) but the difference appears to be the result of an increase in pressure in the controls relative to baseline which is difficult to explain. The RR interval increased significantly (20%) in the HD group at 3 hours (approximately t_{max}) and significantly decreased by 15% at 6 hours relative to controls. QT interval was significantly prolonged by about 12 ms at 3 hours post dosing (approximately t_{max}) but this difference did not persist when QT was corrected for heart rate. There was also an increase in the incidence of AV block in treated dogs. This study did not use the MPB formulation and did not determine pharmacokinetics so the results are difficult to interpret. Nevertheless, vemurafenib appears to have some effects on cardiac parameters.

Study no:	Sponsor – 1040811; Laboratory – (b) (4) 578013
File name:	1040811
Laboratory:	(b) (4)
Study Date:	May 2007
GLP:	Yes
Audited:	Yes
Drug:	PLX4032, Lot no. 02PLE04B-01-79, purity 96.5% This drug product is not the microprecipitated powder
Method	
Species	Beagle dogs, conscious with surgically implanted telemetry devices
N	Four males, 11.8 kg to 16.4 kg
	Doses 0, 30, 100 and 1000 mg/kg 0, 600, 2000 and 20000 mg/m^2
Schedule	Single doses Latin-square cross over design with two day washout between doses
Dose volume	5 mL/kg
Route	PO gavage
Cage Side	Observed at least twice daily
Clinical Obs.	Prior to each dose
Post Dose Obs.	Three hours following each dose
Body weight	Prior to the administration of each dose and SD 37
Cardiac parameters	Baseline recordings were obtained from all animals for approximately 1 hour prior to dosing. Heart rate (derived from arterial waveforms), arterial pressure (systolic, diastolic and calculated mean), body temperature and electrocardiographic (ECG) intervals (PR, QRS, RR, QT and QTCV [Van de Water's correction; QTCV = QT-0.087*(RR-1)]) were acquired using the Dataquest® A.R.T.™ Gold software. Following the administration of each dose of PLX4032, the parameters listed above were recorded for a 30-second interval every 10 minutes for approximately 24 hours.

Respiratory Not done

Results

Drug Analysis Within specifications
Mortality None

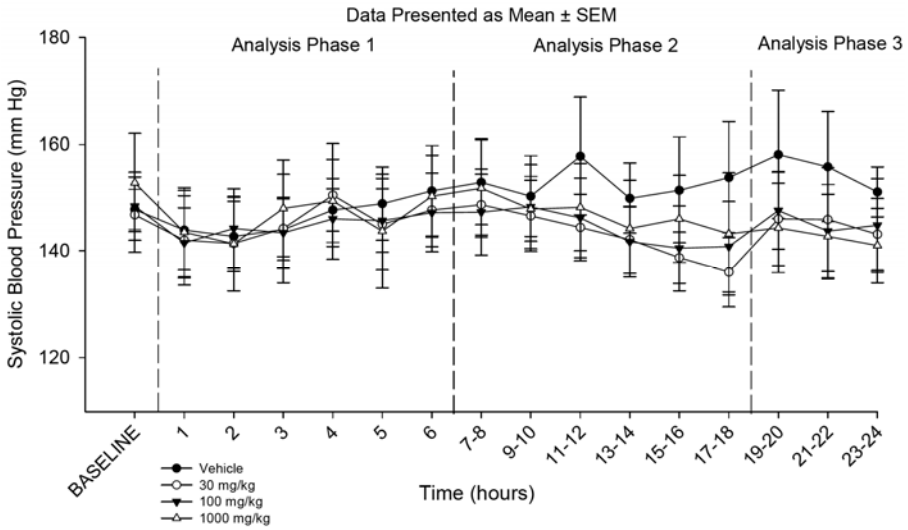
Clinical Observations No toxicologically significant changes

Body Weight Not analyzed

Body Temperature Transient differences of questionable toxicological significance

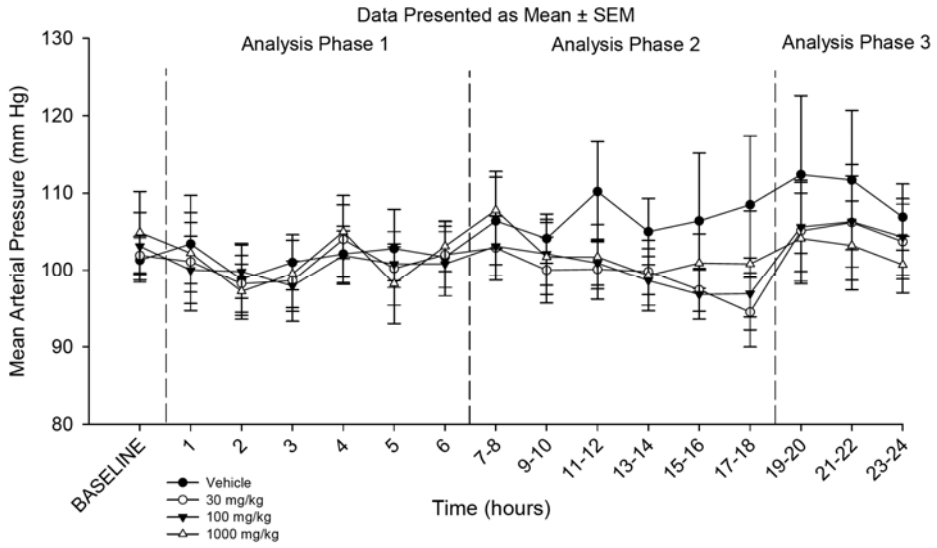
Blood Pressure While there were no statistically significant changes, the systolic pressure in all groups treated with vemurafenib was lower than control values 11 hours after treatment through the end of the experiment (24 hours). The chart below (Figure 15) from the submission shows that this difference is probably toxicologically significant (Figure 15). Diastolic pressure followed a similar pattern (not shown).

Figure 15: Systolic Blood Pressure



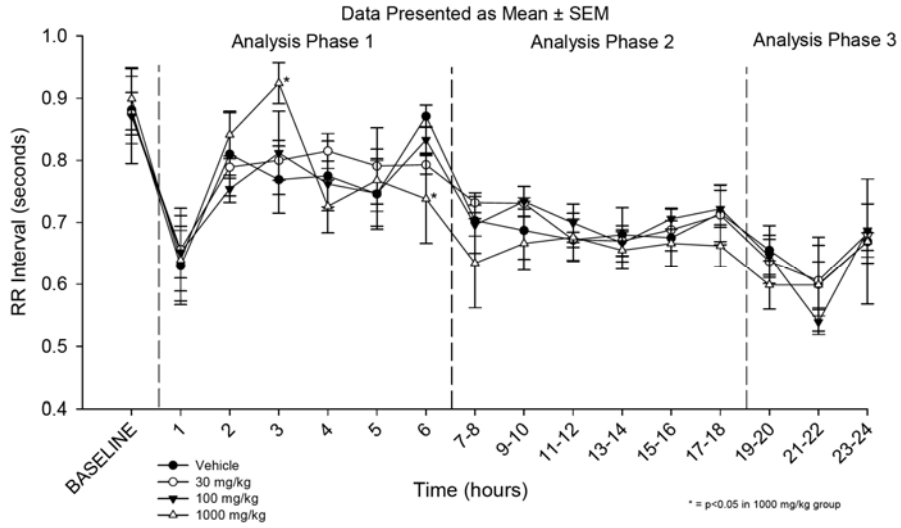
Mean Arterial pressure Like systolic and diastolic pressure, mean arterial pressure was consistently lower in treated dogs between 11 hours after dosing and the end of the monitoring period though this difference did not reach statistical significance. The following graph from the study report shows this result. The difference is most pronounced 17 to 18 hours after dosing (Figure 16).

Figure 16: Mean Arterial Pressure



PR interval No toxicologically significant effects
QRS Complex No toxicologically significant effects
RR interval Significantly increased by 20% in the HD group at 3 hours (approximately t_{max}) and significantly decreased by 15% at 6 hours relative to controls as shown by the chart from the submission (Figure 17).

Figure 17: RR Interval



QT interval Significantly prolonged by about 12 ms at 3 hours post dosing (approximately t_{max}). This difference did not persist when QT was corrected for rate.

ECG findings There was an increase in the incidence AV block in treated dogs as shown by the following table from the study report.

Table 15: Incidence of ECG Findings

Animal No.	Prior To Dosing (Vehicle or Baseline)	30 mg/kg	100 mg/kg	1000 mg/kg
1363	ESC1	-	ESC2	-
1870	ESC1	-	-	-
1365	-	AVB2 (2)	-	PAC (2), AVB2 (6)
1862	-	-	-	-

AVB2 = Atrioventricular block 2nd degree
ESC1 = Single episode of SA block/arrest with escape complex
ESC2 = Multiple episodes of SA block/arrest with escape complex
PAC= Premature atrial contraction
( ) = Number in parentheses indicates the total number of occurrences.

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4) The Acute Central Nervous System Pharmacological Study of RO5185426-002-003 (b-Raf Inhibitor) Following Oral Administration in Rats Using a Modified Functional Observation Battery

Major findings

A single dose of vemurafenib of 6000 mg/m^2 given to male rats caused no toxic signs or symptoms in the Functional Observational Battery. Nevertheless, the formulation used in this test was evidently not the microprecipitated drug product, thus the exposure was probably too low to cause toxicity because of limited bioavailability. The positive control caused the anticipated toxic signs and symptoms.

Study no:	Sponsor – 1026179; Laboratory – (b) (4)-30031
File name:	1026179
Laboratory:	(b) (4)
Study Date:	April 2007
GLP:	Yes
Audited:	Yes
Drug:	RO5185426-002-003, Lot no. 02PLE04B-02-80M, Purity 99.5 %
Method	
Species	Crl:CD(SD)Rats
N	6 males per dose group for FOB
	Doses 0, 30, 100, 1000 mg/kg (0, 180, 600, or 6000 mg/m^2)
Positive Control	Chlorpromazine HCl, 10 mg/kg SC
Schedule	Single dose
Route	PO Gavage
Vehicle	Corn Oil
Dose Volume	5 mL/kg
Cage side	At least twice a day
Clinical Obs	24 hours before and after dosing

FOB	Approximately 1, 2, 4, 6, 12 and 24 hours after dose administration, qualitative motor activity assessment at each time point
Histopathology	Not done

Results

Mortality	None
Clinical Observations	None
Body Weight	No effects
FOB	No toxicologically significant changes

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5) Respiratory Assessment of Orally Administered RO5185426-002-003 (b-Raf Inhibitor) to Plethysmograph- Restrained Male Sprague-Dawley Rats,

Major findings

The respiratory frequency of rats treated with the high dose of 1000 mg/kg was consistently higher than that of controls. This increase reached statistical significance in analysis phase 1 (120 to 160 minutes post dosing). This increase may have been due to the stress of the toxicity of the vemurafenib and not a direct pharmacological effect. Minute volume increased as a result of the increase in rate, but this increase did not reach statistical significance.

Study no:	Sponsor – 1026178; Laboratory – (b) (4)-30030
File name:	1026178
Laboratory:	(b) (4)
Study Date:	April 2007
GLP:	Yes
Audited:	Yes
Drug:	RO5185426-002-003, Lot no. 02PLE04B-02-80M, Purity 99.5 %
Method	
Species	Crl:CD(SD)Rats
N	8 males per dose group, four replicates of 2 animals from each group over a 4-day period
	Doses 0, 30, 100, 1000 mg/kg (0, 180, 600, or 6000 mg/m^2)
Positive Control	baclofen, 20 mg/kg
Schedule	Single dose
Route	PO Gavage
Vehicle	Corn Oil
Dose Volume	5 mL/kg
Cage side	At least twice a day
Clinical Obs	daily
Assessment	Each rat was placed in a head-out, neck-sealed plethysmograph. Respiratory function data were collected for at least 60 minutes prior to dosing and for at least 5 hours post-dosing (beginning approximately 2 hours post-dosing).

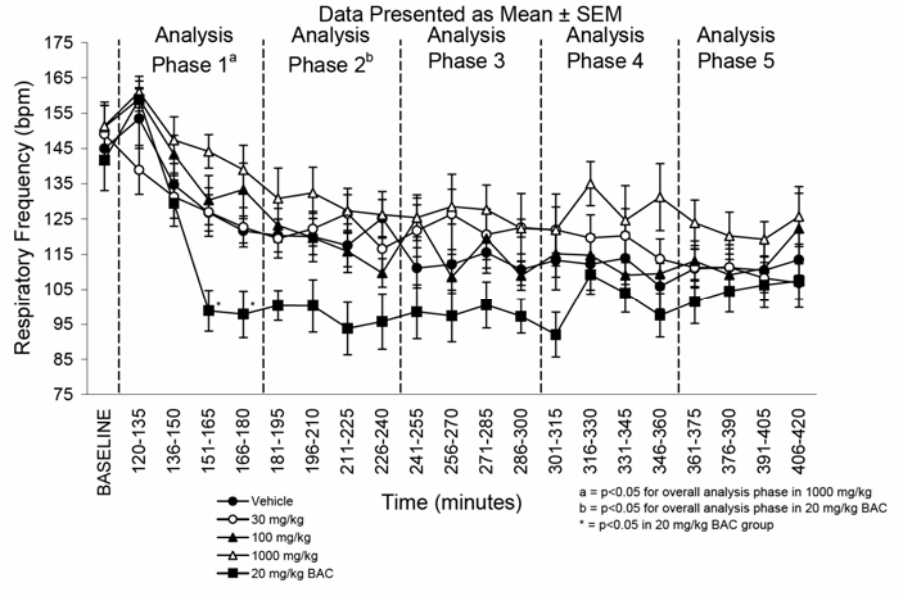
Histopathology Not done

Results

Mortality None
Clinical Observations None
Body Weight No effects
Respiratory parameters

The following graph from the study report (Figure 18) shows that the respiratory frequency of rats treated with the high dose of 1000 mg/kg was consistently higher than that of controls. This increase reached statistical significance in analysis phase 1 (120 to 160 minutes post dosing). This increase may have been due to the stress of the toxicity of the vemurafenib and not a direct pharmacological effect.

Figure 18: Respiratory Frequency



There were no statistically significant differences in tidal volume. In the high dose group minute volume was consistently greater than controls but this difference was small and did not reach statistical significance. This increase probably reflects the increase in respiratory rate in the HD group.

5 Pharmacokinetics/ADME/Toxicokinetics

5.2 Toxicokinetics

1) 26-Week Oral Gavage Toxicity and Toxicokinetic Study with RO5185426-007 (b-Raf Inhibitor) in Rats with a 13-Week Interim Sacrifice and a 12-Week Recovery Phase

Study no: Roche Reference No. 10165; (b) (4) 6131-555
File name: 1025760.pdf
Laboratory: (b) (4)
Study Date: February 2008
GLP: Yes
Audited: Yes
Drug: RO5185426-007, Lot 97617, purity 29.3%
RO5185426-007, Lot 97620, purity 29.5%
The test article was micro-precipitated bulk (MBP) powder containing
drug and "placebo blend" (nominally 70.9% of the total mass containing (b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide)

Methods:

Species: Male and female Crl:CD(SD) rats
6 to 7 weeks old
Body weights 197 to 288 g for males and 136 to 214 g for females

Dose Groups: The following table from the study report shows the dose groups and number of animals in each group. The legend for the table explains the necropsy schedule.

Group	No. of Animals[b] Male	Female	Dose Level[c] (mg/kg/day)	Dose Concentration[d] (mg/mL)
Toxicity Animals				
1 (Control)[a]	36	36	0	0
2 (Low)	36	36	10	1
3 (Mid)	36	36	50	5
4 (High)	36	36	450	45
Toxicokinetic Animals[e]				
5 (Control)[a]	4	4	0	0
6 (Low)	10	10	10	1
7 (Mid)	10	10	50	5
8 (High)	10	10	450	45

a Groups 1 and 5 received control article only.
b Toxicity animals designated for interim (scheduled phase) sacrifice (10 animals/sex in Groups 1, 2, 3, and 4) were sacrificed after 13 weeks (92 days) of dose administration. Toxicity animals designated for dosing phase sacrifice (17 or 18 animals/sex in Groups 1, 2, 3, and 4, dependent on survival) were sacrificed after 26 weeks of dose administration. Toxicity animals designated for recovery phase sacrifice (six to eight animals/sex in Groups 1, 2, 3, and 4, dependent on survival) underwent 12 weeks of recovery following the dosing phase.
c Dose levels represent doses of active pharmaceutical ingredient RO5185426 (free acid).
d The dose volume was 10 mL/kg.
e The first three animals/sex in Group 5 and the first nine animals/sex/group in Groups 6, 7, and 8 were designated for blood collection. The remaining toxicokinetic animals were used as replacements on Days 91, 104, and 182, as appropriate, for animals that died or were sacrificed at an unscheduled interval.

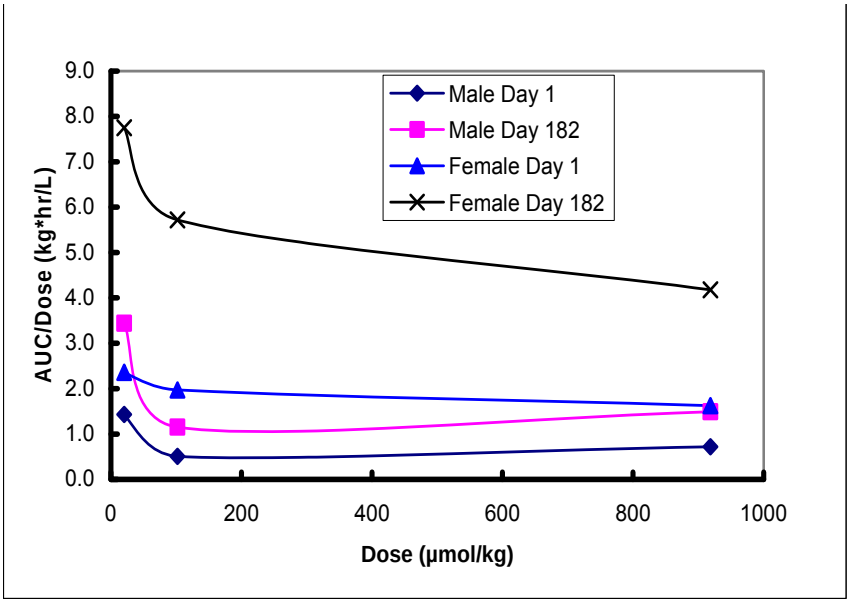
Doses:	0, 10, 50 or 450 mg/kg (0, 60, 300 or 2700 mg/m^2) 450 mg/kg was a maximum feasible dose for this formulation
Formulation:	2% Klucel LF (w/v) in reverse osmosis water adjusted to pH 4 with 1N hydrochloric acid
Placebo	110 mg/mL placebo blend ((b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide) in vehicle corresponding to the placebo blend concentration of the 450 mg/kg formulation
Route	PO Gavage
Toxicokinetics:	Days 1, 91, 104, and 182 predose, 0.5, 1, 2, 4, 8, 12 and 24 hours – two or three animals per time point, analysis by HPLC
Calibration	2.5 to 5000 ng/mL

Results

Male	T_{max} [hours]				C_{max} [µM]				$AUC_{(0-24)}$ µM*hr			
Dose mg/kg	Day 1	Day 91	Day 104	Day 182	Day 1	Day 91	Day 104	Day 182	Day 1	Day 91	Day 104	Day 182
10	2	2	2	2	5.1	9.1	11.9	11.4	29.2	55.1	70.8	70.2
50	1	1	1	1	14.9	29.2	32.0	27.8	51.6	110.2	131.9	117.6
450	2	1	1	1	82.5	120.4	135.1	160.6	661.3	1036.9	1239.0	1367.5
Female	T_{max} [hours]				C_{max} [µM]				$AUC_{(0-24)}$ µM*hr			
Dose mg/kg	Day 1	Day 91	Day 104	Day 182	Day 1	Day 91	Day 104	Day 182	Day 1	Day 91	Day 104	Day 182
10	2	0.5	0.5	2	6.4	15.6	17.3	22.5	48.2	98.8	124.1	158.2
50	2	0.5	2	0.5	23.9	50.0	66.7	81.6	200.8	389.9	471.5	583.8
450	2	2	2	2	143.1	234.7	273.5	300.0	1494.1	2347.3	3469.9	3837.3

Apparent T_{max} with the MBP formulation was reached quickly in rats, usually by hour 2; it did not vary with time on study. The study provided no calculation of volume of distribution or clearance. Exposure in HD females was higher than in HD males. A simple plot of Dose vs C_{max} or AUC appears to demonstrate that the increase in these parameters is linear, but this is illusory. The two low doses are relatively much closer to each other than are the MD and HD so this latter span dominates any liner analysis. Nevertheless, a plot of Dose vs Dose Normalized C_{max} or Dose Normalized AUC (Figure 19) clearly demonstrates non-linearity and a plateau after the MD. This decrease in exposure is likely due to induction of enzyme metabolism or induction of some elimination mechanism.

Figure 19: Dose vs. Dose Normalized AUC in Male and Female Rats



2) 13-Week Oral Gavage Toxicity and Toxicokinetic Study with RO5185426-006 in Dogs with a 4-Week Interim Sacrifice and a 4-Week Recovery Phase

Study no: (b) (4) Study No. 8217797; Roche Reference No. 1032862
File name: 1032862
Laboratory: (b) (4)
Study Date: August 2010
GLP: Yes
Audited: Yes
Drug: RO5185426-006
Lot 120785, Purity 28.8% (99% of label claim)
Lot 120787, Purity 28.9% (99.3% of label claim)
Both these lots are from Batch BS0906SH10
The test article was micro-precipitated bulk powder containing drug and "placebo blend" (nominally 70.9% of the total mass containing (b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide)

Methods:

Species: Beagle dog, 14 to 15 months old, and their body weights ranged from 7.7 to 10.4 kg for males and 7.1 to 8.8 kg for females. Many of these animals had been treated with vemurafenib on a previous study (Roche Reference No. 1033163, *vide infra*). The drug free period was one month.

Dose Groups The following table from the study report shows the dose groups and number of animals in each group. The legend for the table explains the necropsy schedule.

Group[a]	No. of Animals[b] Male	Female	Dose Level[c] (mg/kg/dose)	(mg/kg/day)	Dose Concentration[d] (mg/mL)
1 (Control)	6	6	0	0	0
2 (Low)	9	9	75	150	7.5
3 (High)	9	9	150	300	15

a Group 1 received control article only.
b Animals designated for interim sacrifice (two animals/sex in Group 1 and up to three animals/sex in Groups 2 and 3, dependent on survival) were sacrificed after 4 weeks (29 days) of dose administration. Animals designated for the dosing phase final sacrifice (two animals/sex in Group 1 and up to four animals/sex in Groups 2 and 3, dependent on survival) were sacrificed after 13 weeks (92 days) of dose administration. Animals designated for the recovery phase sacrifice (two animals/sex/group) underwent 4 weeks of recovery following dose administration.
c Dose levels and concentrations represent doses of active pharmaceutical ingredient (free acid).
d Animals were dosed BID, 8 hours ± 30 minutes apart, at a volume of 10 mL/kg for each dose.

Doses	0, 50, 150 mg/kg BID nominal (0, 2000, or 6000 mg/m^2/day)
Schedule	Twice daily administered 8 hours ± 30 minutes apart
Formulation	2% Klucel LF (w/v) in reverse osmosis water adjusted to pH 4 with 1N hydrochloric acid
Dose volume	10 mL/kg
Placebo	36.6 mg/mL of the placebo blend (b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide) in vehicle.
Route	PO Gavage
Toxicokinetics	Days 1, 29, and 92 of the dosing phase prior to the a.m. dose and approximately 1, 3, 7, 9, 11, and 24 hours post-dose (based on the a.m. dose), analysis by HPLC See Toxicokinetics and Pharmacokinetics section above

Results

Table 16-Table 17 present the pharmacokinetic parameters calculated from the measurements taken in this study for male and female dogs.

Table 16: PK Parameters from 13 Week Dog Study—Male

Male	Dose mg/kg/day	Day	T_{max} (hours)	C_{max} (ng/mL)	C_{max} (µM)	$AUC_{(0-24h)}$ ng*hr/mL	$AUC_{(0-24h)}$ µM*hr
G2	150	1	9	25400	51.8	345000	704
G2	150	29	9	28100	57.4	414000	845
G2	150	92	9	23100	47.1	289000	590
G3	300	1	11	43500	88.8	590000	1200
G3	300	29	6	36100	73.7	478000	976
G3	300	92	9	34900	71.2	384000	784

Table 17: PK Parameters from 13 Week Dog Study—Female

Female	Dose mg/kg	Day	T_{max}	C_{max} (ng/mL)	C_{max} (μM)	$AUC_{(0-24h)}$ ng*hr/mL	$AUC_{(0-24h)}$ μM*hr
G2	150	1	9	20600	42	282000	576
G2	150	29	11	27600	56.3	396000	808
G2	150	92	9	22400	45.7	250000	510
G3	300	1	11	42300	86.3	571000	1170
G3	300	29	3	31600	64.5	423000	863
G3	300	92	9	25900	52.9	360000	735

Apparent Tmax is rather long, usually 9 to 11 hours. The study provided no calculation of volume of distribution or clearance. The following graph shows the change in C_{max} in male and female dogs over the course of the 92 days of the experiment.

Figure 20: Time on Study vs C_{max} (μM) in Male and Female Dogs

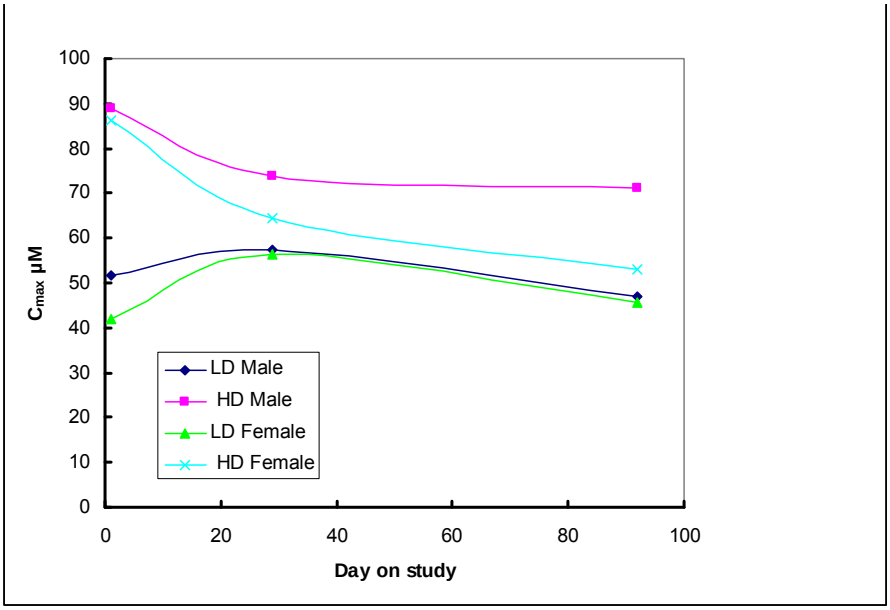
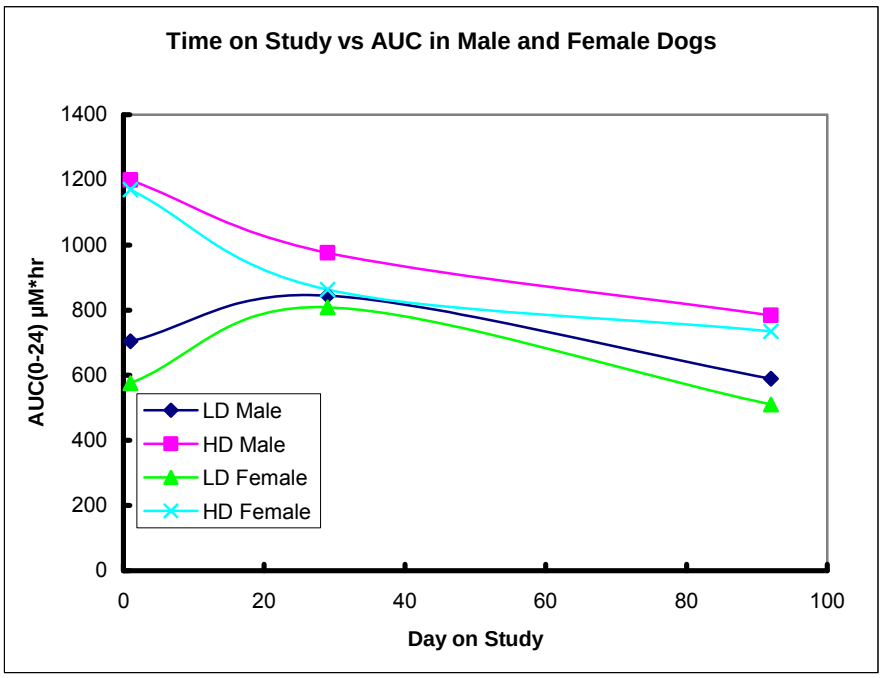


Figure 20 shows that the increase in C_{max} is approximately proportional to the increase in dose on day 1, but is much less than dose proportional on days 29 and 92 as the drug reaches steady state. There is no appreciable difference between males and females in the low dose group, but consistent with the increased toxicity, C_{max} in HD males is somewhat higher than it is in females on days 28 and 92.

The following graph shows the change in $AUC_{(0-24\ hr)}$ in male and female dogs over the course of the 92 days of the experiment.



AUC follows a pattern very similar to that of C_{max}, again demonstrating dose proportionality only on day 1. Both doses appear to converge on almost equivalent steady-state values after by day 29. Again exposure was somewhat higher in the HD males on day 29 consistent with the greater toxicity seen in males in the first month of the study. The decrease in exposure (possibly due to the induction of hepatic metabolism) explains why no dogs died on study after day 31.

6 General Toxicology

6.1 Single-Dose Toxicity

Not reviewed formally reviewed, see Overall Summary

6.2 Repeat-Dose Toxicity

1) 26-Week Oral Gavage Toxicity and Toxicokinetic Study with RO5185426-007 (b-Raf Inhibitor) in Rats with a 13-Week Interim Sacrifice and a 12-Week Recovery Phase

Major findings:

Rats tolerated single daily doses of RO5185426 (micro-precipitated formulation) at doses as high as 450 mg/kg (2700 mg/m^2) for 26 weeks with no drug related mortality or signs of clinical toxicity. The high dose did cause an increase in cholesterol (22% in males and 58% in females). Female rats showed a dose dependent decrease in AST and ALT that remained observable after 13 weeks of recovery. In high dose females,

there was a dose dependent decrease in neutrophils (about 26%) at the end of dosing. Dosing was not associated with microscopic changes. Exposures were unusually high, with C_{max} values reaching 161 µM in HD males and 300 µM in HD females at the end of dosing.

Study no: Roche Reference No. 10165; (b) (4) 6131-555
File name: 1025760.pdf
Laboratory: (b) (4)
Study Date: February 2008
GLP: Yes
Audited: Yes
Drug: RO5185426-007, Lot 97617, purity 29.3%
RO5185426-007, Lot 97620, purity 29.5%
The test article was micro-precipitated bulk powder containing drug and "placebo blend" (nominally 70.9% of the total mass containing (b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide)

Methods:

Species: Male and female Crl:CD(SD) rats
6 to 7 weeks old
Body weights 197 to 288 g for males and 136 to 214 g for females

Dose Groups: The following table from the study report shows the dose groups and number of animals in each group. The legend for the table explains the necropsy schedule.

Group	No. of Animals[b] Male	Female	Dose Level[c] (mg/kg/day)	Dose Concentration[d] (mg/mL)
Toxicity Animals				
1 (Control)[a]	36	36	0	0
2 (Low)	36	36	10	1
3 (Mid)	36	36	50	5
4 (High)	36	36	450	45
Toxicokinetic Animals[e]				
5 (Control)[a]	4	4	0	0
6 (Low)	10	10	10	1
7 (Mid)	10	10	50	5
8 (High)	10	10	450	45

a Groups 1 and 5 received control article only.
b Toxicity animals designated for interim (scheduled phase) sacrifice (10 animals/sex in Groups 1, 2, 3, and 4) were sacrificed after 13 weeks (92 days) of dose administration. Toxicity animals designated for dosing phase sacrifice (17 or 18 animals/sex in Groups 1, 2, 3, and 4, dependent on survival) were sacrificed after 26 weeks of dose administration. Toxicity animals designated for recovery phase sacrifice (six to eight animals/sex in Groups 1, 2, 3, and 4, dependent on survival) underwent 12 weeks of recovery following the dosing phase.
c Dose levels represent doses of active pharmaceutical ingredient RO5185426 (free acid).
d The dose volume was 10 mL/kg.
e The first three animals/sex in Group 5 and the first nine animals/sex/group in Groups 6, 7, and 8 were designated for blood collection. The remaining toxicokinetic animals were used as replacements on Days 91, 104, and 182, as appropriate, for animals that died or were sacrificed at an unscheduled interval.

Doses: 0, 10, 50 or 450 mg/kg (0, 60, 300 or 2700 mg/m^2)
450 mg/kg was a maximum feasible dose for this formulation
Schedule: QD

Formulation:	2% Klucel LF (w/v) in reverse osmosis water adjusted to pH 4 with 1N hydrochloric acid
Placebo	110 mg/mL placebo blend ((b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide) in vehicle corresponding to the placebo blend concentration of the 450 mg/kg formulation
Route	PO Gavage
Clinical signs:	twice daily
Body weights:	Recorded for all animals once during the predose phase, before dosing on Day 1 of the dosing phase, and weekly thereafter
Food cons.:	Measured for toxicity animals weekly during the dosing and recovery phases
Ophthalmology:	Done for all animals once during the predose phase and for toxicity animals during Weeks 13 and 26 of the dosing phase and during Week 12 of the recovery phase
Hematology:	10 animals per sex per dose group at necropsy
Clinical chem.:	10 animals per sex per dose group at necropsy
Necropsy:	See table above
Histopathology:	Adequate battery
Toxicokinetics:	Days 1, 91, 104, and 182 predose, 0.5, 1, 2, 4, 8, 12 and 24 hours – two or three animals per time point, analysis by HPLC

Results

Mortality — The following table from the study report shows the unscheduled deaths during the study. The table shows that treatment with RO5185426 did not affect mortality.

Animal No.	Group/Sex	Description	Day of Death (Dosing Phase)	Death Comment
B71903	1/Male	Moribund	26	Undetermined
B71960	2/Male	Found dead	184	Undetermined
B72001	3/Male	Moribund	41	Inflammation
B72029	4/Male	Accidental	118	Gavage-related death
B72131	2/Female	Moribund	149	Hematopoietic neoplasm, lymphosarcoma
B72136	2/Female	Moribund	20	Inflammation

Clinical Signs — No toxicologically significant or drug related signs

Body Weight — No toxicologically significant changes

Food Consumption — No toxicologically significant changes

Ophthalmology — No toxicologically significant changes

Clinical Chemistry — The following table shows that even the high dose of RO5185426 caused remarkably little toxicity

	Day	Control	10 mg/kg	50 mg/kg	450 mg/kg	Recovery
Male Cholesterol	184	102	6.9%	2.9%	**21.6%**	Normal
Female Cholesterol	184	106	2.8%	1.9%	**58.5%**	Remains elevated
Female ALT	184	108	-15.7%	**-55.6%**	**-46.3%**	Remains decreased
Female AST	184	184	-9.2%	-34.8%	**-31.0%**	Remains decreased

Hematology Likewise changes in hematology values were limited to neutrophils in females

	Day	Control	10 mg/kg	50 mg/kg	450 mg/kg	Recovery
Female Neutrophils	184	0.83	12.0%	**-19.3%**	**-26.5%**	Normal
Female % Neutrophils	184	15.4	18.2%	-11.0%	**-24.7%**	Normal

Organ weights No toxicologically significant findings

Gross Pathology No toxicologically significant findings

Histopathology No toxicologically significant findings

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2) 39-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with RO5185426-006 in Dogs with an 8-Week Recovery Phase

This study was intended to continue for 39 weeks but the high dose of 450 mg/kg BID PO proved intolerable after 10 days. Dosing was suspended at this time in HD animals and two moribund HD dogs (one male, one female) were killed humanely and examined. These dogs were replaced on study and dosing was resumed on day 19 at a lowered dose of 300 mg/kg BID PO in the HD dogs. This dose also proved intolerable and dosing was again suspended on day 37. The study was aborted and the dogs transferred to the subsequent 13 week study (*vide infra*, study1032862).

The HD dogs showed clinical signs of excessive salivation, vomiting, dehydration, soft or malformed feces and hypoactivity. MD (150 mg/kg BID) animals showed these signs less frequently and with less severity. Some dogs in all dosed groups vocalized and struggled during dosing suggesting pain. HD animals, particularly those killed humanely, showed signs of thin appearance, tremor and reddened skin.

Dosing caused dose dependent increases in liver function enzymes and decreases in other parameters consistent with significant liver damage. Nevertheless, this liver damage was not sufficiently severe to have necessitated ending the study. Other, undefined toxicities are probably responsible for the clinical signs that resulted in the termination of the study. Dosing also caused dose dependent neutrophilia with profound eosinophilia consistent with an allergic response.

The two dogs killed humanely were the only ones examined post mortem. In the livers of these two dogs there was minimal scattered hepatocellular degeneration, a mild to moderate increase in the number of Kupffer cells, and the presence of pigment in hepatocytes and Kupffer cells. These findings correlated with increased ALP, ALT, AST and GGT levels in both moribund dogs. In the sternal bone marrow of the moribund female there was moderate pancytic necrosis. There was no finding of necrosis in the femoral bone marrow section or in the spleen.

Study no: (b) (4) Study No. 8203050; Roche Reference No. 1033163
File name: 1033163.pdf
Laboratory: (b) (4)
Study Date: July 2009
GLP: Yes
Audited: Yes
Drug: RO5185426-006 120785 At room temperature 28.8% (99% of label claim)
The test article was micro-precipitated bulk powder containing drug and "placebo blend" (nominally 70.9% of the total mass containing (b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide)

Methods:

Species: Beagle dog,12 to 13 months old, body weights ranged from 7.5 to 9.6 kg for males and 5.9 to 7.8 kg for females.

Dose Groups: The following table from the study report shows the dose groups and number of animals in each group. The legend for the table explains the necropsy schedule.

Group[a]	No. of Animals		Dose Level[b]		Dose Concentration[c]
	Male	Female	(mg/kg/dose)	(mg/kg/day)	(mg/mL)
1 (Control)	6	6	0	0	0
2 (Low)	6	6	50	100	5
3 (Mid)	6	6	150	300	15
4 (High)	6	6	450/300[d]	900/600[d]	45/30[d]
5 (High)[e]	1	1	300	600	30

a Group 1 received control article only.
b Dose levels and concentrations represent doses of active pharmaceutical ingredient (free acid).
c Animals were dosed BID, 8 hours ± 30 minutes apart, at a volume of 10 mL/kg for each dose, unless on dose suspension.
d The high dose was 450 mg/kg/dose (900 mg/kg/day, with a concentration of 45 mg/mL) for Day 1 through the a.m. dose on Day 10 of the dosing phase and 300 mg/kg/dose (600 mg/kg/day, with a concentration of 30 mg/mL) starting on Day 13 of the dosing phase. Dosing of Group 4 was suspended from the p.m. dose on Day 10 through the p.m. dose on Day 18 of the dosing phase.
e Animals in Group 5 were added to the study on Day 16 of the dosing phase and dosed starting on Day 19 of the dosing phase through Day 37 of the dosing phase.

Doses: 0, 50, 150 or 450/300 mg/kg BID Nominal
(0, 2000, 6000 or 18000/12000 mg/m^2/day)
Formulation: 2% Klucel LF (w/v) in reverse osmosis water adjusted to pH 4 with 1N hydrochloric acid
Dose volume 10 ml/kg
Placebo For Days 1 through 12 of the dosing phase, the control article consisted of 110 mg/mL of the placebo blend ((b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide) in vehicle. On Days 13 through 34 of the dosing phase, the control article consisted of 73.3 mg/mL of the placebo blend in vehicle. Placebo blend lot number 120697
Route PO Gavage
Clinical signs: twice daily
Body weights: Body weights were recorded for all animals weekly during the predose phase, before dosing on Day 1 of the dosing phase, and weekly thereafter through Week 5 of the dosing phase, as well as

	on Day 12 of the dosing phase. Body weights were also recorded on Day 19 of the dosing phase for animals in Groups 4 and 5. Beginning Week 6 of the dosing phase and continuing through the end of the dosing phase, body weights were recorded twice weekly.
Food cons.:	Consumption of kibble food was quantitatively assessed for 1 week during the pre-dose phase and weekly through Week 5 of the dosing phase. Additionally, food consumption was recorded twice during Week 2 of the dosing phase for the intervals Days 8 to 12 and Days 12 to 15 of the dosing phase. Beginning Week 6 of the dosing phase and continuing through the end of the dosing phase, food consumption was assessed twice weekly. On Days 21 through 44 of the dosing phase, consumption of canned food, when offered, was qualitatively assessed at the p.m. check prior to commingling the animals; a notation of none, some, or all was recorded for each animal receiving canned food. Starting on Day 45 of the dosing phase, consumption of canned food, when offered, was performed at the a.m. check.
Ophthalmology	Only done predose as the study was aborted
ECG	Only done predose as the study was aborted
Hematology	Blood samples for hematology, clinical chemistry, and coagulation and urine samples for urinalysis were collected from all animals twice during the predose phase (blood tests, once for urinalysis). Blood samples were collected for hematology, coagulation, and clinical chemistry from all surviving animals in Group 4 on Day 12 of the dosing phase, from all surviving animals in Groups 4 and 5 on Day 29 of the dosing phase, and from all surviving animals (Groups 1 through 5) on Days 16 and 38 of the dosing phase. Blood samples were collected for hematology and clinical chemistry from all surviving animals (Groups 1 through 5) on Day 50 of the dosing phase. Blood samples were collected for hematology, coagulation, and clinical chemistry from the two animals sacrificed at an unscheduled interval on Day 10 of the dosing phase.
Clinical chem.	Same as hematology
Necropsy	Only done on the two animals considered moribund All surviving animals were transferred to the subsequent Hoffmann-La Roche study1032862 (13 week study, (b) (4) Study No. 8217797) on Day 63 of the dosing phase.
Histopathology	Only done on the two animals considered moribund
Toxicokinetics	Days 1, 91, 104, and 182 predose, 0.5, 1, 2, 4, 8, 12 and 24 hours – two or three animals per time point, analysis by HPLC See Toxicokinetics and Pharmacokinetics section above

Results

Dose analysis	Dose analysis results indicated that on Days 1 through 10 of the dosing phase, the doses administered to the high-dose group

were 78.7 and 75.7% of the intended concentration corresponding to doses of approximately 708 and 681 mg/kg/day. The concentrations were within specifications thereafter in the HD group. The concentrations for the LD and MD groups were within specifications throughout dosing period.

Mortality — Two animals, one male and one female given 900 mg/kg/day and were killed humanely due to moribund condition on Day 10 of the dosing phase.

The male vomited on 6 of the first 10 days, the female on 7 of the first 10 days of dosing. During this time they excreted soft or unformed feces. The veterinarian determined that they were gaunt and lethargic and that the male was slightly dehydrated. Prior to the a.m. dose on Day 10 of the dosing phase, the male was laterally recumbent and had tremors. The male had a fever of 39.5°C, the female 40.2°C. They tightened muscles in avoidance of pain (splinting) and vocalized upon abdominal palpation. Both animals were given fluids with buprenorphine intravenously for dehydration and pain. After approximately 2 hours of treatment and continued monitoring, the animals did not show improvement and were sacrificed. No macroscopic observations were noted for the male at necropsy. The female had duodenal discoloration.

Clinical Signs — Some LD, MD and HD animals vocalized and struggled during dosing. Controls did not show this behavior.
Dose dependent excessive salivation, vomiting, hypoactivity, dehydration and abnormal feces in MD and HD animals
Thin appearance, tremor and reddened skin in the HD group

The veterinarian examined four HD males and four HD females on Day 8 of the dosing phase due to body weight-loss and low food consumption. As a result the investigators suspended the p.m. dose on Day 8 for two of the males and the four females, and the animals were given canned food and subcutaneous fluids (including the two dogs described above under ‘mortality’). Dosing was resumed for these animals on Day 9. Dosing was again suspended for the high dose group on Day 10 due intolerable toxicity. On Day 19, dosing was resumed but at 300 mg/kg BID (600 mg/kg/day) in the HD group. One male and one female were added to the high dose group on Day 16 to maintain group sample size and also began receiving RO5185426-006 at 300 mg/kg BID (600 mg/kg/day) on Day 19.

During the dosing suspension the HD animals regained some of their lost body weight but lost weight again on the resumption of dosing. The HD subsequently proved intolerable and dosing was suspended for all groups on Day 38. All animals were taken off study on Day 63.

Body Weight — HD males lost about 5% and HD females lost about 7% of body weight between days 1 and 12. Surviving animals recovered their weight when dosing was stopped but began loosing weight again after dosing resumed the second time. The HD dogs gained weight normally after dosing stopped. Body weights of LD and MD animals were stable.

Food Cons. Decreased in HD animals requiring canned food supplementation
Decreased in MD animals but did not result in decreased body weight over the short period of this study

Ophthalmology Not done

Clinical Chem. The values in the following table demonstrate significant progressive dose related liver damage and diminished thyroid function. These toxicities began to resolve after dosing ceased.

Parameter	Day	Control	LD	MD	HD	Recovery
Male Albumin	38	3.5	2.9%	0.0%	**-11.4%**	Normal
Female Albumin	38	3.5	5.7%	0.0%	**-22.9%**	Normal
Male Globulin	38	2.2	0.0%	**18.2%**	**13.6%**	Remains elevated
Female Globulin	38	Normal				
Male AG ratio	38	1.6	6.2%	**-12.5%**	**-25.0%**	Remains decreased
Female AG ratio	38	1.6	12.5%	-12.5%	**-18.8%**	Normal
Male cholesterol	38	150	2.0%	**75.3%**	**48.0%**	Remains elevated
Female cholesterol	38	185	**34.1%**	**47.6%**	-6.5%	Normal
Male Triglycerides	38	47	46.8%	274.5%	48.9%	Normal
Female Triglycerides	38	95	-17.9%	**211.6%**	22.1%	Normal
Male AST (value)	12				108	
Male AST	16	29	3.4%	**86.2%**	3.4%	
Male AST	38	32	-9.4%	**34.4%**	46.9%	Normal
Female AST (value)	12				32	
Female AST	16	28	0.0%	**89.3%**	128.6%	
Female AST	38	26	23.1%	**65.4%**	**188.5%**	Normal
Male ALT (value)	12				272	
Male ALT	16	29	20.7%	**362.1%**	**320.7%**	
Male ALT	38	34	2.9%	**267.6%**	132.4%	Normal
Female ALT (value)	12				129	
Female ALT	16	25	8.0%	**344.0%**	**232.0%**	
Female ALT	38	24	350.0%	**466.7%**	608.3%	Normal
Male ALP	38	51	5.9%	82.4%	92.2%	Normal
Male ALP	12	44			202.3%	
Female ALP	38	39	89.7%	**148.7%**	123.1%	Normal
Female ALP	12	54			179.6%	
Male GGT	38	5	-20.0%	**60.0%**	**40.0%**	Normal
Male GGT	12	4			125.0%	
Female GGT	38	3	100.0%	100.0%	200.0%	Elevated
Female GGT	12	5			60.0%	
Male Amylase	38	744	8.9%	19.2%	20.7%	Normal
Male Amylase	12	840			15.5%	
Female Amylase	38	629	-8.9%	-7.3%	33.2%	Normal
Female Amylase	12	579			56.0%	
Male Lipase	38	44	11.4%	36.4%	122.7%	Normal
Female Lipase	38	65	-4.6%	-26.2%	27.7%	Normal
Females T3	38	81	2.5%	1.2%	-17.3%	Normal
Females T3	12	93			-21.5%	
Male T4	38	2.1	14.3%	0.0%	-19.0%	Normal
Male T4	12	2			20.0%	
Female T4	38	3.1	-12.9%	-19.4%	-29.0%	Normal
Female T4	12	2.6			-15.4%	

Hematology The following table shows that dosing was associated elevated white cell parameters, particularly eosinophils and decreased clotting times.

Male	Day	Control	LD	MD	HD	Recovery
WBC	38	10.01	2.6%	-3.3%	**55.9%**	Normal
WBC	12				9.7%	Normal
Neutrophils	38	6.42	1.2%	-8.9%	**47.2%**	Normal
Neutrophils	12				-1.7%	Normal
Lymphocytes	12				51.1%	Slightly elevated
Monocytes	38	0.54	-9.3%	3.7%	**63.0%**	Normal
Eosinophils	38	0.32	-25.0%	50.0%	**478.1%**	Elevated
APTT	38	12.2	**-6.6%**	**-17.2%**	**-9.0%**	Not done
APTT	12				**-8.5%**	
Female	Day	Control	LD	MD	HD	Recovery
WBC	38	11.49	-20.5%	-2.8%	23.0%	Normal
WBC	12				53.5%	Normal
Neutrophils	38	7.01	-20.7%	1.6%	**12.4%**	Normal
Neutrophils	12				70.2%	Normal
Lymphocytes	12				48.0%	Normal
Monocytes	38	0.5	-8.0%	10.0%	**68.0%**	Elevated
Monocytes	12				65.9%	Elevated
Eosinophils	38	0.37	-24.3%	-8.1%	**343.2%**	Elevated
Lucocytes	38	0.03	-33.3%	-66.7%	**-66.7%**	Normal
APTT	38	12.1	-5.0%	-9.1%	**-10.7%**	Not done
APTT	12	11.7			-2.6%	

Organ weights: No changes in the two moribund dogs

Gross Pathology: Duodenal discoloration in the moribund female

Histopathology:

In the liver of the two moribund dogs there was minimal scattered hepatocellular degeneration, a mild to moderate increase in the number of Kupffer cells, and the presence of pigment in hepatocytes and Kupffer cells. These findings correlated with increased ALP, ALT, AST and GGT levels in both moribund dogs.

In the sternal bone marrow of the moribund female there was moderate pancytic necrosis. There was no finding of necrosis in the femoral bone marrow section or in the spleen.

3) 13-Week Oral Gavage Toxicity and Toxicokinetic Study with RO5185426-006 in Dogs with a 4-Week Interim Sacrifice and a 4-Week Recovery Phase

Major findings

A dose of 300 mg/kg BID of vemurafenib resulted in the early death of two male dogs on Days 23 and 31. A female given the low dose of 150 mg/kg BID also became moribund by day 31. These dogs were included in the assessment of the 28 day interim necropsy. These dogs had lost weight, appeared thin, refused canned food, vomited and vocalized during handling suggesting pain. All three had histomorphological changes in the liver including of necrosis, perivascular mixed infiltrates, and Kupffer cell increases. The damage in the liver was not sufficient to account for the animal's demise.

All these dogs had been treated with vemurafenib one month earlier in a previous study. The previous exposure likely contributed to the death of these dogs.

Animals that survived to the Day 28 interim necropsy also showed signs of vomiting, vocalizing and weight loss in males. LD females actually weighed more than controls by 17%. No dogs died after day 31 and their condition appeared to improve somewhat with further dosing. Dogs necropsied on Day 28 (and the dogs that died early) showed signs of hepatic damage including increased liver function enzymes, decreased serum protein and microscopic damage noted above. Liver damage as measured by ALT was somewhat worse in females. This damage was present at Day 93 after the end of dosing but was less severe than the changes seen at the interim necropsy because exposure decreased as the study progressed (see above in the toxicokinetics section). Cholesterol and triglycerides were elevated on day 15 possibly because of a direct effect on kinases involved in their synthesis. Cholesterol remained elevated in males at the end of the study but was normal in females. Amylase was elevated suggesting pancreatic damage but glucose was actually low. BUN was low probably reflecting reduced protein metabolism, decreased dietary protein or both. Hematological analysis showed a dose dependent neutrophilia that was more severe in males during all parts of the study. This was accompanied by an unusual eosinophilia and basopenia. The changes resemble those seen with an allergic response. All these changes showed signs of recovery or partial recovery four weeks after the end of dosing.

On Day 28, liver weight was low relative to body weight in males and females and remained somewhat low in females at the end of the study. On Day 28, there was a dose dependent increase in spleen weight in males. Spleen weight in males was only slightly elevated at Day 93. In females at Day 28, there was a decrease in spleen weight in both LD and HD dogs but by Day 93 spleen weight was increased relative to controls (~50%). On Day 28 thymus weight increased significantly in LD males but decreased in HD males, it was well below normal (30% or more) in both groups at the end of dosing. In females, thymus weight was low (~40%) in LD dogs on Day 28 but only slightly elevated in HD dogs. Thymus weight was low in HD females on Day 28. Adrenal weights were low in both sexes on Day 28. It is difficult to interpret some of these results because the sample sizes were so low, but the severity of change and the correlation with the unusual hematology argues for some real drug related effects probably secondary to the inhibition of kinases other than the target.

HD males had statistically significant prolonged mean corrected QT (QT_c) interval and QT interval (15 msec for both) on Day 24. The mean QT interval was increased 3 ms on Day 87 and 13 ms on Day 23 of recovery but these increases did not reach statistical significance. This finding correlates with the finding of a 15 ms increase in human patients. The QT interval was not prolonged in females. In HD males a ~9% increase in PR interval was observed on all days of dosing, but this increase did not reach significance due to sample size. This suggests some effects on conduction in the AV node.

Study no: (b) (4) Study No. 8217797; Roche Reference No. 1032862
File name: 1032862.pdf
Laboratory: (b) (4)
Study Date: August 2010

GLP: Yes
Audited: Yes
Drug: RO5185426-006
120785 At room temperature 28.8% (99% of label claim)
120787 At room temperature 28.9% (99.3% of label claim)
The test article was micro-precipitated bulk powder containing drug and "placebo blend" (nominally 70.9% of the total mass containing (b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide)

Methods:

Species: Beagle dog, 14 to 15 months old, and their body weights ranged from 7.7 to 10.4 kg for males and 7.1 to 8.8 kg for females. Many of these animals had been treated with vemurafenib on a previous study (Roche Reference No. 1033163, *vide supra*). The drug free period was one month.

Dose Groups The following table from the study report shows the dose groups and number of animals in each group. The legend for the table explains the necropsy schedule.

	No. of Animals[b]		Dose Level[c]		Dose Concentration[d]
Group[a]	Male	Female	(mg/kg/dose)	(mg/kg/day)	(mg/mL)
1 (Control)	6	6	0	0	0
2 (Low)	9	9	75	150	7.5
3 (High)	9	9	150	300	15

a Group 1 received control article only.
b Animals designated for interim sacrifice (two animals/sex in Group 1 and up to three animals/sex in Groups 2 and 3, dependent on survival) were sacrificed after 4 weeks (29 days) of dose administration. Animals designated for the dosing phase final sacrifice (two animals/sex in Group 1 and up to four animals/sex in Groups 2 and 3, dependent on survival) were sacrificed after 13 weeks (92 days) of dose administration. Animals designated for the recovery phase sacrifice (two animals/sex/group) underwent 4 weeks of recovery following dose administration.
c Dose levels and concentrations represent doses of active pharmaceutical ingredient (free acid).
d Animals were dosed BID, 8 hours ± 30 minutes apart, at a volume of 10 mL/kg for each dose.

Doses 0, 75, 150 mg/kg BID nominal (0, 3000, or 6000 mg/m^2/day)
Schedule Twice daily administered 8 hours ± 30 minutes apart
Formulation 2% Klucel LF (w/v) in reverse osmosis water adjusted to pH 4 with 1N hydrochloric acid
Dose volume 10 mL/kg
Placebo 36.6 mg/mL of the placebo blend ((b) (4) polymer hydroxypropyl methylcellulose acetate succinate and (b) (4) silicon dioxide) in vehicle.
Route PO Gavage
Clinical signs: twice daily
Body weights: Body weights were recorded twice during the predose phase, before dosing on Day 1 of the dosing phase, and weekly through Week 3 of the dosing phase. Body weights were recorded twice weekly starting Week 4 of the dosing phase and continuing through the recovery phase.
Food cons. Weekly
Ophthalmology Once during the predose phase, during Weeks 5 and 13 of the dosing phase, and during Week 4 of the recovery phase

ECG and BP	Recorded for unanaesthetized animals predose, Day 24, Day 87 and at the end of the recovery phase. ECGs were done approximately 3 hours after the p.m. dose. Routine measurements of ECGs were performed using Leads I, II, aVF, CV_5RL, and CV_6LL. A rate correction for the QT interval (QT_c) was calculated using the Fridericia method.
Hematology	Twice during the predose phase (blood tests, once for urinalysis), on Days 15 and 57 of the dosing phase, on Day 15 of the recovery phase, and on days of scheduled necropsy
Clinical Chem.	Same as hematology
Necropsy	After four weeks (day 29) interim group After 13 weeks (92 days) terminal group After 17 weeks (120) recovery group
Histopathology	Adequate battery, electron microscopy and frozen sections done on liver samples
Toxicokinetics	Days 1, 29, and 92 of the dosing phase prior to the a.m. dose and approximately 1, 3, 7, 9, 11, and 24 hours post-dose (based on the a.m. dose), analysis by HPLC See Toxicokinetics and Pharmacokinetics section above

Results

Dose analysis	Within acceptable specifications (mean concentration within +10% of the theoretical concentration and mean values for each location within +7% of the overall mean)
Mortality	The table below shows the mortality in this study. All dogs that became moribund had been on the previous study (1033163).

Animal number	Sex	Dose mg/kg/day	Description	Dose level in previous study	Day of death
H03910	Male	300	Moribund	Mid dose (300 mg/kg)	31
H03911	Male	300	Moribund	Low dose (100 mg/kg)	23
H03930	Female	150	Moribund	Low dose (100 mg/kg)	31
H03931	Female	150	Moribund	Naïve	9

Prior to their demise these animals vomited after dosing, the vomitus was frequently white in color. They frequently excreted discolored feces.

Animal No. H03930 lost weight and appeared thin beginning on day 20 of the dosing phase. Her consumption of kibble was low and she frequently refused canned food. On day 31 her body weigh was 21% below day 1 body weight. No remarkable findings were noted at necropsy. Histomorphological changes consisted of minimal individual hepatocellular degeneration.

Animal No. H03910 had lost weight and appeared thin on day 20. His consumption of kibble was low and he frequently refused canned food. Clinical pathology findings on day 31 included decreased albumin, total protein, and calcium. On Day 31 the animal was found recumbent and reluctant to stand. Upon veterinary examination, the dog was dehydrated. As a result, the animal was treated with subcutaneous fluids. No

remarkable findings were noted at necropsy. Histomorphological changes in the liver consisted of minimal necrosis, minimal perivascular mixed infiltrates, and minimal Kupffer cell increases.

Animal No. H03911 had lost weight and appeared thin on Days 22 and 23. His consumption of kibble was low and he frequently refused canned food. On Day 23 serum ALT (~ 8x), AST (~ 2x), ALP (~ 7x), and GGT (~ 5x) and total bilirubin (~ 4x) were elevated compared with predose phase values. Upon veterinary examination on Day 23, the animal was dehydrated and subcutaneous fluids were administered. At necropsy, multiple red discolored regions of the mucosa were noted in colon, duodenum, jejunum, and ileum; the gallbladder was enlarged, and the pancreas had light red discoloration. Histomorphological changes in the liver included minimal individual hepatocellular degeneration (correlating with increases in serum chemistry parameters), the presence of minimal amounts of pigment in hepatocytes and Kupffer cells, and minimal perivascular mixed infiltrates. Previous exposure likely contributed to the deaths of these three dogs.

The investigators did not consider the death of animal no. H03931 to be drug related. Gross and microscopic examination showed lung damage consistent with inhalation pneumonia, secondary to episodes of vomiting.

Clinical signs

HD females and males had a marked increased incidence of interdigital cage sores or reddened skin relative to controls. Four of nine males appeared thin. Three of nine LD and 4 of nine HD males struggled during dosing, some of these dogs also vocalized suggesting pain. The incidence of this sign was similar in females. One female developed ataxia. Five of nine LD and HD males salivated excessively prior to dosing. The incidence was similar in females but some three controls salivated also. The incidence of vomiting was high in both males and females in the LD and HD groups. There were fecal abnormalities in all groups including controls probably associated with excretion of the vehicle. These signs mostly resolved during recovery. One HD male developed what was called a “wart like growth” in the front left paw.

Body Weight

See organ weight tables below.

Food Cons.

Dose related decrease in males and females, significant only in Week 1 in HD males. It increased somewhat during recovery.

Ophthalmology No toxicologically significant changes

ECG

HD males had statistically significant prolonged mean corrected QT (QT_c) interval and QT interval (15 and 15 msec, respectively) on Day 24. The mean QT interval was

increased 3 ms on day 87 and 13 ms on Recovery Day 23, but these increases did not reach statistical significance. In the LD animals QT was slightly increased on Day 24 and Recovery Day 23 but the differences did not reach significance. The changes on Day 87 and Recovery Day 23 for HD males and the changes in LD males were not apparent when the QT was corrected for heart rate. QT prolongation did not occur in females. PR interval was increased relative to controls (about 9%) at all three time-points in HD males but this increase did not reach significance. There were smaller increases on Day 24 of the dosing phase and Day 23 of the recovery phase in LD males all suggesting dose dependent delayed conduction in the AV node. PR interval did not change in females.

Blood Pressure No toxicologically significant changes

Hematology The following table shows the toxicologically significant changes caused by RO5185426

Male	Day	Control	LD	HD	Recovery
Reticulocytes	57	55.9	-16.1%	-59.7%	Rebound day 15
WBC	93	7.98	**18.7%**	**81.1%**	Slightly elevated
Neutrophils	93	5.2	19.2%	70.6%	Slightly elevated
Lymphocytes	93	2.04	14.2%	**56.4%**	Slightly elevated
Monocytes	93	0.34	14.7%	79.4%	Normal
Eosinophils	30	0.29	55.2%	248.3%	Normal
Eosinophils	93	0.31	54.8%	**448.4%**	Normal
Basophils	15	0.1	-30.0%	**-50.0%**	Normal
Basophils	30	0.1	**-40.0%**	**-50.0%**	Normal
Leucocytes	15	0.02	-50.0%	**-50.0%**	Rebound
PT	15	9.4	4.3%	**11.7%**	Indeterminant
PT	57	9.9	**5.1%**	**17.2%**	Indeterminant
PT	93	10.2	3.9%	**11.8%**	Indeterminant
APTT	15	11.3	**-7.1%**	**-16.8%**	Somewhat low
APTT	30	11.7	-6.8%	**-14.5%**	Somewhat low
APTT	93	11.9	**-7.6%**	**-13.4%**	Somewhat low
Female	Day	Control	LD	HD	Recovery
WBC	93	9.85	-18.9%	43.1%	Normal
Neutrophils	93	6.22	-24.0%	27.0%	Normal
Lymphocytes	93	2.75	-15.3%	20.4%	Normal
Monocytes	93	0.37	-5.4%	64.9%	Normal
Eosinophils	30	0.31	22.6%	142.3%	Normal
Eosinophils	93	0.44	15.9%	388.6%	Low
Basophils	15	0.12	**-41.7%**	-41.7%	Slightly low
Basophils	30	0.11	**-45.5%**	-36.4%	Slightly low
Leucocytes	15	0.03	-33.3%	-33.3%	Rebound
Leucocytes	93	0.02	0.0%	250.0%	Rebound
PT	No changes				
APTT	15	10.8	**-8.3%**	**-9.3%**	Somewhat low
APTT	30	11.5	**-7.8%**	**-13.0%**	Somewhat low
APTT	93	11.5	-7.8%	-4.3%	Somewhat low

The results demonstrate a progressive neutrophilia with lymphocytosis. Monocytes are elevated and eosinophils are strikingly increased. This unusual pattern has the appearance of an allergic reaction. The effects are more pronounced in males than in females. The increase in PT is consistent with decreased hepatic function. Nevertheless, the decrease in APTT while small is consistent across time, seen in both

sexes statistically significant and extremely unusual. It is possibly due to an overexpression of a particular factor in the clotting cascade.

Clinical Chemistry

The following tables show the major changes in clinical chemistry parameters at different time points in this study.

Male	Day	Control	LD	HD	Recovery
Glucose	30	95	-8.4%	-10.5%	Low (81 day 29)
Glucose	57	99	-7.1%	-14.1%	
Glucose	93	93	**-14.0%**	-19.4%	
BUN	93	15	-13.3%	**-13.3%**	Normal
BUN	93	17	-17.6%	-17.6%	
Creatinine	All	Normal			
Total Protein	All	Normal			
Albumin	93	3.6	2.8%	**-16.7%**	Normal
Globulin	All	Normal			
A/G ratio	15	1.6	-6.3%	**-12.5%**	Low (1.4 day 29)
A/G ratio	57	1.5	-6.7%	**-20.0%**	
A/G ratio	15	1.6	**-12.5%**	**-25.0%**	
Cholesterol	15	184	28.8%	**59.8%**	Normal
Cholesterol	57	168	13.7%	**33.9%**	
Cholesterol	15	176	15.9%	27.8%	
Triglycerides	15	35	0.0%	**31.4%**	Normal
Total Bilirubin	All	Normal			
AST	All	Minor elevations did not reach significance			
ALT	15	32	**278.1%**	**481.3%**	Normal
ALT	57	31	**306.5%**	96.8%	
ALT	93	33	**203.0%**	21.2%	
ALP	15	43	37.2%	**174.4%**	Normal
ALP	57	38	36.8%	**139.5%**	
ALP	93	28	**92.9%**	**185.7%**	
GGT	15	4	0.0%	**100.0%**	Normal
GGT	57	4	0.0%	**50.0%**	
GGT	93	3	33.3%	**100.0%**	
Amylase	15	589	**26.1%**	**44.0%**	Elevated 30% in LD
Amylase	57	640	**18.8%**	**32.3%**	
Amylase	93	584	**35.8%**	**62.5%**	
Lipase	All	Mild dose dependant elevations 2X or less, continued in recovery			
Ca^{++}	All	Normal			
PO_4	All	Normal			
Na^{+}	15 and 57	Mild decreases in HD			
K^{+}	All	Normal			
Cl^{-}	15 and 57	Mild decreases in HD and LD			
TSH	All	Normal			
T3	All	Normal			
T4	All	Normal			

Females	Day	Control	LD	HD	
Glucose	30	95	-6.3%	**-16.8%**	Normal
Glucose	57	96	-7.3%	**-15.6%**	
Glucose	93	90	-10.0%	**-17.8%**	
BUN	93	14	-7.1%	0.0%	Normal
BUN	93	15	-20.0%	-6.7%	
Creatinine	All	Normal			
Total Protein	57	5.7	5.3%	**-14.0%**	Normal
Total Protein	93	5.7	8.8%	**-14.0%**	
Albumin	57	3.4	5.9%	-20.6%	Normal
Albumin	93	3.5	5.7%	**-25.7%**	
Globulin	All	Normal			
A/G ratio	15	1.7	-5.9%	-5.9%	Normal
A/G ratio	57	1.5	-6.7%	-20.0%	
A/G ratio	15	1.6	**-6.3%**	**-31.3%**	
Cholesterol	15	197	**54.3%**	**58.4%**	Normal
Cholesterol	57	200	**50.5%**	12.0%	
Cholesterol	93	218	**33.9%**	3.2%	
Triglycerides	15	30	**30.0%**	**56.7%**	Normal
Triglycerides	57	30	56.7%	**83.3%**	
Triglycerides	93	29	48.3%	**89.7%**	
Total Bilirubin	All	Normal			
AST	All	Mild dose related elevations less than 2X			
ALT	15	25	**588.0%**	**980.0%**	Low in all groups including controls
ALT	30	29	**455.2%**	**306.9%**	
ALT	57	29	**534.5%**	72.4%	
ALT	93	29	**500.0%**	10.3%	
ALP	15	39	64.1%	**102.6%**	Normal
ALP	57	32	118.8%	56.3%	
ALP	93	31	196.8%	67.7%	
GGT	15	4	**50.0%**	**25.0%**	Normal
GGT	57	3	100.0%	33.3%	
GGT	93	4	100.0%	0.0%	
Amylase	15	548	-5.5%	4.9%	Normal
Amylase	57	615	-7.5%	20.3%	
Amylase	93	557	6.8%	**52.8%**	
Lipase	All	Mild dose dependant elevations 2X or less, continued in recovery			
Ca^{++}	All	Normal			
PO_4	All	Normal			
Na^{+}	15 and 57	Mild decreases in HD			
K^{+}	All	Normal			
Cl^{-}	All	Normal			
TSH	All	Normal			
T3	All	Normal			
T4	All	Normal			

The decreases in blood glucose and BUN are small and are possibly due to decreased food consumption. Decreases in albumin, and increases in ALT, GGT and AST are consistent with dose dependent liver damage and diminished liver function. Cholesterol biosynthesis is controlled in the liver by several kinases that require ATP. Phosphorylation of several enzymes in the synthetic pathway limits the *de novo* synthesis of cholesterol. It is likely that vemurafenib is inhibiting one of these kinases and releasing cholesterol from feedback control thus causing its concentration to rise. Increases in amylase and Alkaline Phosphatase are consistent with pancreatic damage.

Urinalysis In males there was no consistent change in urine parameters. In females, urine pH tended to be lower in treated dogs than in

controls at most observations times. This decrease showed a dose dependence and was statistically significant on days 15 and 93. One day 30 urine pH was higher in treated animals than in controls.

Organ weights

The following table shows the toxicologically significant changes in organ weights. Many of these changes did not reach statistical significance only because the number of dogs in each group was small. The fact that many of them show dose-dependent and unusual changes over time signifies their toxicological significance. Where weight changes were significant for only one sex, I have included the values for the other sex for comparison. Values in red indicate a possible bell shaped dose response curve.

Male	Day	Control	LD	HD	Recovery HD Group
Body weight	28	8.8	**6.8%**	-4.5%	-7.5%
Brain	28	77.06	5.3%	0.8%	Normal
Brain/body weight	28	0.886	-1.2%	4.3%	
Heart	28	84.25	-4.5%	-2.6%	-12.9%
Heart/body weight	28	0.964	-10.8%	1.3%	
Liver	28	230.8	4.4%	-14.6%	-15.2%
Liver/body weight	28	2.67	-3.0%	-12.4%	
Kidney	28	43.2	-7.9%	-2.5%	Normal
Kidney/body weight	28	0.497	-14.3%	0.6%	
Spleen	28	28.28	43.9%	89.9%	20.6%
Spleen\body weight	28	0.325	35.1%	95.7%	
Thymus	28	5.897	**36.1%**	-21.3%	-53.3%
Thymus/Body weight	28	0.065	**29.2%**	-15.4%	
Thyorid	28	0.631	18.1%	5.4%	Normal
Thyroid/body weight	28	0.007	14.3%	14.3%	
Adrenals	28	1.343	-22.8%	-16.2%	Normal
Adrenals/body weight	28	0.015	-26.7%	-13.3%	
Testes	28	13.7	**-29.9%**	-14.6%	-28.2%
Testes/Body weight	28	0.158	**-33.5%**	-11.4%	
Female	Day	Control	LD	HD	Recovery HD Group
Body weight	28	6.9	**17.4%**	2.9%	-1.3%
Brain	28	80.5	-9.6%	-14.9%	Normal
Brain/body weight	28	1.164	**-22.3%**	**-16.8%**	
Heart	28	63.6	2.0%	-6.9%	Normal
Heart/body weight	28	0.925	-12.9%	-9.6%	
Liver	28	214.5	-4.5%	-15.2%	5.4%
Liver/body weight	28	3.11	**-18.2%**	**-17.3%**	
Kidney	28	34.69	-1.4%	-21.0%	Normal
Kidney/body weight	28	0.506	-16.0%	-23.5%	
Spleen	28	52.96	-37.5%	-44.1%	-54.3%
Spleen/body weight	28	0.758	-45.6%	-45.6%	
Thymus	28	5.726	**-37.8%**	9.0%	Normal
Thymus/Body weight	28	0.084	**-47.6%**	2.4%	
Thyorid	28	0.585	6.0%	21.4%	-12.3%
Thyroid/body weight	28	0.009	-11.1%	11.1%	
Adrenals	28	1.345	5.6%	-20.3%	Normal
Adrenals/body weight	28	0.02	-10.0%	-25.0%	
Uterus	28	Not done			

Male	Day	Control	LD	HD
Body weight	93	8.5	2.4%	-5.9%
Heart	93	77.14	1.9%	-6.9%
Heart/body weight	93	105.5	5.2%	-12.7%
Spleen	93	32.16	10.6%	4.2%
Spleen/body weight	93	0.38	7.9%	9.2%
Thymus	93	8.12	-44.2%	-32.8%
Thymus/Body weight	93	0.097	-45.4%	-28.9%
Thyorid	93	0.822	3.8%	-22.4%
Thyroid/body weight	93	0.01	0.0%	-20.0%
Testes	93	13.14	-29.9%	-7.7%
Female				
Body weight	93	8.2	-2.4%	-9.8%
Liver	93	208.2	-8.2%	6.8%
Liver/body weight	93	2.545	-6.0%	**18.9%**
Spleen	93	24.8	48.0%	51.0%
Spleen/body weight	93	0.306	46.7%	65.0%
Thymus	93	5.213	-0.6%	-24.2%
Thymus/Body weight	93	0.067	1.5%	-19.4%
Thyorid	93	0.558	31.4%	26.5%
Thyroid/body weight	93	0.007	28.6%	42.9%
Uterus	93	Not done		

Male	Day	Control	LD	HD
Body weight	120	9.3	6.5%	-7.5%
Heart	120	83.644	1.2%	-12.9%
Heart/body weight	120	0.905	-5.2%	-5.9%
Liver	120	240.288	4.4%	-15.2%
Liver/body weight	120	2.6	-2.4%	-8.6%
Spleen	120	31.57	14.0%	20.6%
Spleen/body weight	120	0.34	86.2%	32.4%
Thymus	120	7.96	-14.1%	-53.3%
Thymus/Body weight	120	0.087	-21.8%	-50.6%
Thyorid	120	0.759	-1.6%	7.2%
Thyroid/body weight	120	0.008	0.0%	12.5%
Testes	120	15.423	-3.0%	-28.2%
Testes/Body weight	120	0.168	-9.5%	-23.8%
Female				
Body weight	120	8	-5.0%	-1.3%
Liver	120	224	-12.9%	5.4%
Liver/body weight	120	2.811	-8.3%	6.7%
Spleen	120	50.811	-27.2%	-54.3%
Spleen/body weight	120	0.629	-22.9%	-52.9%
Thymus	120	6.782	-20.6%	-2.3%
Thymus/Body weight	120	0.082	14.6%	3.7%
Thyorid	120	0.709	1.3%	-12.3%
Thyroid/body weight	120	0.009	0.0%	-11.1%
Uterus	28	Not done		

Histopathology

The following table shows the toxicologically significant histopathological changes in the animals necropsied on day 28. The most pronounced damage was in the liver

consistent with the increases in LFTs and the decreases in serum proteins. The damage to the thymus is also consistent with the weight changes seen in that organ.

Male	Control	LD	HD
N	2	4	5
LIVER			
- Perivasc mixed infiltrations		2	5
- Kupffer cell increase			3
- Kupffer cell & hepatic pigmentation		3	3
GALLBLADDER			
- Secretions present		1	4
PITUITARY GLAND			
- Cyst	1	1	3
KIDNEYS			
- Papillary Mineralization	1	3	4
SPLEEN			
- Hemosiderin Deposits	2	4	3
THYMUS			
- Atrophy	1	4	3
HEART			
- Mononuclear Infiltrates		2	
- Arteritis/Periarteritis		1	
Female			
N	2	5	4
LIVER			
- Necrosis		1	
- Perivascular mixed infiltrates		3	4
- Scattered hepatic degeneration		1	
- Kupffer cell increase		1	2
- Kupffer cell & hepatic pigmentation		1	3
GALLBLADDER			
- Secretions present		1	2
KIDNEYS			
- Mononuclear Infiltrates		1	1
- Tubular Vacuolation	1	3	4
- Papillary Mineralization	1	3	4
- Tubular Pigment	1	3	2
THYMUS			
- Atrophy		4	4

The following table shows the microscopic damage observed in animals necropsied on day 93. Again the most pronounced finding is damage to the liver in both sexes.

Male N	2	3	2
LIVER - Perivascular mixed infiltrates - Scattered hepatic degeneration - Kupffer cell increase - Kupffer cell & hepatic pigmentation		 3 2 1 1	 1 1 1 2
GALLBLADDER - Secretions present		 2	 2
Female N	2	2	3
LIVER - Perivasc mixed inf - Kupffer c & hep pigm:		 1	 2 3
GALLBLADDER - Secretions present		 1	 1
THYMUS - Atrophy			 1

Animals killed after four weeks of recovery showed only minimal damage in the liver indicating progressive recovery.

7 Genetic Toxicology

1) Rat Bone Marrow Erythrocytes Micronucleus Test Following a Single Oral Administration of RO5185426-004

Major findings

Under the conditions of the assay, a single oral gavage dose of vemurafenib at doses up to and including 800 mg/kg (the maximum feasible dose) did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in either male or female Hsd:SD rats.

Study no: Sponsor – 1026332; Laboratory – AC04VW.125 (b) (4)
File name: 1026332
Laboratory: (b) (4)
Study Date: October 2007
GLP: Yes
Audited: Yes
Drug: RO5185426-004, Lot number X537RWFP-07-001M, Purity 99.6 %
MPB formulation as identified by the sponsor

Method:
- Species: Sprague Dawley rats (Hsd:SD rats)
- N: Five males and five females per dose group
- Doses: 0, 30, 150 or 800 mg/kg (180, 900 or 4800 mg/m^2)
  The highest dose is a maximum feasible dose
  The following table from the report shows the different dose groups and allocation of animals.

Treatment (20 mL/kg)	Number of Rats/Sex Dosed	Number of Rats/Sex Used for Bone Marrow Collection at	
		24 hrs post-dose	48 hrs post-dose
Negative Control: Placebo	10	5	5
Test Article: RO5185426-004			
Low dose (30 mg/kg)	5	5	0
Mid dose (150 mg/kg)	5	5	0
High dose (800 mg/kg)	15*	5	5
Positive Control: CP (40 mg/kg)	5	5	0

*Including 5 replacement rats/sex to ensure the availability of five rats for micronucleus analysis.

Route	PO gavage
Schedule	Single dose
Vehicle	2% Klucel LF adjusted to pH 4.0,
Dose volume	20 mL/kg
Positive control	Cyclophosphamide
Marrow Collection	24 and 48 hours post dosing
Analysis	Incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes for each rat and per 10,000 PCEs for each treatment group

Results

Mortality	None
Clinical signs	None
Analysis	

There were no appreciable reductions in the ratio of polychromatic erythrocytes to total erythrocytes in the vemurafenib groups relative to the respective vehicle control groups, suggesting that the vemurafenib did not inhibit erythropoiesis.

There was no statistically significant increase in the number of micronucleated polychromatic erythrocytes in vemurafenib groups relative to the respective negative control groups in male or female rats at 24 or 48 hours after dose administration ($p > 0.05$, Kastenbaum-Bowman Tables).

There was a statistically significant increase in the incidence of micronucleated PCEs ($p \leq 0.05$, Kastenbaum-Bowman Tables) in both male and female rats given cyclophosphamide, the positive control. The number of micronucleated PCEs in the negative control groups did not exceed the historical vehicle (negative) control range. This indicates that all criteria for a valid test were met as specified in the protocol.

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Not required and not submitted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Not required and not submitted

9.2 Embryonic Fetal Development

1) A Dose Range-Finding and Toxicokinetic Study of the Effects of RO5185426 on Embryo/Fetal Development in Rats

Key study findings:

- At 800 mg/kg, there was decreased maternal food consumption and body weight gain; however, there was no evidence of embryo-fetal toxicity
- At 800 mg/kg, clinical signs of red material around the mouth were noted in all females.
- At 150 mg/kg and 800 mg/kg, both AUC and Cmax dose ratios showed that the plasma exposure (AUC0-24hr and Cmax) increased less than dose-proportionally.
- There was slight accumulation of plasma exposure after multiple days of dosing at all dose levels.
- The highest dose tested, 800 mg/kg/day, was considered the NOAEL for embryo/fetal development while the next lower dose, 150 mg/kg/day, was considered the NOAEL for maternal toxicity.

Study No.	1026029
Volume #, and page #:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 19, 2007
GLP compliance:	No
QA report:	yes () no (X)
Drug, lot #, and % purity:	RO5185426-004, ZG-37427-217E, F, and D, 91%

Methods

Doses:	0, 30, 150, and 800 mg/kg/day
Species/strain:	Rat/ Crl:CD(SD)
Number/sex/group or time point:	8 females/group
Route, formulation, volume, and infusion rate:	Oral gavage, 2% hydroxypropylcellulose (Klucel LF) in deionized water adjusted to pH 4 (± 0.2) with 1 N HCl; 20 mL/kg based on daily body weight
Age:	Approximately 70 days old
Weight:	219 g to 266 g at Day 0 pc
Satellite groups:	6/group (TK) on gestation days 6 and 17 at 0 (pre-dose), 0.5, 1, 2, 4, 8, 12, and 24 hours post-dose
Study design:	▪ The microprecipitated bulk powder (MBP) formulation was NOT used in this study

- Dose Range to determine toxicity and toxicokinetics.
- F0 females dosed from Day 6-17 post coital (pc).
- Females euthanized on Day 20 pc
- Toxicokinetics on Gestation Days 7 and 21

Population	Parameters and Endpoints
F0 females	Clinical observations daily, body weight, food consumption, gross necropy, and toxicokinetics (on gestation days 6 and 17 at 0 (pre-dose), 0.5, 1, 2, 4, 8, 12, and 24 hours post-dose)
F1 litters	Implantations, resorptions, live and dead fetuses; fetal weight, sex, placental and fetal gross morphology

Results

Mortality (dams):

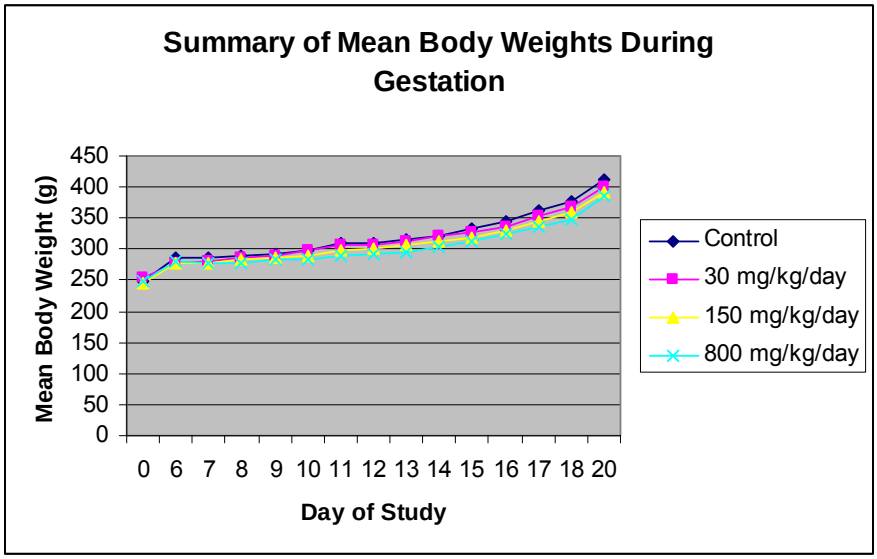
Animal No.	Dose (mg/kg)	Dose (mg/m2)	Day of death	Clinical signs / In-life observations
70264	800	4800	GD11	▪ Small movable mass on the right forelimb ▪ Yellow material on urogenital and anogenital areas and base of tail ▪ Red material on nose and mouth on GD9-10 ▪ Necropsy showed intubation error was cause of death and not drug related.

Clinical signs (dams):

- The presence of red material around the mouth was noted in all 8 females approximately 1 hour following dose administration during gestation days 7-16 from the 800 mg/kg/day dose group.
- Other observations included hair loss, yellow or clear material findings on various body surfaces, red material around the nose, and gasping. These signs were observed in all dose groups including controls.

Body weight (dams):

- At 800 mg/kg there was a decrease in the amount of mean body weight change through the entire treatment period (gestation days 6-9, 9-12, 12-18, and 6-18) with statistically significant decreases during gestation days 6-18 (See table below).
- The high percent change seen during gestation days 6-9 was attributed to other variables and not considered drug related.
- At 800 mg/kg during the entire treatment period (gestation days 6-18), mean body weight gain was 28% lower than controls (See table below).



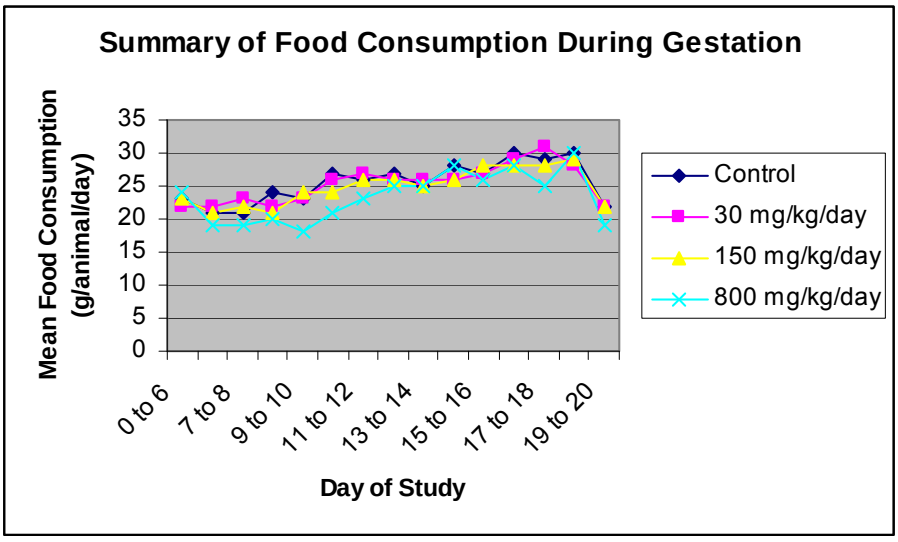
Change in Body Weight Over Specified Period of Gestation					
Period of Gestation	Control	Low Dose 30 mg/kg	Mid Dose 150 mg/kg	High Dose 800 mg/kg	Percent Change (HD from Control)
6-9 PC	8	10	6	1	↓87%
9-12 PC	17	17	17	10	↓41%
12-18 PC	66	63	58	55	↓17%
6-18 PC**	91	90	81	66	↓27%

** = difference was statistically significant ($p<0.01$) using Dunnett's test

Overall Average Total Body Weight Gain Adjusted for Uterine Weight (GD 6-18)			
Control	Low Dose 30 mg/kg	Mid Dose 150 mg/kg	High Dose 800 mg/kg
74.8	60.3	64.9	53.9

Food consumption (dams):

- At the 800 mg/kg dose, mean food consumption was lower than the control group on gestation days 6-9, 9-12, and when the entire treatment period (gestation days 6-18) was evaluated with statistically significant decreases during gestation days 9-12 and 6-18 (See table below).



Food Consumption Over Specified Period of Gestation (g/animal/day)					
Period of Gestation	Control	Low Dose 30 mg/kg	Mid Dose 150 mg/kg	High Dose 800 mg/kg	Percent Change (HD from Control)
6-9 PC	22	22	22	19	↓13%
9-12 PC**	26	25	25	21	↓19%
12-18 PC	28	28	27	26	↓7%
6-18 PC**	26	26	25	23	↓11%

** = difference was statistically significant ($p<0.05$ or $p<0.01$) using Dunnett's test

Terminal and necroscopic evaluations:

	Dose Level (mg/kg/day)			
	0	30	150	800
Number Corpora Lutea-Mean/♀	16.1	16.7	15.8	16.3
Number Implantations-Mean/♀	15.6	15.7	14.6	15.6
Percent Pre-Implantation Loss	3.0	5.6	8.1	4.2
Post-Implantation Loss- Mean/♀				
Early	6.0	5.0	4.0	6.0
Late	0.0	0.0	0.0	0.0
Dead	0.0	0.0	0.0	0.0
Total	6.0	5.0	4.0	6.0
Percent Post-Implantation Loss	4.7	4.7	3.1	5.4
Number of Live Fetuses/♀	119	105	113	103
Percent Live Males	44.1	42.5	49.6	42.9
Live Birth Index (%) (live/implants)	762.8	668.7	773.9	660.2
Mean Fetal Weight				
Female	3.9	3.8	3.7	3.8
Male	4.1	3.9	4.0	4.0
Gravid Uterine Weight	90.0	86.4	82.6	86.4

Offspring (malformations, variations, etc.):
No fetal malformations or variations were noted in this study.

Toxicokinetics (dams):

Toxicokinetic Study of the Effects of RO5185426 on Embryo/Fetal Development in Rats				
Dose (mg/kg)	**0**	**30**	**150**	**800**
No of animals	**6**	**6**	**6**	**6**
AUC0-24 hr (ng hr/mL)				
GD6	NA	182	460	1680
GD17	NA	279	790	3120
Cmax (ng/mL)				
GD6	NA	18.3	45.1	95.0
GD17	NA	40.7	68.3	169

Note:
NA = not applicable
GD = gestation day

- At 150 mg/kg and 800 mg/kg, both AUC and Cmax showed that the plasma exposure increased less than dose-proportionally.
- There was slight drug accumulation after multiple days of dosing at all dose levels based on AUC and Cmax.

2) An Oral (Gavage) and Toxicokinetic Study of the Effects of RO5185426 on Embryo/Fetal Development in Rats

Key study findings:
- Maternal and embryo/fetal developmental toxicity were not observed at any dose levels tested.
- There was drug accumulation (up to 2-fold based on AUC and Cmax) at all dose levels.
- Mean fetal levels at 4 hours post dose on Gestation Day 20 increased with increasing dose.
- The highest dose tested, 250 mg/kg/day, was considered the NOAEL for maternal and embryo/fetal development.

Study No. 1028543
Volume #, and page #: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: February 21, 2008
GLP compliance: Yes
QA report: yes (X) no ()
Drug, lot #, and % purity: RO5185426-007, 97620, 97%

Methods

Doses:	0, 30, 100, and 250 mg/kg/day
Species/strain:	Rat/ Crl:CD(SD)
Number/sex/group or time point:	25 females/group
Route, formulation, volume, and infusion rate:	Oral gavage, 2%

	hydroxypropylcellulose (Klucel LF) in deionized water adjusted to pH 4 (± 0.2) with 1 N HCl; 10 mL/kg based on daily body weight
Age:	12 weeks
Weight:	225 g to 290 g at Day 0 pc
Satellite groups:	3/group (TK) on Gestation Days 6 and 17 at 0 (predose), 0.5, 1, 2, 4, 8, 12, and 24 hours post dose.
Study design:	▪ The microprecipitated bulk powder (MBP) formulation was used in this study ▪ F0 females dosed from Day 6-17 post coital (pc). ▪ Females euthanized on Day 20 pc ▪ Toxicokinetics on Gestation Days 7 and 21

Parameters and endpoints evaluated:

Population	Parameters and Endpoints
F0 females	Clinical observations (twice daily, detailed—Day 6, 7, 18, and 21 pc), body weight (gestation days 0, 6-18, and 20), food consumption (gestation days 0, 6-18 and 20), gross necropsy, and toxicokinetics (on Gestation Days 6 and 17 at 0 (predose), 0.5, 1, 2, 4, 8, 12, and 24 hours post dose)
F1 litters	Implantations, resorptions, live and dead fetuses; fetal weight, sex, gross morphology, and skeletal alterations

Results

Mortality (dams): No drug-related deaths

Clinical signs (dams):
- Findings including hair loss on the forelimbs and red material around the nose were observed in all dose groups including controls and were not considered drug-related.

Body weight (dams): No drug related changes in maternal body weights and gravid uterine weights.

Food consumption (dams): No drug related changes in mean food consumption.

Terminal and necroscopic evaluations:

Dose Group Number of ♀(Litters if Different) mg/kg/day	Control 25 0	Low 25 30	Mid 25 100	High 25 250
Number Corpora Lutea-Mean/♀	17.4	18.4	17.0	17.2
Number Implantations-Mean/♀	16.3	16.6	16.0	16.2
Percent Pre-Implantation Loss	6.2	7.8	5.1	5.6

Dose Group Number of ♀(Litters if Different) mg/kg/day	Control 25 0	Low 25 30	Mid 25 100	High 25 250
Post-Implantation Loss- Mean/♀				
Early	16.0	26.0	15.0	18.0
Late	1.0	0	0	0
Dead	0.2	0	0	0
Total	17	26.0	15.0	18.0
Percent Post-Implantation Loss	4.3	9.9	3.7	4.9
Number Live Fetuses-Mean/♀	15.6	15.6	15.4	15.4
Percent Live Males	52.2	50.8	50.6	48.9
Live Birth Index (%) (live/implants)	95.7	93.9	96.2	95
Mean Fetal Weight				
Female	3.5	3.4	3.5	3.5
Male	3.7	3.6	3.7	3.7
Gravid Uterine Weight	88.4	86.9	86.5	86.7

Offspring (malformations, variations, etc.):
No fetal malformations or variations were noted in this study.

Toxicokinetics (dams):

Toxicokinetic Study of the Effects of RO5185426 on Embryo/Fetal Development in Rats				
Dose (mg/kg)	**0**	**30**	**100**	**250**
No of animals	**6**	**6**	**6**	**6**
AUC0-24 hr (ng hr/mL)				
GD6	NA	132000	328000	789000
GD17	NA	187000	487000	159000
Cmax (ng/mL)				
GD6	NA	21000	40400	68100
GD17	NA	23000	55700	99100
Cmax4hr (ng/mL)				
GD20 (F0 female)	NA	11700	39100	71100
GD20(fetus)	NA	359	1460	3930

Note:
NA = not applicable
GD = gestation day

- At 30 mg/kg and 100 mg/kg, both AUC and Cmax showed that the plasma exposure (AUC0-24hr and Cmax) increased less than dose-proportionally.
- In general, there was drug accumulation (up to 2-fold based on AUC and Cmax) at all dose levels.
- Mean fetal drug levels at 4 hours following dose administration on Gestation Day 20 was 3.1% (0.36 ug/mL), 3.6% (1.46 ug/mL), and 5.5% (3.93 ug/mL) of the maternal plasma drug levels at the 30, 100, and 250 mg/kg/day dose levels, respectively.

3) A Dose Range-Finding and Toxicokinetic Study of the Effects of RO5185426 on Embryo/Fetal Development in Rabbits.

Key study findings:

- At 450 mg/kg/day, all females were found dead, euthanized and/or euthanized due to administration difficulties on Gestation Days 9-11. No fetuses were examined for this dose.
- Drug accumulation was observed after multiple days of dosing at 30 and 150 mg/kg/day dose.
- There was an increase in exposure after repeated dosing due to the slow clearance of the drug.
- The drug appeared to be slowly absorbed from the GI tract.
- Since deaths occurred in animals at 450 mg/kg/day and fetal data is available, the next highest dose of 150 mg/kg/day was considered the NOAEL for maternal and embryo/fetal development.

Study No.	1026033
Volume #, and page #:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 29, 2007
GLP compliance:	No
QA report:	yes () no (X)
Drug, lot #, and % purity:	RO5185426-004, ZG-37427-217E, F, and D, 91%

Though the sponsor states that the study was not audited by the (b) (4), they did generally follow GLP standards.

Methods

Doses:	30, 150, and 450 mg/kg/day
Species/strain:	Hra:(NZW)SPF rabbits
Number/sex/group or time point:	6 females/group
Route, formulation, volume, and infusion rate:	Oral gavage, 2% hydroxypropylcellulose (Klucel LF) in deionized water adjusted to pH 4 (± 0.2) with 1 N HCl, 10 mL/kg daily
Age:	5 months
Weight:	2902 – 4261 g
Satellite groups:	3/group (TK) on Gestation Days 7 and 20 at 0 (predose), 0.5, 1, 2, 4, 8, 12, and 24 hours post dose.
Study design:	▪ The microprecipitated bulk powder (MBP) formulation was NOT used in this study ▪ Dose Range to determine toxicity and toxicokinetics. ▪ Rabbits were dosed daily on Gestation Days 7-20. ▪ Scheduled sacrifice on Gestation

Day 29.
- Toxicokinetics on Gestation Day 7 and 20

Population	Parameters and Endpoints
F0 females	Clinical signs (twice daily and detailed from Gestation Days 1 to 29) body weight (Day 0, 4, daily on Days 7 to 21, 24, and 29), and food consumption (daily on Gestation Days 4-29), gross necropsy and toxicokinetics (on Gestation Days 7 and 20 at 0 (predose), 0.5, 1, 2, 4, 8, 12, and 24 hours post dose)

Results

Mortality (dams):

Animal #	Dose (mg/kg)	Day of Death	Observations	
			Reason for Removal	General
50695	450	9	Found dead	Gravid; incubation error cause of death
50709	450	10	Found Dead	Nongravid; incubation error was cause of death.
50693	450	11	Terminated	Gravid; Terminated due to administration difficulties
No ID	450	9-11	Euthanized	Due to intubation error
No ID	450	9-11	Euthanized	Due to intubation error
No ID	450	9-11	Euthanized	Due to intubation error

Note:
- According to the sponsor, all females that died were the result of forced administration, using a 12-gauge steel cannula to administer the dose. The forced administration led to the esophageal perforations noted for five of six animals in this group at the time of necropsy. The other female was terminated due to administration difficulties.

Clinical signs (dams):

Index	**No. of animals affected**			
Dose (mg/kg/day)	**0**	**30**	**150**	**450**
No. of animals	**6**	**6**	**6**	**6**
▪ Decreased defecation	3	5	4	2
▪ Soft stool	6	4	5	3
▪ Brown material on tail	2	2	3	2
▪ Brown material urogenital area	5	4	2	1
▪ Head held high				2
▪ Labored respiration				2
▪ Perforated esophagus				5*
▪ White contents/fluid in thoracic cavity				3*
▪ Dark red lungs				2*
▪ Dark red areas stomach lining				1*
▪ White material on skin				2*

Note: * indicates that observations were resulted form administration difficulties

Body weight (dams):

- Decreases in mean maternal body weight gain were noted at 450 mg/kg/day during gestation days 7-11. The differences, however, were not statistically significant when gestation days 7-10 and 10-11 were evaluated.
- Further evaluation of body weight data at 450 mg/kg/day was precluded by the early euthanasia of this group on gestation day 11. Due to the dose difficulties, it is unclear if the changes in body weight observed were test article-related.

Food consumption (dams):

- Decreases in mean food consumption decreases were noted at 450 mg/kg/day during gestation days 7-10. This decrease, however, was not statistically significant.
- Further evaluation of food consumption data at 450 mg/kg/day was precluded by the early euthanasia of this group on gestation day 11. Due to the dose difficulties, it is unclear if the changes in food consumption observed were test article-related.

Terminal and necroscopic evaluations:

Dose Group Number of ♀(Litters if Different) mg/kg/day	Control 6 0	Low 6 30	Mid 5 150	High 0 450
Number Corpora Lutea-Mean/♀	10.0	11.2	10.0	NA
Number Implantations-Mean/♀	8.8	10.0	7.8	NA
Percent Pre-Implantation Loss	12.8	10.0	23.5	NA
Post-Implantation Loss- Mean/♀				
Early	2.0	1.0	0	NA
Late	0	0	0	
Dead	0	0	0	
Total	2.0	1.0	0	
Percent Post-Implantation Loss	3.8	1.9	0	NA
Number Live Fetuses-Mean/♀	8.5	9.8	7.8	NA
Percent Live Males	52.2	50.8	50.6	NA
Live Birth Index (%) (live/implants)	96.5	98	100	NA
Mean Fetal Weight	42.6	38.6	44.0	NA
Gravid Uterine Weight	489.1	508.0	455.2	NA

NA = At 450 mg/kg/day, evaluation of laparohysterectomy parameters in the 450 mg/kg/day group was precluded by mortality and early euthanasia of this group on gestation days 9 and 11. This group was discontinued from further evaluation.

Toxicokinetics (dams):

Toxicokinetic Study of the Effects of RO5185426 on Embryo/Fetal Development in Rabbits				
Dose (mg/kg)	**0**	**30**	**150**	**450**
No of animals	**3**	**3**	**3**	**3**
AUC0-24 hr (ng hr/mL)				
GD7	BLQ	74600	272000	605000
GD20	BLQ	222000	477000	ND
Cmax (ng/mL)				
GD7	BLQ	5100	20400	37000

GD20	BLQ	13300	29000	ND
Tmax (hours)				
GD7	BLQ	8	4	4
GD20	BLQ	4	4	ND

Note:
BLQ = these samples were below levels that were quantified.
ND = not determined
GD = gestation day
Gestation Day 20 samples at 450 mg/kg/day were not obtained due to premature termination of the group due to dosing problems.

- Exposure increased less than dose proportionally to increases in doses at 150 and 450 mg/kg/day.
- Drug accumulation was observed after 14 days at both 30 and 150 mg/kg/day.
- An increase in exposure after repeated dosing was noted due to slow clearance of the drug.
- Drug appeared to be slowly absorbed from the GI tract based on Tmax values.

Offspring (malformations, variations, etc.):
There were no malformations or developmental variations observed in any of the groups evaluated.

4) An Oral (gavage) Toxicokinetic Study of the Effects of RO185426 on Embryo/Fetal Development in Rabbits

Key study findings:
- Non-drug related mortality in 1 control and 1 HD (450 mg/kg/day) animal on Gestation Days 22-24.
- At 450 mg/kg, there was decreased food consumption and body weight gain throughout treatment period.
- At 450 mg/kg, there was decreased food consumption and body weight gain during the entire treatment period (GD 7-20).
- Drug exposure increased less than proportionally to increases in dose.
- There was drug accumulation (up to 3-fold based on AUC and Cmax) at all dose levels.
- Mean fetal levels at 4 hours post during gestation increased with increasing dose.
- The highest dose tested, 450 mg/kg/day, was considered the NOAEL for embryo/fetal development while the next lower dose, 150 mg/kg/day, was considered the NOAEL for maternal toxicity.

Study No. 1028544
Volume #, and page #: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: March 11, 2008
GLP compliance: Yes
QA report: yes (X) no ()
Drug, lot #, and % purity: RO5185426-007, 97620, 97%

Methods

Doses:	30, 150, and 450 mg/kg/day
Species/strain:	Hra:(NZW)SPF rabbits
Number/sex/group or time point:	22 females/group
Route, formulation, volume, and infusion rate:	Oral gavage, RO5185426 2% hydroxypropylcellulose (Klucel LF) in deionized water adjusted to pH 4 (± 0.2) with 1 N HCl, 10 mL/kg daily
Age:	5 months
Weight:	2900 to 4500 g
Satellite groups:	3/group (TK) on Gestation Days 7 and 21 at 0 (predose), 0.5, 1, 2, 4, 8, 12, and 24 hours post dose.
Study design:	▪ The microprecipitated bulk powder (MBP) formulation was used in this study ▪ Rabbits were dosed daily on Days Gestation Days 7-20. ▪ Females euthanized on Day 21 pc ▪ Toxicokinetics on Gestation Days 7 and 21

Population	Parameters and Endpoints
F0 females	Clinical signs (twice daily and detailed from Gestation Days 1 to 29) body weight (Day 0, 4, daily on Days 7 to 21, 24, and 29), and food consumption (daily on Gestation Days 4-29), gross necropy and toxicokinetics (on Gestation Days 7 and 20 at 0 (predose), 0.5, 1, 2, 4, 8, 12, and 24 hours post dose).
F1 litters	Implantations, resorptions, live and dead fetuses; fetal weight, sex, gross morphology, and skeletal alterations

Results

Mortality (dams):

Mortality was ruled out as being drug related since clinical findings and changes in body weight and food consumption parameters were similar between control and the one high dose female at 450 mg/kg/day.

Animal #	Dose (mg/kg)	Day of Death	Observations	
			Reason for Removal	General
54361	Control	GD22	Euthanized in extremis	▪ Black or brown material on the base of the tail, decreased defecation, soft stool noted generally beginning 10 days prior to euthanasia ▪ Body weight loss of 13.8% of gestation day 7 body weight ▪ Decreased food consumption starting on Gestation days 13-14
54286	450	GD24	Euthanized in extremis	▪ black or brown material on the base of the tail, decreased defecation, soft stool noted generally beginning 10 days prior to euthanasia, and swollen urogenital

Animal #	Dose (mg/kg)	Day of Death	Observations	
			Reason for Removal	General
54361	Control	GD22	Euthanized in extremis	▪ Black or brown material on the base of the tail, decreased defecation, soft stool noted generally beginning 10 days prior to euthanasia ▪ Body weight loss of 13.8% of gestation day 7 body weight ▪ Decreased food consumption starting on Gestation days 13-14
				and anogenital areas ▪ Body weight loss of 8.2% of gestation day 7 body weight ▪ Decreased food consumption starting on Gestation days 14-15

Clinical signs (dams):

- Clinical observations included soft stool, decreased defecation, small feces, and brown and/or black material around the base of tail. These signs were observed in all dose groups including controls.

Body weight (dams):

- Lower mean body weight gains or body weight losses were observed at 450 mg/kg/day throughout the treatment period (gestation days 7-10, 10-13, 13-21, and 7-21) when compared to controls. Only the mean body weight loss on gestation day 13-14 achieved statistical significance ($p<0.05$).
- Mean body weight gain for the entire treatment period (gestation days 7-21) was reduced by approximately 48% and considered adverse.
- During the post-treatment period (gestation days 21-29), mean body weight gains at 450 mg/kg/day were higher than the placebo control group reaching statistical significance ($p<0.01$) during gestation days 21-24 and 21-29.

Change in Body Weight Over Specified Period of Gestation					
Period of Gestation	Control	Low Dose 30 mg/kg	Mid Dose 150 mg/kg	High Dose 450 mg/kg	Percent Change (HD from Control)
7-10 PC	69	73	81	39	↓43%
10-13 PC	21	53	1	-8	↓138%
13-21 PC*	109	147	129	71	↓36%
7-21 PC	199	273	211	102	↓48%
21-29 PC**	71	92	147	191	↑169%

* = difference was statistically significant ($p<0.05$) using Dunnett's test
** = difference was statistically significant ($p<0.01$) using Dunnett's test

Food consumption (dams):

- Mean food consumption was slightly reduced at 450 mg/kg/day compared to the controls during gestation days 7-10. Reduced food consumption was noted during gestation days 10-13, 13-21, and when the entire treatment period (gestation days 7-21) was evaluated (low food consumption was noted in 7 of 22 females in this group, which excludes the female that was euthanized in extremis, during the treatment

period). The differences at these intervals were generally significant ($p<0.05$) and correlated to the body weight gain effects.

- During the post-treatment period (gestation days 21-29), mean food consumption at the 450 mg/kg/day dose level was higher than controls. This increase was not statistically significant.

Change in Food Consumption Over Specified Period of Gestation					
Period of Gestation	Control	Low Dose 30 mg/kg	Mid Dose 150 mg/kg	High Dose 450 mg/kg	Percent Change (HD from Control)
7-10 PC	166	179	172	152	↓8%
10-13 PC**	154	171	147	123	↓20%
13-21 PC**	132	162	135	98	↓26%
7-21 PC**	148	168	146	115	↓22%
21-29 PC	116	111	128	137	↑18%

** = difference was statistically significant ($p<0.05$) using Dunnett's test

Terminal and necroscopic evaluations:

Dose Group	Control	Low	Mid	High
Number of ♀(Litters if Different)	6	6	5	0
mg/kg/day	0	30	150	450
Number Corpora Lutea-Mean/♀	10	10.5	10	10.6
Number Implantations-Mean/♀	9.1	9.1	9.4	9.6
Percent Pre-Implantation Loss	8.8	11.8	5.9	11.0
Post-Implantation Loss- Mean/♀				
Early	3	11	7	4
Late	0	1	4	3
Dead	0	0	0	0
Total	3	12	11	7
Percent Post-Implantation Loss	1.5	5.6	5.0	3.7
Number Live Fetuses-Mean/♀	9.0	8.6	8.9	9.3
Percent Live Males	52.7	49.5	51.3	52.5
Live Birth Index (%) (live/implants)	98.9	94.5	94.7	96.9
Mean Fetal Weight	40.3	40.2	40.9	39.9
Gravid Uterine Weight	493.1	483.5	508.4	505.4

Toxicokinetics (dams):

Toxicokinetic Study of the Effects of RO5185426 on Embryo/Fetal Development in Rabbits				
Dose (mg/kg)	**0**	**30**	**150**	**450**
No of animals	**6**	**6**	**6**	**6**
AUC0-24 hr (ng hr/mL)				
GD7	BLQ	61500	194000	342000
GD20	BLQ	194000	577000	674000
Cmax (ng/mL)				
GD7	BLQ	5700	15300	194000
GD20	BLQ	13400	32100	577000
Cmax4hr (ng/mL)				

GD21 (F0 female)	BLQ	12700	29600	31400
GD21 (fetus)	BLQ	232	756	1820

Note:
BLQ = these samples were below levels that were quantified.
ND = not determined
GD = gestation day

Offspring (malformations, variations, etc.): There were no treatment-related malformations or developmental variations observed in any of the groups evaluated.

Embryo-fetal Toxicity Studies

Study # (GLP)	Species	Route/ duration	N/sex/ dose	mg/kg/ day	mg/m^2	Significant findings
10260 29/No	Rat	Oral gavage Daily on GD 6-17	8 Female /group /6 (TK)	30 150 800	180 900 4800	**30 mg/kg/day:** No noteworthy findings **150 mg/kg/day:** No noteworthy findings **800 mg/kg/day**: Clinical signs included: red material around the mouth, statistically significant lower mean body weight gain and reduced food consumption during treatment period. **F1 litters at all doses:** No noteworthy findings
10285 43/Yes	Rat	Oral gavage Daily on GD 6-17	25 Female /group /3 (TK)	30 100 250	180 600 1500	**30 mg/kg/day:** No noteworthy findings **100 mg/kg/day:** No noteworthy findings **250 mg/kg/day**: No noteworthy findings **F1 litters at all doses:** No noteworthy findings
10260 33/No	Rabbit	Oral gavage Daily on GD 7-20	6 Female /group /3 (TK)	30 150 450	360 1800 5400	**30 mg/kg/day:** No noteworthy findings **150 mg/kg/day:** No noteworthy findings **450 mg/kg/day**: Morbidity and group termination due to administration difficulties due to intubation errors on gestation days 9-11. Clinical signs included: soft stool, decreased defecation and brown

Embryo-fetal Toxicity Studies

Study # (GLP)	Species	Route/ duration	N/sex/ dose	mg/kg/ day	mg/m^2	Significant findings
						material around urogenital area and base of tail. Lower mean body weight and good consumption prior to death/euthanasia. **F1 litters at 450 mg/kg/day:** Evaluation of fetal morphology in this group was precluded by mortality and early euthanasia on gestation days 9-11.
10285 44/Yes	Rabbit	Oral gavage Daily on GD 7-20	6 Female /group /3 (TK)	30 150 450	360 1800 5400	**30 mg/kg/day:** No noteworthy findings **150 mg/kg/day:** No noteworthy findings **450 mg/kg/day:** Lower mean body weight gain, some individual body weight losses, and reduced mean food consumption noted during treatment period. **F1 litters at all doses:** No noteworthy findings

Note: Both GLP rat and rabbit studies (Study No. 10285 and 102854) used the microprecipitated bulk powder (MBP) formulation of RO5185426
NA = not applicable.
GD = Gestation day

9.3 Prenatal and Postnatal Development

Not required and not submitted.

10 Special Toxicology Studies

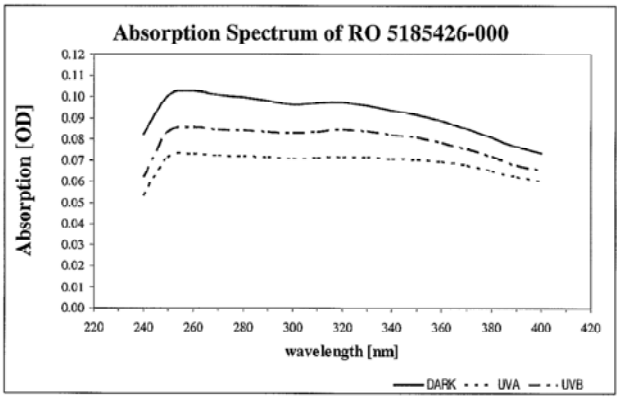
1) Phototoxicity Study *in vitro*

Study Number	Sponsor 1030720, Laboratory 1020S08
File name	1030720
Laboratory	F. Hoffmann-La Roche Ltd., Basle, Switzerland
Study Date	July 2008
GLP	No
Audited	No
Drug	RO5185426, Batch ABRO7607, purity not specified
Methods	
Cells	3T3 fibroblasts clone A 31
Concentrations	0.004 to 9 µg/mL
Solvent	3% DMSO in PBS
Incubation time	1 hour
Analysis	Neutral Red uptake by living cells read at 540 nm after extraction
Radiation source	For UV-A : Sol 500 with filter
	Main spectrum – 315-690 nm
	Irradiance – ~ 1.67 mW/cm^2
	Radiation dose – ~ 5 J/cm^2
Positive Control	Chlorpromazine-HCl

Results

The following spectrograph from the study report shows that vemurafenib has an unusual broad absorption through the near and far UV. The investigators did not specify the concentration of vemurafenib so absorptivity cannot be calculated from the graph. The graph also shows that irradiation of the test solution results in dose and wavelength dependent photodegradation.

Figure 21: UV absorption spectrum of RO5185426-000 (PLX4032) alone or after irradiation with approx. 5 J/cm^2 UVA or with approx. 0.05 J/cm^2 UVB



In the dark (not irradiated) the IC_{50} value for cell death in the presence of vemurafenib was greater than 9 µg/mL (18.4 µM). Cells irradiated with UV-A light died at significantly lower concentrations with an IC_{50} value of 0.197 µg/mL (0.40 µM). Vemurafenib is phototoxic *in vitro* to cultured cells.

2) Determination of Systemic Phototoxicity in the Hairless Rat

Study Number	Sponsor 1032674, Laboratory C51293
File name	1032674
Laboratory	F. Hoffmann-La Roche Ltd., Basle, Switzerland
Study Date	October 2009
GLP	No
Audited	No
Drug	RO5185426-007 (MBP), Batch 97620, purity 29.1%
Methods	
Animals	Female Ico:OFA-hr/hr hairless rat 158.2 – 238.1 g (groups 1 to 4) 202.7 – 285.6 g (groups 6 and 7), group 8 not given The following table from the report shows the doses and animal allocation in this study. The table includes no group 5, a positive control with 8-MOP, because the treatment (0.2 ml/kg i.p.) was fatal to these animals.

	NUMBER OF ANIMALS PER GROUP	ANIMAL NUMBERS PER GROUP		
1 30 mg/kg	6	1		6
2 150 mg/kg	6	7		12
3 450 mg/kg	6	13	-	18
4 Control group (placebo)	6	19	-	24
6 Positive control (8-MOP at 5 mg/kg)	6	37	-	42
7 Positive control (8-MOP at 2 mg/kg)	6	43	-	48
8 450 mg/kg	6	49	-	54

Doses	0, 180, 900, 2700 mg/m^2
Schedule	Single dose
Route	PO gavage
Formulation	Klucel LF in water, pH 4
Incubation time	1 hour
Analysis	Neutral Red uptake by living cells read at 540 nm after extraction
Radiation source	(b) (4) tubes
Spectrum	Main spectral output 350-400 nm; peak 370 nm
Radiation Dose	5, 10, 15, 20, 25, 30 and 35 J/cm^2 (UV-A)
Irradiance	Main test with 5.85 to 6.22 mW/cm^2 Positive control test with 5.47 to 6.34 mW/cm^2
UV-meter	UVA/UVB radiometer (b) (4)
Positive Control	8-methoxypsoralen (8-MOP)
Necropsy	Not done

Procedure

Approximately 1.5 hours after the last administration of vemurafenib (30 minutes following the 8-MOP) the animals were anesthetized with a pentobarbitone (IP). They were

then covered on their back with a stencil with seven uniform holes of 2 cm^2. The exposed seven skin areas of were irradiated with increasing doses of UVA light in steps of 5 J/cm^2 (range 5 J/cm^2 to 35 J/cm^2). The animals were visually assessed for dermal erythema then examined after injection of Evans blue at 24 hours.

Results

Except for group 5 all of which probably died due to an experimental error all animals survived to the end of the experiment. None showed any clinical signs of toxicity.

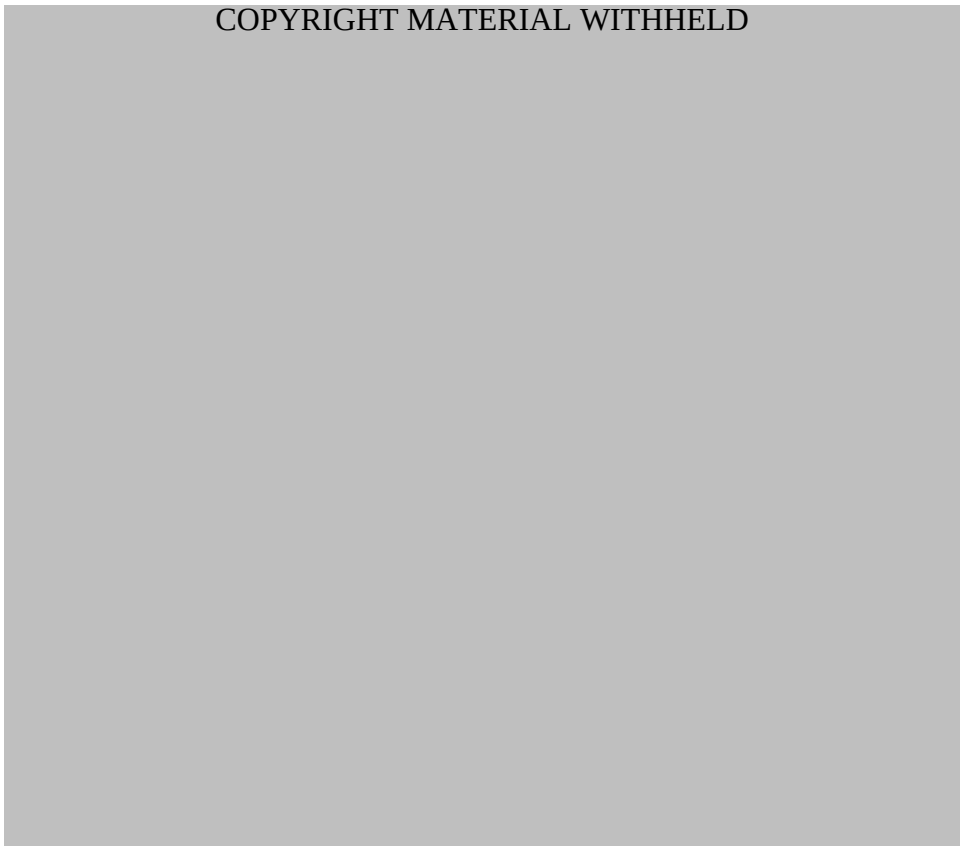
No skin responses developed in any of the treated groups or negative control. The positive controls developed dose dependent erythematous skin reactions. The concentrations of vemurafenib in skin at these doses were probably too low to cause phototoxicity.

11 Integrated Summary and Safety Evaluation

Mechanism of Action

BRaf is a cytosolic serine/threonine-protein kinase. In a well established kinase signal cascade, BRaf is phosphorylated and thus activated by KRas. Activated BRaf phosphorylates MEK which in turn phosphorylates ERK. ERK translocates to the nucleus whereby it stimulates cell growth and division.[7] The following schematic diagram (Figure 22) depicts this cascade.[8]

[7] P. I. Poulikakos and Neal Rosen, , January 18, 2011, Cancer Cell 19:11-15
[8] E. Halilovic and D. B. Solit Current Opinion in Pharmacology 2008, 8:419–426



This simplification of a complex system of stimulatory and inhibitory signals involved in cellular and organ homeostasis forms the basis for the hypotheses on which vemurafenib was developed.

In normal cells, BRaf is quiescent until an extracellular growth factor such as epidermal growth factor binds to its cell surface receptor resulting in the phosphorylation of the upstream kinase RAS thereby initiating the signal cascade that results in cell growth and division. In malignant cells the gene that encodes BRaf, *BRAF*, frequently has a mutation resulting in a valine to glutamic acid substitution at residue 600; the resulting protein is, for this reason, designated $BRaf^{V600E}$. Nevertheless, other amino acid residues can substitute for valine, such as arginine (V600R), aspartic acid (V600D) and lysine (V600K). Numerous cancers, especially malignant melanoma, express one or more of these mutated forms of BRaf. These mutant forms of BRaf are constitutively active, that is they do not need to be phosphorylated by RAS in order to initiate the signal cascade that results in cell division. Vemurafenib was chosen from among other drug candidates because it binds to the ATP binding site of these mutant BRaf kinases and wild type BRaf at relatively low concentrations. Nevertheless, no one has done any formal enzymatic kinetic analysis on this interaction. The nature of the binding has been characterized only by X-ray crystallography.[9]

Pharmacology

In an initial experiment, vemurafenib inhibited the activity of the isolated catalytic region of BRaf wild type protein with an IC_{50} of 140 nm. It inhibited the activity of the isolated catalytic region of $BRaf^{V600E}$ with an IC_{50} of 50 nM indicating only a three fold difference in binding between the wild-type and mutated enzyme. In other studies, vemurafenib was not specific for

[9] G. Bollag et al. Nature. 2010 September 30; 467(7315): 596–599

$BRaf^{V600E}$ (IC_{50} = 10 nM) as it inhibited seven other mutant BRaf kinases at IC_{50} concentrations between 7 and 31 nM including $BRaf^{V600K}$ (IC_{50} = 7 nM), a mutant erroneously detected by the sponsor's clinical assay to determine the patient's BRAF mutational status. When tested against a battery of other kinases, vemurafenib was not specific for $BRaf^{V600E}$ protein. It inhibited the phosphorylation activity of at least 28 other wildtype kinases at IC_{50} values less than 2 µM. These included CRaf, ARaf, SRMS, ACK1, MAP4K5 and FGR, all with IC_{50} values of 100 nM or less as well as wild-type BRaf (IC_{50} = 39 nM in this assay). At steady state, the clinical C_{min} for vemurafenib ranges from 108 to 122 µM, a concentration 2 to 3 orders of magnitude greater than the IC_{50} for inhibition *in vitro*. The clinical concentrations are necessarily much higher than the *in vitro* concentrations because the binding of vemurafenib to human serum albumin is 99.5% (Site IIIA, K_d 3.2 µM). Thus at a total human serum concentration of 100 µM the concentration of free drug would be less than 1 µM.

In various *in vitro* experiments, vemurafenib inhibited human tumor cell growth at IC_{50} concentrations ranging between 0.04 and 25.3 µM. Most cancer cell lines with mutations at the V600 position of BRAF had IC_{50} values that were lower than those of cells with wild-type BRAF. The IC_{50} value for growth inhibition of normal (non-cancerous) human kidney cells was 19 µM.

In 10 cell lines expressing a mutation of BRAF at V600, vemurafenib inhibited cell growth at IC_{50} values less than 1 µM. Cells expressing a mutation at some site other than V600 or wild type BRAF had IC_{50} values that ranged between about 4 and 20 µM except for three that had mutations at NRAS or KRAS where the IC_{50} value was higher. No lines with NRAS or KRAS mutations had IC_{50} values less than 1 µM suggesting that these cells may not require the BRaf pathway for growth stimulation. Inhibition of MEK and ERK by vemurafenib in LOX cells derived from an invasive human melanoma line expressing the $BRAF^{V600E}$ mutation was concentration dependent. The inhibition of MEK was consistently greater than that of ERK.

In vivo in athymic mice, vemurafenib inhibited the growth of LOX, Colo829, and A375 xenografts implanted subcutaneously. All of these invasive human melanoma cell lines express the $BRAF^{V600E}$ mutant. Treated mice survived longer than controls and some achieved complete tumor cure. In mice bearing LOX xenografts, a dose of 100 mg/kg of vemurafenib caused decreases in the phosphorylation of MEK and ERK in tumor tissue. Phosphorylation returned toward normal levels as vemurafenib was cleared from plasma and tumor. Again, MEK phosphorylation was more completely inhibited than ERK phosphorylation suggesting ERK phosphorylation by pathways other than RAF-MEK-ERK or a nonlinear effect.

In a series of studies designed to examine mechanisms of resistance to the inhibition of vemurafenib *in vitro*, investigators developed six strains of cells derived from a parental A375 melanoma line by clonal selection under the pressure of increasing concentrations of vemurafenib. They showed that the parental line was sensitive not only to inhibition by vemurafenib but also to two other drugs targeting RAF, and to drugs targeting MEK, CDK, PI3K, or mTOR but not to a drug targeting AKT. The results for the three RAF inhibitors are notable as the values span two orders of magnitude. In the resistant clones, vemurafenib had IC_{50} values 90 to 120 fold greater than those seen in the parental line. The six resistant clones were also relatively resistant to MEK inhibition but became more sensitive to one RAF inhibitor, a CDK inhibitor, a dual inhibitor of PI3K and mTOR, and an AKT inhibitor. The selection mechanism appears to increase expression of CRAF and to some extent BRaf. Addition of vemurafenib suppressed the phosphorylation of ERK in sensitive cells but had little effect on ERK phosphorylation in one resistant line. The selection process also increased the phosphorylation of AKT in this cell line. Further experiments suggested that Pgp may contribute to the resistance mechanism as may CRAF upregulation. Unlike the parental line, all six clones had elevated expression of activated RAS and all six had an uncommon, activating mutation (K117N) in the *KRAS* gene. Knocking down KRAS expression with siRNA constructs had no effect on the vemurafenib sensitivity of the parental A375 cells, but caused increased sensitivity

in the resistant cells, with 2.3-fold and 5.8-fold decreases in IC_{50} values in resistant cell lines R1 and R6, respectively. PI Poulikakos and N Rosen have reviewed other possible mechanisms for the development of resistance to vemurafenib.[7]

In clinical trials, vemurafenib stimulates the development and growth of cutaneous squamous cell carcinoma (SCC). To investigate potential mechanisms for this finding, the sponsor conducted xenograft experiments with human SCC cells in nude mice to determine the effects of treatment with various doses of vemurafenib, R05212054 (another compound selected to inhibit $BRaf^{V600E}$), or RO5068760 (a compound selected to inhibit MEK). Treatment with vemurafenib caused a dose dependent acceleration of the growth of the xerographs in these mouse models. Treatment with R05212054 had no effect. Since RO5212054 was also selected to inhibit the ATP binding site of $BRaf^{V600E}$, this result suggests that the effect of vemurafenib on the development of SCC in melanoma patients is not mediated by inhibition of the mutant kinase but is rather an off target effect. Treatment with the MEK inhibitor alone resulted in significantly decreased tumor growth. Lastly treatment with both vemurafenib and the MEK inhibitor in combination resulted in tumor growth suppression that depended on the dose of the MEK inhibitor suggesting that the effect of vemurafenib is upstream of that kinase. The highest doses tested in this series of experiments caused some weight loss or decreased weight gain indicating that they were at or close to the MTD.

In a separate *in vivo* experiment conducted in nude mice implanted subcutaneously with human squamous cell carcinoma tumors, treatment with doses of vemurafenib of 75 mg/kg bid for two weeks caused a slight increase in invasive tumor growth and inflammatory cell infiltration relative to untreated controls suggesting that the mechanism by which vemurafenib stimulates SCC growth may involve inhibition of some immune mediated mechanism controlling the growth of these cells, but these experiments were not definitive. Vemurafenib did not cause any discernable increase in stain intensity for phosphorylated MAPK or MEK1 in immunostained tumor sections again suggesting that the mechanism may not involve that pathway. The mechanism by which vemurafenib increases the incidence of the development of SCC clinically is still unclear, but may possibly involve MEK activation through an alternative pathway up-regulated by BRAF inhibition.

Secondary Pharmacology

A study report of experiments designed to identify off-target pharmacological activity was inadequate for comprehensive review, but the report states that the drug had no activity at concentrations up to 10 μM at any of the 63 pharmacologically active non-kinase receptors or channels tested *in vitro*. Nevertheless, toxicological evidence suggests that vemurafenib may target numerous kinase pathways *in vivo* and none can be singled out as the sole mechanism by which it exerts its clinical efficacy.

Safety Pharmacology

Vemurafenib inhibited the delayed rectifier cardiac potassium current (I_{Kr}) in human embryonic kidney cells expressing hERG with an IC_{50} of 1.24 μM and a Hill Coefficient of 1.7.

A standard study of the effects of vemurafenib at on isolated dog Purkinje fibers at concentrations of 0.2, 2 μM and 20 μM was difficult to interpret because only two of the experiments at a nominal concentration of 20 μM had measurable drug in the perfusate. The drug evidently had limited or highly variable solubility under the conditions of this assay. The two valid tests at determined concentrations of 7.26 and 7.86 μM demonstrated a clear decrease in V_{max} of about 50% suggesting the potential for conduction delay. Visual inspection

of the action potentials suggests a dose dependent delay in repolarization consistent with the QT prolongation seen clinically. This study is inadequate to characterize the potential for cardiac toxicity associated with vemurafenib but it is strongly suggestive of such toxicity.

In conscious dogs, single oral doses as high as 1000 mg/kg (20000 mg/m^2) appeared to caused a relative decrease in systolic, diastolic and mean arterial blood pressure between 11 and 24 hours (difference about 20 mm in systolic pressure at 18 hours relative to control) but the difference appears to be the result of an increase in pressure in the controls relative to baseline which is difficult to explain. The RR interval increased significantly (20%) in the HD group at 3 hours (approximately t_{max}) and significantly decreased by 15% at 6 hours relative to controls. QT interval was significantly prolonged by about 12 ms at 3 hours post dosing (approximately t_{max}) but this difference did not persist when QT was corrected for heart rate. There was also an increase in the incidence of AV block in treated dogs. This study did not use the MPB formulation and did not determine pharmacokinetics so the results are difficult to interpret. Nevertheless, vemurafenib appears to have some effects on cardiac parameters.

The respiratory frequency of rats treated with the high dose of 1000 mg/kg was consistently higher than that of controls. This increase reached statistical significance between 120 to 160 minutes post dosing, approximately T_{max} in the rat. This increase may have been due to the stress of the toxicity of vemurafenib and not a direct pharmacological effect.

In the standard Functional Observational Battery test a single dose of vemurafenib of 6000 mg/m^2 given to male rats caused no toxic signs or symptoms. Nevertheless, the formulation used in this test was evidently not the microprecipitated drug product, thus the exposure was probably too low to cause toxicity because of limited bioavailability.

Toxicology

The two single dose studies of vemurafenib in rat and dog were exploratory and designed to determine the pharmacokinetics of the drug. The highest doses in these studies, 300 mg/kg in the dog and 1000 mg/kg in the rat, were too low to cause toxicity. The pharmacokinetic sampling was too sparse to yield accurate parameters. Nevertheless, the studies did demonstrate that rats and dogs eliminate vemurafenib much more rapidly than do humans.

In an early study using crystalline vemurafenib, rats tolerated single daily doses as high as 1000 mg/kg (6000 mg/m^2) per day for 28 days with no mortality. Cholesterol increased about 33% in males and females and triglycerides increased 100% in males in the HD group. Triglycerides were decreased in females in all dose groups about 40%; the decrease was not dose dependent. Relative to body weight, the average weights of the heart (~10%), liver (~7% in males) and spleen (~10%) increased in the HD group. Uterine weight increased about 31% in mid dose animals (600 mg/m^2) and about 34% in HD animals. Microscopically the increased weight in the heart correlated with the finding of chronic inflammation in 5 of 10 males and 3 of 10 females. In the liver there were mononuclear infiltrates (7/10 females, 10/10 males). In the pancreas, there was hyperplasia of the islet cells in 8 of 10 HD females. In the Jejunum there was lymphangiectasis. All these organs showed signs of recovery. The exposure of rats to vemurafenib based on AUC on day 21 of the study relative to the AUC at steady state in humans was about the same in males but only about 70% that of humans in females.

In another early study using crystalline vemurafenib, dogs tolerated daily doses as high as 1000 mg/kg (20000 mg/m^2) per day for 28 days with no mortality, but the incidence of emesis increased sharply with increasing dose. In HD males, there was a 33% decrease in heart rate at the end of the study, but QT_c was unchanged. Females showed a 17% increase in heart rate. Creatine kinase increased 23% in HD males and 100% in HD females. In males, absolute liver weight increased 20% in HD animals and the kidney/brain weight ratio increased 7%; both

these changes were statistically significant. In females, absolute and relative heart weight increased with increasing dose and the difference was significant in the HD group. A 7% increase in relative liver weight was also significant. Relative kidney weight increased with dose to as much as 19% in HD females; in the HD group the increase was significant. One HD male had vacuolization of the renal tubular epithelium. There were no other histopathological correlates. The exposure of dogs to vemurafenib was very low relative to the human exposure.

Rats tolerated single daily doses of vemurafenib (micro-precipitated formulation) as high as 450 mg/kg (2700 mg/m^2) for 26 weeks with no drug related mortality or signs of clinical toxicity. This was a maximum feasible dose for this formulation. The high dose caused an increase in cholesterol (22% in males and 58% in females). Female rats showed a dose dependent decrease in AST and ALT that remained observable after 13 weeks of recovery suggesting chronic liver injury. In high dose females, there was a dose dependent decrease in neutrophils (about 26%) at the end of dosing. Dosing was not associated with microscopic changes. Maximum concentrations reached in this study were unusually high, with C_{max} values reaching 161 µM in HD males and 300 µM in HD females at the end of dosing. This is greater than the mean C_{max} seen in clinical studies, 126 µM. Rats are relatively insensitive to vemurafenib toxicity because they clear the drug much more rapidly, probably through oxidative metabolism.

A long term study in dogs was intended to continue for 39 weeks but the high dose of 450 mg/kg BID PO proved intolerable after 10 days. Dosing was suspended at this time in HD animals and two moribund HD dogs (one male, one female) were killed humanely and examined. These dogs were replaced on study and dosing was resumed on day 19 at a lowered dose of 300 mg/kg BID PO in the HD dogs. This dose also proved intolerable and dosing was again suspended on day 37. The study was aborted and the dogs transferred to the subsequent 13 week study (*vide infra*, study1032862).

The HD dogs showed clinical signs of excessive salivation, vomiting, dehydration, soft or malformed feces and hypoactivity. MD (150 mg/kg BID) animals showed these signs less frequently and with less severity. Some dogs in all dosed groups vocalized and struggled during dosing suggesting pain. HD animals, particularly those killed humanely, showed signs of thin appearance, tremor and reddened skin.

Dosing caused dose dependent increases in liver function enzymes and decreases in other parameters consistent with significant liver damage. Nevertheless, this liver damage was not sufficiently severe to have necessitated ending the study or to cause the death of the two dogs. Other, undefined toxicities are probably responsible for the clinical signs that resulted in the termination of the study. Dosing also caused dose dependent neutrophilia with profound eosinophilia consistent with an allergic response.

The two dogs killed humanely were the only ones examined post mortem. In the liver of these two dogs there was minimal scattered hepatocellular degeneration, a mild to moderate increase in the number of Kupffer cells, and the presence of pigment in hepatocytes and Kupffer cells. These findings correlated with increased ALP, ALT, AST and GGT levels in both moribund dogs. In the sternal bone marrow of the moribund female there was moderate pancytic necrosis. There was no finding of necrosis in the femoral bone marrow section or in the spleen.

After 28 days recovery the dogs from the previous study and some additional dogs were placed on a 13 week study. A dose of 300 mg/kg BID of vemurafenib resulted in the early death of two male dogs on days 31 and 23. A female given the low dose of 150 mg/kg BID also became moribund by day 31. These dogs had lost weight, appeared thin, refused canned food, vomited and vocalized during handling suggesting pain. All three had histomorphological changes in the liver including of necrosis, perivascular mixed infiltrates, and Kupffer cell increases. The damage in the liver was not sufficient to account for the animal's demise. All these dogs had been treated with vemurafenib one month earlier in a previous study.

Animals that survived to the day 28 interim necropsy also showed signs of vomiting, vocalizing and weight loss in males. LD females actually weighed more than controls by 17%. No dogs died after day 31 and their condition appeared to improve somewhat with further dosing consistent with the decreasing exposure to the drug seen in the pharmacokinetic study. Dogs necropsied on day 28 (and the dogs that died early) showed signs of hepatic damage including increased liver function enzymes, decreased serum protein and microscopic damage noted above. Liver damage as measured by ALT was somewhat worse in females. This damage was present at day 93 after the end of dosing but was less severe. Cholesterol and triglycerides were elevated on day 15 possibly because of a direct effect on kinases involved in their synthesis. Cholesterol remained elevated in males at the end of the study but was normal in females. Amylase was elevated suggesting pancreatic damage but glucose was actually low. BUN was low probably reflecting reduced protein metabolism, decreased dietary protein or both. Hematological analysis showed a dose dependent neutrophilia that was more severe in males during all parts of the study. This was accompanied by an unusual eosinophilia and basopenia. The changes resemble those seen with an allergic response. All these changes showed signs of recovery or partial recovery four weeks after the end of dosing.

On Day 28, liver weight was low relative to body weight in males and females. It remained somewhat low in females at the end of the study. On Day 28, there was a dose dependent increase in spleen weight in males. Spleen weight in males was only slightly elevated in males at Day 93. In females at Day 28, there was a decrease in spleen weight in both LD and HD dogs but by Day 93 spleen weight was increased to the level seen in dogs on day 28 (~50%). On Day 28 thymus weight increased significantly in LD males but decreased in HD males, it was well below normal (30% or more) in both groups at the end of dosing. In females, thymus weight was low (~40%) in LD dogs on Day 28 but only slightly elevated in HD dogs. Thymus weight was low in HD females on Day 28. Adrenal weights were low in both sexes on Day 28. It is difficult to interpret some of these results because the sample sizes were so low, but the severity of change and the correlation with the unusual hematology argues for some real drug related effects probably secondary to the inhibition of kinases other than the target.

HD males had statistically significant prolonged mean corrected QT (QT_c) interval and QT interval (15 msec for both) on Day 24. The mean QT interval was increased 3 ms on Day 87 and 13 ms on Day 23 of recovery but these increases did not reach statistical significance. This finding correlates with the finding of a 15 ms increase in human patients. The QT interval was not prolonged in females. In HD males a ~9% increase in PR interval on all days of dosing, but this increase did not reach significance due to sample size. This suggests some effects on conduction in the AV node.

Females in this study had consistently lower exposures than males, but only by a small amount. The exposure in the HD group was only slightly greater than that in the LD group once the drug had reached steady state. Exposure decreased between Days 28 and 93 suggesting induction of metabolism. Exposure based on AUC was consistently less than half that seen clinically suggesting that despite more rapid clearance dogs are much more sensitive to vemurafenib toxicity than are humans.

Reproductive Toxicology

The sponsor conducted a series of dose-range finding (non-GLP) and definitive (GLP) embryo-fetal developmental toxicity studies in pregnant Sprague-Dawley rats and New Zealand White rabbits. vemurafenib was administered by oral gavage during organogenesis in rats (gestation days 6 through 17) and in rabbits (gestation days 7 through 20), followed by caesarian sections conducted on gestation days 20 and 29, respectively. As recommended in

ICHS9, no specific studies in rats and rabbits were conducted to evaluate the potential effects of vemurafenib on fertility; however, in repeat-dose toxicology studies in both rats and dogs there were no remarkable histopathological findings on reproductive organs in males and females. For the definitive embryo-fetal toxicity studies, the microprecipitated bulk powder formulation of vemurafenib was used.

In the initial dose-range finding study, pregnant rats were given oral doses of vemurafenib of 0, 30, 150, or 800 mg/kg/day (0, 180, 900 or 4800 mg/m^2/day). Clinical signs included red material around the mouth in all females at the high dose of 800 mg/kg/day. Maternal toxicities such as decreased food consumption and reduced body weight gain of up to 12 and 28%, respectively, during gestation days 6-18 were also observed at this dose. There was no evidence of embryo-fetal toxicity or teratogenicity at this or any other dose tested. Toxicokinetic data showed that at 150 mg/kg and 800 mg/kg, both AUC and C_{max} increased less than dose-proportionally with increasing dose. As a result of the reduced body weight gain at 800 mg/kg/day, the NOAEL for maternal toxicity was 150 mg/kg/day, however, the NOAEL for embryo/fetal development was the high-dose of (800 mg/kg/day). In the GLP definitive study, pregnant female rats were administered vemurafenib at 0, 30, 100, or 250 mg/kg/day (0, 180, 600 or 1500 mg/m^2/day). There was no mortality nor any adverse effects in the dams or the fetuses treated with vemurafenib at doses up to 250 mg/kg/day. Toxicokinetic data showed that drug accumulation (up to 2-fold based on AUC and C_{max}) was observed across all dose levels and mean fetal levels at 4 hours post-dose at necropsy increased with increasing dose. Thus, the NOAEL for embryo-fetal development toxicity in rats was 250 mg/kg/day, the highest dose tested.

In an initial range-finding study in pregnant rabbits with doses of 0, 30, 150, or 450 mg/kg/day (0, 360, 1800 or 5400 mg/m^2/day), all HD animals were killed humanely due to administration difficulties with the corn-oil formulation. No significant adverse effects on the dams or fetuses were observed in the LD or MD groups. Toxicokinetic data showed drug accumulation after multiple days of dosing at both 30 and 150 mg/kg/day dose. Furthermore, there was an increase in exposure after repeated dosing due to the slow clearance of the drug. Since deaths occurred in animals at 450 mg/kg/day and fetal data was not available, it was unclear whether this dose affected the fetuses. Therefore, the mid-dose level of 150 mg/kg/day was considered the NOAEL for maternal and embryo/fetal development.

In a subsequent definitive GLP study, pregnant female rats were administered vemurafenib at 0, 30, 150, and 450 mg/kg/day. In this study the sponsor used the MBP formulation making it was feasible to give the HD. Maternal toxicities (reduced food consumption and decreased body weight gain of up to 22 and 50%, respectively, during gestation days 7-21) were observed at 450 mg/kg/day, but the HD did not cause any signs of embryo-fetal toxicity or teratogenicity. Toxicokinetic data showed drug exposure increased less than proportionally to increases in dose and there was some drug accumulation (up to 3-fold based on AUC and C_{max}) at all dose levels. Mean fetal concentrations at 4 hours post-dose at necropsy increased with increasing dose. Due to the changes in body weight and food consumption parameters, the highest dose tested, 450 mg/kg/day, was considered the NOAEL embryo/fetal development while the next lower dose, 150 mg/kg/day, was considered the NOAEL for maternal toxicity.

Because vemurafenib showed no evidence of teratogenicity in rat embryo/fetuses at doses up to 250 mg/kg/day (approximately 1.3 times the human clinical exposure based on AUC) or rabbit embryo/fetuses at doses up to 450 mg/kg/day (approximately 0.6 times the human clinical exposure based on AUC), the sponsor recommended pregnancy category (b) (4). The description of a Pregnancy category (b) (4) in the CFR, however, is based on the assumption that complete battery of non-clinical tests as specified by the ICH document M3 were conducted. Vemurafenib was developed under the exceptions to ICH M3 described in ICH S9,

thus the sponsor was not required to complete the full battery of non-clinical reproductive toxicology studies and the full potential for vemurafenib to cause developmental damage remains unclear. Additionally, the doses in the reproductive toxicology studies yielded exposure multiples of only 1.3 in the rat and 0.6 in the rabbit. These exposure multiples were relatively low to reliably predict embryo-fetal toxicity. Furthermore, variations in BRAF signaling and studies in BRAF knock out mice have been associated with serious embryo-fetal toxicity. Therefore based on this information, Pregnancy category D is recommended.

Genotoxicity

Vemurafenib did not cause an increase in reverse mutations in the Ames assay. Neither did it cause chromosomal aberrations in an *in vitro* test using fresh human peripheral blood lymphocytes. *In vivo*, vemurafenib did not cause chromosomal damage in the rat bone marrow erythrocytes micronucleus test. Nevertheless, in the clinical studies vemurafenib dosing was associated with the rapid development and growth of cutaneous squamous cell carcinoma. In these studies three patients developed new primary melanomas.

Correlation with Clinical Toxicity

The sponsor selected the dose for clinical development based on nonclinical data and clinical efficacy and safety observed in the dose escalation phase of study PLX06-02. By the sponsor's interpretation, *in vitro* studies demonstrated cell cycle arrest at lower vemurafenib concentrations and apoptosis only at higher concentrations. Similarly, in xenograft models, tumor stasis was observed at lower vemurafenib concentrations ($AUC_{0\text{-}24}$hr of 100-200 μM*hr), and tumor shrinkage was observed only at higher vemurafenib concentrations ($AUC_{0\text{-}24}$hr > 400 μM*hr). Higher vemurafenib concentrations were associated with greater tumor shrinkage and longer survival. The sponsor thus concluded that vemurafenib exposures in patients would need to exceed an $AUC_{0\text{-}24hr}$ of 400 μM*hr to observe tumor regression. Furthermore, there was considerable heterogeneity in tumor response to treatment with vemurafenib, depending on the tumor cell line used. Plasma exposures greater than 2000 μM*hr were required for xenograft tumor growth inhibition of some tumor cell lines (e.g. HT29 this data was not submitted to the NDA). The sponsor considered that the maximum tolerated dose determined in the Phase 1 trial would afford the maximum therapeutic index for metastatic melanoma.

The dose escalation study for vemurafenib, PLX06-02, evaluated doses ranging from 160 mg b.i.d. to 1120 mg b.i.d. with the microprecipitated bulk powder. Consistent with nonclinical findings, tumor regressions in PLX06-02 were first observed in the dose range of 240 mg b.i.d. to 360 mg b.i.d. that on average exceeded the target exposure threshold $AUC_{0\text{-}24h}$ of 400 μM*hr. The investigators considered response to be dose proportional. Tumor responses were detected in patients with Stage M1a disease, whereas other patients in these initial dose cohorts progressed. Consistent and more pronounced tumor regression in metastatic disease at sites such as liver, lung, and bone, was observed in the dose range of 720 mg b.i.d. to 1120 mg b.i.d. Nevertheless, DLTs, primarily Grade 3 rash and Grade 3 fatigue, were observed in 4 of 6 patients at 1120 mg b.i.d., indicating that this dose could not be tolerated. Therefore, the maximum tolerated dose of 960 mg b.i.d., representing the approximate midpoint between 720 mg b.i.d. and 1120 mg b.i.d., was selected for further clinical development. The following table correlates the toxicities seen clinically at an incidence of 10% or more with toxicities seen in non-clinical models.

Toxicity	Humans	In Vitro	Mice	Rats	Dogs
Cutaneous Squamous Cell Carcinoma	~ 29%		Modeled in Athymic Mice		
Photosensitivity	~ 40 %	Cytotoxic		Exposure too low	
Rash	~ 64 %				erythema
Fatague	~ 52 %				Lethargy
Arthralgia	~ 49 %				Some indication of pain during handling
Liver Toxicity	~ 24%				Hepatocellular damage and increased LFTs
QT Prolongation	12 -15 ms	Positive hERG assay			15 ms

In clinical studies at steady state, daily fluctuations in the concentration time profiles were small with C_{max} values approximately 1.2–fold higher than pre-dose values. C_{min} vemurafenib concentrations averaged 108 to 122 µM across studies and remained constant for patients continuing on the treatment regimen. Study NP25163 demonstrated that vemurafenib exposure increased proportional to increasing dose over the dose range of 240, 480, 720, and 960 mg b.i.d., with an overlap in the ranges of individual exposures between doses. A population PK analysis utilizing 458 patients from NO25026, NP22657 and NP25163 estimated the elimination half-life to be approximately 57 hours.

Pharmacokinetic data collected in clinical study NP22676 was designated by the clinical pharmacology reviewer as the data that should be used to make comparisons between clinical and non-clinical exposures and is represented in the following table.

N = 21	C_{max} (µM)	T_{max} (hr)	AUC_{0-8hr} (µM*hr)	AUC_{0-24hr} (µM*hr)	AUC_{0-12hr} (µM*hr)
Mean	**126**	**6.86**	**861**	**2400**	**1227**
SD	35	9.53	247	751	347
Min	34	0	251	649	363
Median	129	3.1	898	2425	1253
Max	185	26.1	1296	3609	1798
CV%	27.9	139	28.7	31.3	28.3

Data from the same trial suggested that treatment with vemurafenib could affect the metabolism of drugs that were substrates for cytochrome P450 enzymes 1A2 and 3A4 but not 2C19, 2D6, or 2C19. In all clinical studies and at all dose levels evaluated, vemurafenib exposure was highly variable among individual patients.

The relative proportions of vemurafenib and its metabolites were characterized in a mass balance study following oral administration of a single dose of ^{14}C-vemurafenib to melanoma patients. In this study, the majority of the parent molecule and metabolites were eliminated in the feces, accounting for an average of 94% of the radioactivity dose. Renal elimination accounted for < 1% of the input radioactive dose. The parent compound was the predominant component in all analyzed plasma samples with metabolites representing less than 5% of the total chromatographic radioactivity. Over the first 96 hours, potential metabolites each accounted for less 0.5% of the total administered dose in urine and ≤ 6% of the total administered dose in feces. Based on mass spectrometry, no new metabolites have been

detected in plasma and feces in this study that have not been observed in *in vitro* studies with rat and human hepatocytes and *in vivo* studies in rats and dogs.

There are no outstanding pharmacology issues and no post-marketing requirements or commitments. There are no non-clinical findings of unacceptable toxicity that would prevent the approval of vemurafenib for this indication.

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