CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203341Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Bosulif (bosutinib)

Date: August 15, 2012
To: File for NDA 203341
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review of Dr. Luan Lee and labeling and secondary memorandum provided by Dr. Saber. I concur with Dr. Saber's conclusion that Bosulif may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
08/15/2012

MEMORANDUM

Date: August 9, 2012
From: Haleh Saber, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology Oncology Products (OHOP)
Re: Approvability for Pharmacology and Toxicology
NDA: 203341
Drug: BOSULIF® (bosutinib) tablets for oral administration
Indication: indicated for the treatment of chronic, accelerated, or blast phase Ph+ chronic myelogenous leukemia (CML) in adult patients with resistance, or intolerance to prior therapy
Applicant: Wyeth Pharmaceuticals, Inc.

Bosutinib is a small molecule tyrosine kinase inhibitor (TKI) developed for the treatment of CML. Bosutinib inhibits the Bcr-Abl kinase and the Src-family kinases, including Src, Lyn, and HCK as demonstrated *in vitro* by Invitrogen Z-Lyte assay. Bosutinib inhibited several mutant forms of Bcr-Abl; however, it did not inhibit the T315I mutant *in vitro* as demonstrated by IC50s greater than 100-fold for T315I-Abl compared to those for Src and c-Abl. In *in vitro* studies, bosutinib inhibited proliferation of CML cell lines. Bosutinib treatment reduced the size of CML tumors growing in nude mice and inhibited growth of murine myeloid tumors expressing several imatinib-resistant forms of Bcr-Abl; very low or minimal inhibition was seen for cells containing the T315I mutation in the panel of mutants examined. The pharmacologic class assigned to bosutinib is "kinase inhibitor" consistent with other drugs of the same class, such as imatinib, dasatinib, and nilotinib.

Pharmacology, safety pharmacology, pharmacokinetic/ADME (absorption, distribution, metabolism and excretion), and toxicology studies were conducted in *in vitro* systems and/or in animal species. Animal toxicology studies were conducted in appropriate species, using the administration route and dosing regimens that adequately addressed safety concerns in humans. In general, the toxicity profile was similar in rodent and nonrodent species. The main drug-related toxicities in toxicology studies were in the GI tract and the hematologic system. GI toxicities included hemorrhage and erosion and was considered the cause of deaths in animals. Hematologic toxicities may be secondary to the GI bleeding and the resulting inflammation; effects included increases in white blood cells and differentials, changes in red blood cell parameters, and increased platelet counts. Adverse findings in the liver (e.g. increased ALT and AST) were reported in patients; however, hepatobiliary toxicity was of low incidence in animals and was mainly seen upon histopathology examination with no correlating changes in clinical chemistry parameters. In a safety pharmacology study, bosutinib inhibited the hERG channel current at an IC_{50} value of 0.3 μM and may be hence considered a moderate potency blocker. In a safety pharmacology study in dogs, transient increases in blood pressure and a secondary reduction in heart rate were observed for 2 minutes after IV infusion of

bosutinib. The effect of a single dose of bosutinib at 500 mg was investigated in 70 healthy subjects. No significant changes in the QTc were observed. However, QTc prolongation was reported in patients treated with bosutinib. See the label for additional information.

Bosutinib was not mutagenic or clastogenic when tested in the battery of genotoxicity studies. A unique human metabolite, metabolite M2, was tested to assess its genotoxicity potential. M2 was not genotoxic in the studies conducted, the Ames test and the chromosomal aberration assay in human peripheral blood lymphocytes. A 2-year carcinogenicity study was conducted in Sprague-Dawley rats. The study was negative for drug-induced neoplasms.

In embryofetal developmental (EFD) studies in rats and rabbits, bosutinib was administered orally to pregnant animals. There was no maternal toxicity or adverse embryo-fetal developmental toxicity in rats treated with bosutinib up to 10 mg/kg/day. It appears that this study did not expose pregnant rats to enough bosutinib to fully evaluate adverse outcomes. The inadequacy of dosing in rats is supported by the following observations: lack of maternal toxicities in the rat EFD study, presence of bosutinib-derived radioactivity in the fetus in a distribution study indicating that bosutinib and/or its metabolites can cross the placenta, and the embryonic toxicities observed in the fertility and early embryonic developmental study when female rats were dosed with 30 mg/kg/day of bosutinib.

Adverse embryofetal effects were evident in rabbits. At the maternally-toxic dose of 30 mg/kg/day of bosutinib in rabbits, there were fetal anomalies (fused sternebrae, and various visceral observations), and an approximate 6% decrease in fetal body weight. Due to the adverse embryofetal findings, a pregnancy Category D has been assigned to bosutinib.

In a fertility and early embryonic developmental study conducted in rats, drug-treated males were mated with untreated females, or untreated males were mated with drug-treated females. When males treated with bosutinib were mated with females, the number of pregnancies was reduced. When treated female rats were mated with untreated males, the number of pregnancies was unaffected but there were increased embryonic resorptions, decreased implantations, and reduced number of viable embryos.

In a distribution study, lactating rats were administered a single oral dose of radioactive bosutinib. Significant amount of radioactivity was detected in the milk of lactating rats. In addition, the level of radioactivity in suckling offspring was significant (8-fold the radioactivity in the plasma of lactating rats). The presence of radioactivity in the (b) (4) (b) (4) plasma of suckling offspring has been added to Section 8.3 of the label, Nursing Mothers.

The nonclinical studies were reviewed by Dr. Shwu-Luan Lee. The nonclinical findings are summarized in the "Executive Summary" of the NDA review and reflected in the

product label. The carcinogenicity study was reviewed by Dr. Shawna Weiss and archived separately. For product labeling, see the approved label.

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

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/s/

HALEH SABER
08/09/2012

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

CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

Reviewer:	Shawna L. Weis, PhD
ECAC Meeting Date:	26 June 2012
NDA #:	203341
Drug Code:	SKI-606, WAY-173606
CAS#:	380843-75-4
Division(s):	Division of Hematology and Oncology Toxicology in support of the Division of Hematology Products
Drug Names:	Bosutinib®
Applicant:	Pfizer Global Research and Development
Conducting Laboratory:	Until Week 36: Wyeth, Chazy, NY
	Thereafter: (b) (4)
Carcinogenicity Final	
Study Report Date:	24 May 2012

Therapeutic Category/MOA:

Indicated for the treatment of chronic , accelerated, or blast phase Ph+ chronic myelogenous leukemia (CML) in adult patients with resistance, or intolerance to prior therapy via Inhibition of Src family kinase and Abl protein tyrosine kinase

Pharmacological Classification: Kinase inhibitor

Mutagenicity/Genotoxicity: No. Bosutinib was negative in Ames, and in vitro chromosome aberration both with and without S9 activation, as well as in the in vivo micronucleus assays in rats.

The following were the conclusions of the Executive Carcinogenicity Assessment Committee on 17 July 2012:

- The Committee agreed that the study was acceptable, but noted that moving an ongoing carcinogenicity study is undesirable and rendered historical control data difficult to interpret due to numerous differences between the two sites.
- The Committee concurred that the study was negative for drug-induced neoplasms.

RAT CARCINOGENICITY STUDY: Standard two-year bioassay

Rat study duration (weeks):	91-100 weeks with early termination due to mortality
Study starting date:	16 July 2009
Study ending date:	23 March 2010
Rat strain:	Sprague-Dawley CD®
Route:	Oral gavage Dosing
Comments:	None

Number of rats used: 360/sex (Main Study) + 9/sex TK

Table 1: Study Design

Toxicology Group	Dose (mg/kg/day)	# / Sex	Toxicokinetic Groups	Dose (mg/kg/day)	# / Sex
1 (water) †	0	60 M / 60 F	10	0	3 M / 3 F
2 (vehicle) §	0	60 M / 60 F	11	2.5	9 M
3 (vehicle) §	0	60 M / 60 F	12	1.5	9 F
4	2.5	60 M	13	7.5	9 M
5	1.5	60 F	14	5.0	9 F
6	7.5	60 M	15	25 / 15	9 M
7	5.0	60 F	16	15	9 F
8	25 / 15	60 M			
9	15	60 F			

†Group 1 = water

§Groups 2, 3 = vehicle (0.5% CMC, 2.0% TWEEN 80, 0.06% acetic acid and water)

Basis for doses selected:

The high dose selection was based on the results of the 6-month rat toxicology study. Decreased body weight, food consumption and survival were observed at doses of 100 / 70 mg/kg/day. Animals tolerated 30 mg/kg/day bosutinib in that study.

Prior FDA dose concurrence:

Yes. Dose selection was per CAC recommendation (16 June 2009).

Rat Carcinogenicity:

No. There were no tumors or combinations of tumors that met the criteria for statistical significance in this study. As indicated in the **Table 2 and Table 3** below (Males and Females, respectively), there were tumors that met criteria for acceptance by either trend or pairwise comparison; however, none met both criteria. Moreover, when the high dose groups were excluded from the analysis due to early mortality, the positive trends were no longer statistically significant.

Table 2: Rat tumor findings: Males

Site	Tumor	Common or Uncommon	Incidence in controls	Trend		Pairwise (treated vs control)		
				Excluding High	Including High	Low	Mid	High
Pituitary (pars distalis)	Adenoma	Common	52.5%	0.0271	0.0979	0.6961	0.0338	0.2074
	Adenoma and Carcinoma	Common	54.2%	0.0486	0.1424	0.7843	0.0596	0.2899
Thyroid	Follicular cell carcinoma	Common	1.7%	0.2494	0.0278	1.0000	0.3735	0.1133
Skin	Fibroma	Common	3.3%	0.3297	0.9297	0.0094	0.4028	1.0000
	Fibroma and fibrosarcoma	Common	5.8%	0.6669	0.9573	0.0194	0.7927	0.9516
	Malignant schwannomas	Rare	0.0%	0.1764	0.0307	0.3333	0.3182	0.0696

Table 3: Tumor findings (females)

Site	Tumor	Common or Uncommon	Incidence in controls	Trend	Pairwise (treated vs control)		
					Low	Mid	High
Adrenal glands	Pheochromocytoma (benign)	Rare	0.0%	0.7852	0.0289		
Skin	Fibroma	Common	1.7%	0.3931	0.6768	0.0381	0.6768
	Keratocanthoma and squamous cell tumors	Rare	0%	0.0483	0.3204		0.0963
Pituitary (pars distalis)	Adenomas and carcinomas	Common	72.5%	0.1377	0.1459	0.0398	0.1685

Rat Study Comments

- **Mid-Study Relocation and Applicability of Historical Controls**: This study originated at the Wyeth facility in Chazy, NY and was relocated during Week 36 to (b) (4)

- **Dose Modifications and Early Termination**: Dose reductions, suspensions, and terminations were implemented during the dosing phase for males and females in the high dose group, as described below:
    - **Males**: During Week 78, Group 9 Male doses were reduced from 25 to 15 mg/kg/day; however due to ongoing mortality, dosing was suspended during week 79, and the group was terminated during week 86. All remaining male groups were terminated during week 91.
    - **Females**: Dosing was suspended for High Dose females during week 92. All females were terminated during Weeks 97-100. Preterminal cessation of dosing may have impacted the assessment of non-neoplastic findings in High Dose animals due to potential for recovery.

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Study title: BOSUTINIB: TWO YEAR ORAL (GAVAGE)

Study no.:	10-2185 (SPONSOR #09_0837)
Study report location:	(b) (4)
Conducting laboratory and location:	Until Week 36: Wyeth, Chazy, NY Thereafter: (b) (4)
Date of study initiation:	16 July 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SKI-606 Monohydrate (WAY-173606) Batch #MR090034, purity: 97.3%
CAC concurrence:	Yes

Key Study Findings

- Daily oral (gavage) administration of bosutinib decreased survival, body weight and food consumption in males and females at the highest dose level of 25/15 mg/kg/day for males and 15 mg/kg/day for females.
- There were no increases in tumor incidences related to bosutinib administration. A complete tabulation of tumors observed in this study is provided in Table 12, whereas a tabulation of those observed in animals that survived to scheduled termination is provided in Table 13.
- The systemic exposures achieved at the high dose level of 25 mg/kg/day in the male and 15 mg/kg/day in the female, represent an approximately 1.4-2.9-fold multiple (AUC_{0-24}) of the therapeutic exposure achieved at a dose of 500 mg/day.
- However, as the high dose of 25/15 or 15 mg/kg/day in males and females, respectively, exceeded the MTD for 2 years of repeated-dosing, exposure at the tolerable dose level of 7.5 or 5 mg/kg/day in males and females, respectively, represents an exposure multiple of 0.25-0.52X of those achieved clinically at the 500 mg/day dose level.
- This study was relocated during week 36 of the dosing phase. Animals were transferred by truck from the Sponsor site in Chazy, NY to (b) (4). An analysis of the number of deaths during the weeks preceding and following the move indicates that, although variable, the treatment groups were comparable to control groups in overall mortality rates, and in the percentage of decedents that were found to have tumors at termination or death. .

Non-Neoplastic Findings.

The target organs in this study were the GI tract (stomach, small and large intestine). Summaries of the treatment-related, non-neoplastic findings are provided in Sponsor **Text Tables 10-12**, below. Other histopathological changes attributed to bositinib administration were observed in the kidneys of males and females, lymphatic vessel, lymph nodes, and exocrine pancreas. These findings were considered non-adverse by the Sponsor's pathologist.

Text Table 10: Incidence of test article related non-neoplastic findings in the forestomach of rats dosed with bosutinib for up to 2 years

	Males						Females					
Bosutinib (mg/kg/day)	0[w]	0[v]	0[v]	2.5	7.5	25/15	0[w]	0[v]	0[v]	1.5	5	15
# animals examined	60	60	60	60	60	60	60	60	60	60	60	60
Limiting ridge: Epithelial hyperplasia/hyperkeratosis (slight)	1	1	2	3	6	7	0	2	2	2	2	8
Forestomach epithelial hyperplasia/hyperkeratosis												
minimal	1	0	0	0	0	0	0	0	0	0	0	4
slight	2	3	3	4	5	4	4	9	5	11	4	9
moderate	0	0	0	0	0	0	0	0	1	0	1	2
Total incidence	3	3	3	4	5	4	4	9	6	11	5	15
Erosions/ulceration												
minimal	2	1	3	2	3	5	0	1	1	2	0	4
slight	0	2	3	0	2	0	3	1	2	3	1	2
moderate	1	1	0	1	1	1	0	2	1	1	0	1
marked	2	1	1	1	0	0	0	1	0	1	2	2
Total incidence	5	5	7	4	6	6	3	5	4	7	3	9
Inflammation/edema/hemorrhage												
minimal	1	0	0	2	1	5	0	2	4	0	1	4
slight	1	5	3	4	4	2	4	4	3	9	2	7
moderate	3	1	2	1	1	0	0	2	1	2	3	2
Total incidence	5	6	5	7	6	7	4	8	8	11	6	13

w: water control, v: vehicle control

Text Table 11: Incidence of hyaline change in the stomach and small intestine of rats dosed with bosutinib for up to 2 years

	Males						Females					
Bosutinib (mg/kg/day)	0[w]	0[v]	0[v]	2.5	7.5	25/15	0[w]	0[v]	0[v]	1.5	5	15
# examined	60	60	60	60	60	60	60	60	60	60	60	60
Stomach: collagen deposition (slight)	1	0	0	1	1	5	0	0	1	0	0	0
# examined	58	58	58	60	58	58	59	60	60	59	60	60
Duodenum: collagen deposition												
minimal	0	0	0	27	15	8	0	0	0	16	28	6
slight	0	0	0	5	28	29	0	0	0	0	26	46
moderate	0	0	0	0	3	7	0	0	0	0	0	6
Total incidence	0	0	0	32	46	44	0	0	0	16	54	58
# examined	58	59	59	60	58	57	57	60	60	59	58	60
Jejunum: collagen deposition												
minimal	0	0	0	0	7	16	0	0	0	0	15	24
slight	0	0	0	0	2	12	0	0	0	0	3	26
moderate	0	0	0	0	0	3	0	0	0	0	0	0
Total incidence	0	0	0	0	9	31	0	0	0	0	18	50
# examined	57	60	59	60	58	58	59	59	60	56	60	60
Ileum: collagen deposition												
minimal	0	0	0	0	4	6	0	0	0	0	13	24
slight	0	0	0	0	1	8	0	0	0	0	0	12
Total Incidence	0	0	0	0	5	14	0	0	0	0	13	36

w: water control, v: vehicle control

Text Table 12: Incidence of erosions/ulceration/necrosis and mucosal congestion/hemorrhage in the small and large intestine of rats dosed with bosutinib for up to 2 years

	Males						Females					
Bosutinib (mg/kg/day)	0[w]	0[v]	0[v]	2.5	7.5	25/15	0[w]	0[v]	0[v]	1.5	5	15
# animals examined	59	60	60	60	59	60	60	60	60	60	60	60
Erosions/ulceration/necrosis												
Total incidence*	0	0	0	0	1	8	0	0	0	0	2	4
Mucosal congestion/hemorrhage												
Total incidence*	0	0	0	0	1	9	0	0	0	0	0	1

*Incidence represents the total number of animals with the finding in one or more regions of the small and/or large intestine.

w: water control, v: vehicle control

Adequacy of Carcinogenicity Study

Design of this rat carcinogenicity study was based upon feedback from the CAC. The study was conducted in accordance with the protocol and provided sufficient histopathological assessment of the designated organs and tissues to evaluate both the non-neoplastic and neoplastic effects of bosutinib at all dose levels including effects in control animals.

The MTD was achieved in this study. Decreases in food consumption, absolute body weight and body weight gain, relative to pooled vehicle control groups was observed for both males and females in the high dose groups. Moreover, a positive dose-related trend ($p < 0.001$) in male mortality (see Sponsor **Text Table 3.3-1** was observed in treated groups versus pooled vehicle control groups that was not positive when the high dose group was excluded. No such trend was evident for females.

Text Table 3.3-1: Percentage of animals surviving at terminal sacrifice. [a]

Dose (mg/kg/day)	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 2.5 F: 1.5	M: 7.5 F: 5	M:25/15 F: 15
Treatment	Water	Vehicle	Vehicle	Bosutinib	Bosutinib	Bosutinib
Males	33.3	43.3	51.7	41.7	40.0	25.0[b]
Females	33.3	26.7	33.3	31.7	30.0	28.3

[a]Initial on-test number of animals = 60/sex/group; terminal sacrifice commenced from Week 86 for Group 9 males (25/15 mg/kg/day) and Week 90-91 for remaining males; females were euthanized Week 97-100.

[b] Survival was statistically significantly lower than the control groups based on the trend test and the pair-wise comparison.

M: Male F: Female

Appropriateness of Test Models

The animal model (Sprague-Dawley rat) is commonly used in carcinogenicity studies to assess human tumor risk, and the oral route of administration reflects the intended clinical route.

Evaluation of Tumor Findings

Methods	
Doses (active moiety):	Males:0 , 0, 0, 2.5, 7.5, 25/15[a] mg/kg/day Females: 0, 0, 0, 1.5, 5, 15 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Daily oral gavage (10 mL/kg)
Formulation/Vehicle:	0.5% methylcellulose (4000 cps) (w/v), 2.0% polysorbate 80, NF (w/v), 0.06% glacial acetic acid, NF (w/v) and distilled water
Basis of dose selection	MTD from chronic study
Species/Strain:	CD® (Sprague-Dawley derived) [Crl:CD® (SD) IGS BR]
Number/Sex/Group:	60/sex/group
Age	~ 7 weeks (Males: 234-289 g; Females: 161-205 g)
Animal Housing	1-2 rats/cage in plastic solid bottom cages
Paradigm for dietary restriction	None
Dual control employed	Yes, 3 control groups: Group 1 = water; Groups 2-3 = Vehicle
Interim sacrifice	No
Satellite groups	TK animals (9/sex in Groups 11-16 + 3/sex in Vehicle control Group 10)
Deviation from study protocol	None that significantly impact study interpretation.
Special Features	*Study relocated during Week 36*

[a]dose reduction for males occurred during week 78; termination of group week 79d

Observations and Results	
Clinical signs:	Daily beginning Day -8
Body weight:	Twice pretest, then once weekly through week 13, then every 4 weeks thereafter
Food consumption	pretest, then once weekly through week 13, then every 4 weeks thereafter
Ophthalmoscopy	Not conducted
Hematology	Not conducted
Clinical chemistry	Not conducted
Urinalysis:	Not conducted

Gross pathology	Complete macroscopic examinations included the following parameters: examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass for the presence of macroscopic morphologic abnormalities.
Organ weights	Not conducted
Histopathology:	A complete histopathological assessment was performed on a comprehensive list of tissues collected from all main study animals. All collected tissues were microscopically examined.
Toxicokinetics	Day 182, 6 timepoints/group, 3 animals/timepoint. Control groups: 1 timepoint/group; 3 animals/timepoint. Samples were collected into tubes containing the anticoagulant, dipotassium-EDTA. Samples were collected from the jugular vein under CO_2/O_2 anesthesia. Terminal samples were collected from the caudal vena cava or abdominal aorta.

Survival:

There were a large number of preterm deaths on this study, the majority of which were attributable to tumors of the pituitary and mammary glands; however, within a sex, there were no significant differences between the groups in the number tumors observed (see Sponsor **Text Table 3.3-2**). Kaplan-Meier Survival Curves are provided for Males and Females, in **Figure 1** and **Figure 2**, respectively.

Text Table 3.3-2: Major causes of death in animals administered control articles or bosutinib

	Males						Females					
Bosutinib (mg/kg/day)	0[v]	0[w]	0[w]	2.5	7.5	25/15	0[v]	0[w]	0[w]	1.5	5	15
# animals examined	40	34	29	35	36	45	40	44	40	41	42	43
Pituitary tumor	18	10	10	15	14	12	23	32	21	25	27	29
Mammary tumor	0	0	0	0	0	0	11	6	14	10	7	4
Undetermined	11	13	6	12	13	18	1	1	0	1	4	4

w: water control, v: vehicle control

Figure 1: Kaplan-Meier Survival Function for Males

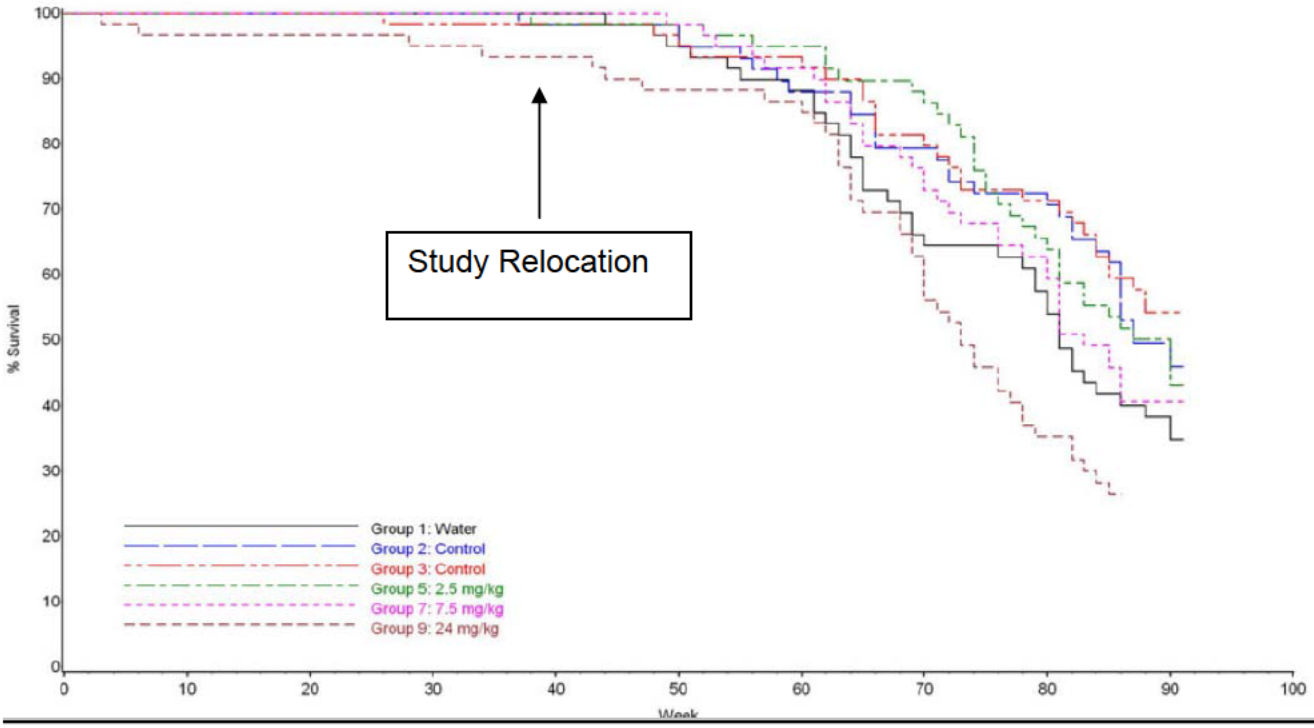
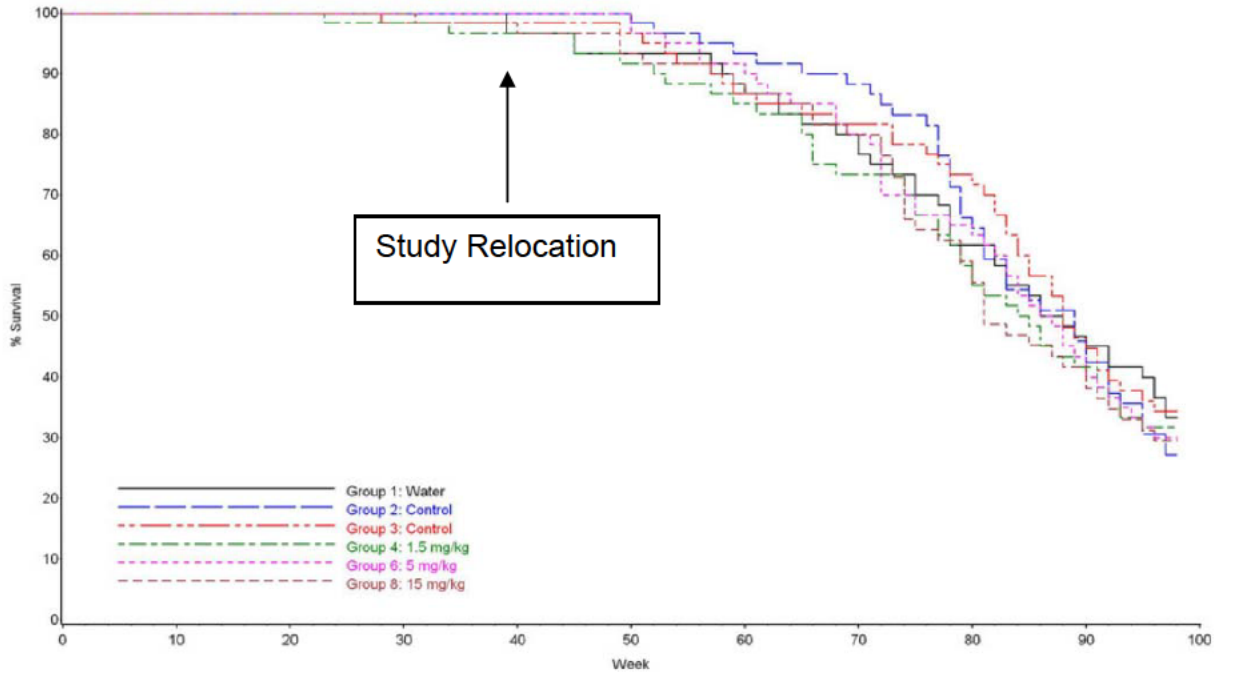


Figure 2: Kaplan-Meier Survival Function for Females



Disposition:

Tables 4 and 5 summarize the disposition of all males and females, respectively. Although timing of unscheduled deaths may have differed between groups, here were no appreciable differences between the groups in the overall number of unscheduled deaths (i.e. aggregate of humane and moribund sacrifices), suggesting that factors other than bosutinib toxicity were responsible for some preterm deaths. It is unclear whether the number of preterm deaths observed in Groups 1-3 (water and vehicle controls), exceed historical controls for the Wyeth and Huntingdon facilities.

Table 4 Disposition (Males)

	Group 1*	Group 2**	Group 3**	Group 5	Group 7	Group 9
Dose (mg/kg/day)	0	0	0	2.5	7.5	25/15
Scheduled Euthanasia	21	26	31	25	24	15
	Mortality					
Found Dead	20	19	14	14	18	24
Unscheduled†	19	15	15	21	18	21
	Cause of Death					
Undetermined	11	13	6	12	13	18
Accidental	2	3	2	2	1	2

*water

**vehicle (0.5% methylcellulose (4000 cps) (w/v), 2.0% polysorbate 80, NF (w/v), 0.06% glacial acetic acid, NF (w/v) and distilled water)

†Humane + Moribund euthanasia

Table 5 Disposition (Females)

	Group 1*	Group 2**	Group 3**	Group 4	Group 6	Group 8
Dose (mg/kg/day)	0	0	0	1.5	5	15
Scheduled Euthanasia	20	16	20	19	18	17
	Mortality					
Found Dead	8	12	12	11	13	12
Unscheduled†	32	31	27	30	29	29
	Cause of Death					
Undetermined	1	1	0	1	4	4
Accidental	0	1	1	0	0	2

*water

**vehicle (0.5% methylcellulose (4000 cps) (w/v), 2.0% polysorbate 80, NF (w/v), 0.06% glacial

acetic acid, NF (w/v) and distilled water)

†Humane + Moribund euthanasia

Effect of Study Relocation on Primary Endpoints:

Relocation of this study occurred during week 36 of the dosing phase. Animals were transported by truck from the Sponsor site in Chazy, NY to the [redacted] (b) (4).

Morality rates were highly variable between groups, however, the percentage decedents in treatment groups was generally comparable to those of concurrent control groups (both water and vehicle). Moreover, among the preterm decedents that succumbed in the interval before and immediately following the move, there was no relationship between dose level and tumor status at death or termination (**Table 6**).

Table 6: Move Impact on Mortality

Date of Death	Animal Number	Disposition	Tumor	Treatment group
239	720	AD	N	9
258	141	FD	N	2
260	470	FD	N	5
266	566	AD	N	7
268	83	HE	Y	1
270	38	AD	N	1

AD: accidental death; FD: found dead; HE: humane euthanasia

In addition, in the week immediately following the move, the group mean body weight loss in treatment groups were comparable to those of controls over the interval immediately following the move (**Table 7**). Animals in treated and control groups lost between 1.0-1.7% of body weight following the move, compared to Week 36 baseline measurement. Similarly, over the 4-week period preceding and following the move, there was no net decrement in body weight gain across treatment groups (**Table 7**).

Table 7: Move Impact on Animal Body Weights

Group		**1**	**2**	**3**	**4**	**5**	**6**	**7.0**	**8.0**	**9**
Dose (mg/kg/day)		**0**	**0**	**0**	**1.5**	**3**	**5**	**7.5**	**15.0**	**25/15**
	Week	Weight (g)								
	33	837.7	840	835.7	*	864.6	*	856.0	*	801.8
	36 - before	854.4	859	854.6	*	888.7	*	875.8	*	826.3
	36 - after	840.2	848.6	840.4	*	876.1	*	862.3	*	813
	%Δ	**-1.7**	**-1.2**	**-1.7**		**-1.4**		**-1.5**		**-1.6**
	37	854.3	858	851.8	*	889.3	*	877.6	*	823.6
Male	**%Δ***	**2.0**	**2.1**	**1.9**		**2.9**		**2.5**		**2.7**
Female	33	460.1	461.3	451.8	467.1	*	458.5	*	447.6	*
	36 - before	473.7	471.4	465.9	482.3	*	472.2	*	460.7	*

	36 - after	469.1	466.3	460.8	477.6	*	467.5	*	456.0	*
	%Δ	**-1.0**	**-1.1**	**-1.1**	**-1.0**	*	**-1.0**	*	**-1.0**	*
	37	472.1	468.7	464.5	481.6	*	473.1	*	459.4	*
	%Δ*	**2.6**	**1.6**	**2.8**	**3.1**	*	**3.2**	*	**2.6**	*

* = Overall % gain relative to baseline measurement on Week 33

Clinical Signs

Clinical signs:

Clinical signs exhibiting an apparent treatment-relationship, as evaluated by increased incidence in treated males and/or females relative to concurrent controls, include: red pigment around eyes, oral/nasal salivation, alopecia, hunched posture, thin appearance, pale appearance, ptosis, swollen limbs, anogenital staining, decreased food consumption, and decreased fecal volume.

Treatment Distribution of Tumors:

The frequency of tumors was generally greater in females than males irrespective dose, however within genders, there was no dose-related increase in the frequency of tumors observed. When the frequency of tumors is evaluated by mode of disposition (found dead, unscheduled euthanasia or terminal sacrifice), no increase in the percentage of tumors was observed with dose. Indeed, among early decedents, there was a decrease in the frequency of tumors observed at the highest dose level (particularly in males), which suggests that animals at the high dose levels succumbed prior to tumor formation (see **Figure 1** and **Figure 2**, Kaplan-Meier plots).

Table 8: Distribution of Tumors (total) by Treatment Group

Males						
Group	**1**	**2**	**3**	**5**	**7**	**9**
Dose (mg/kg/day)	0	0	0	2.5	7.5	25/15
N (# animals with Masses	24	18	22	17	15	15
% mass incidence per group	40% (24/60)	30% (18/60)	37% (22/60)	28% (17/60)	25% (15/60)	25% (15/60)
FEMALE						
Group	**1**	**2**	**3**	**4**	**6**	**8**
Dose (mg/kg/day)	0	0	0	1.5	5	15
N (# animals with Masses	43	43	45	41	40	20
% mass incidence per group	72% (43/60)	72% (43/60)	75% (45/60)	68% (41/60)	67% (40/60)	33% (20/60)

Table 9: Percentage of Tumors by Mode of Disposition

Disposition (Male)						
Group	**1**	**2**	**3**	**5**	**7**	**9**

Dose (mg/kg/day)	0	0	0	2.5	7.5	25/15
AD	0/2 (0%)	0/3 (0%)	0/2 (0%)	0/2 (0%)	0/1 (0%)	1/2 (50%)
FD	8/20 (40%)	5/19 (26%)	2/14 (14%)	4/14 (29%)	5/18 (28%)	2/24 (8%)
UE	7/17 (41%)	4/12 (33%)	7/13 (54%)	8/19 (42%)	2/17 (12%)	5/19 (26%)
TS	9/21 (43%)	9/26 (35%)	13/31 (42%)	5/25 (20%)	8/24 (33%)	7/15 (50%)
Disposition (Female)						
Group	**1**	**2**	**3**	**4**	**6**	**8**
Dose (mg/kg/day)	0	0	0	1.5	5	15
AD	--	0/1	1/1 (100%)	--	--	1/2 (50%)
FD	4/8 (50%)	7/12 (58%)	8/12 (67%)	7/11 (%)	9/13 (69%)	5/12 (42%)
UE	21/32 (66%)	22/31(71%)	20/27 (74%)	20/30 (67%)	18/29 (62%)	8/29 (28%)
TS	18/20 (90%)	14/16 (87%)	16/20 (80%)	14/19 (74%)	13/18 (72%)	6/17 (35%)

FD = Found Dead; UE = Unscheduled Euthanasia (i.e. moribund euthanasia + humane euthanasia); TS = Terminal Sacrifice

Body Weights

Mean body weight: Statistically significant differences in mean body weights were observed in Group 6 animals versus controls, which began during week 6 in males and week 53 in females. There was no decrease in absolute baseline adjusted body weight during the dosing interval for either sex.

Figure 3: Group Mean Male Body Weights

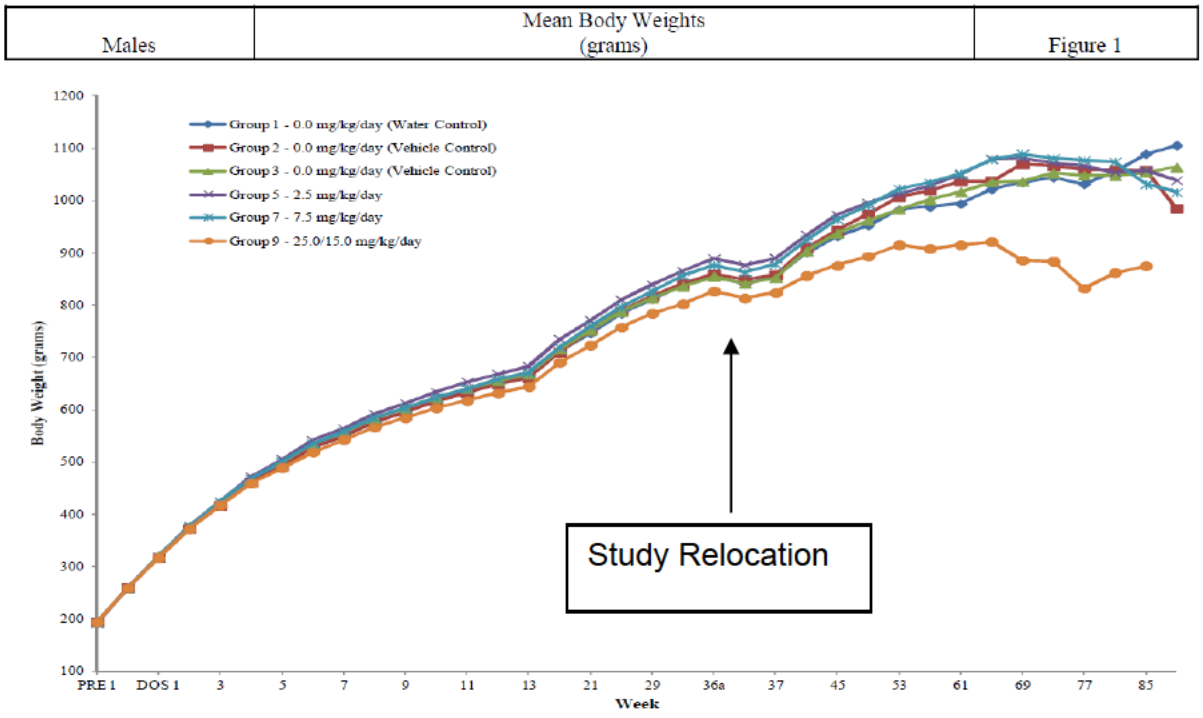
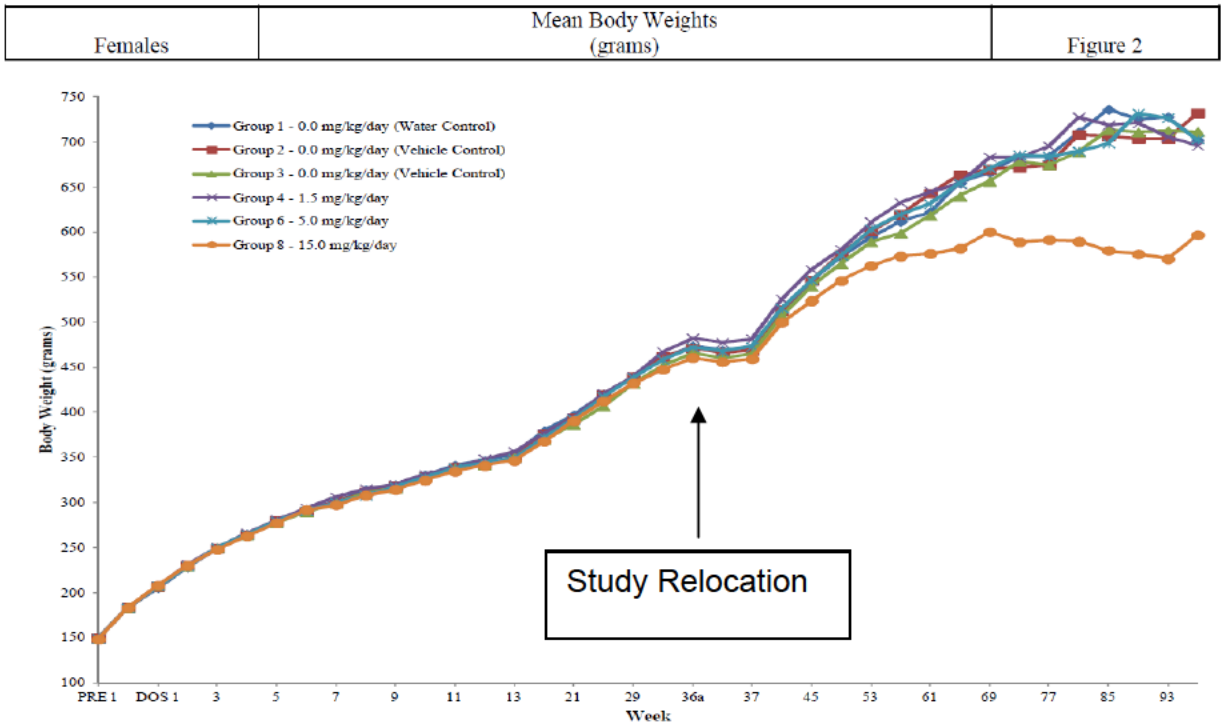


Figure 4: Group Mean Female Body Weights



Feed Consumption

A statistically significant reduction in food consumption was observed for male and female animals in the highest dose groups.

Figure 5: Male Group Mean Food Consumption

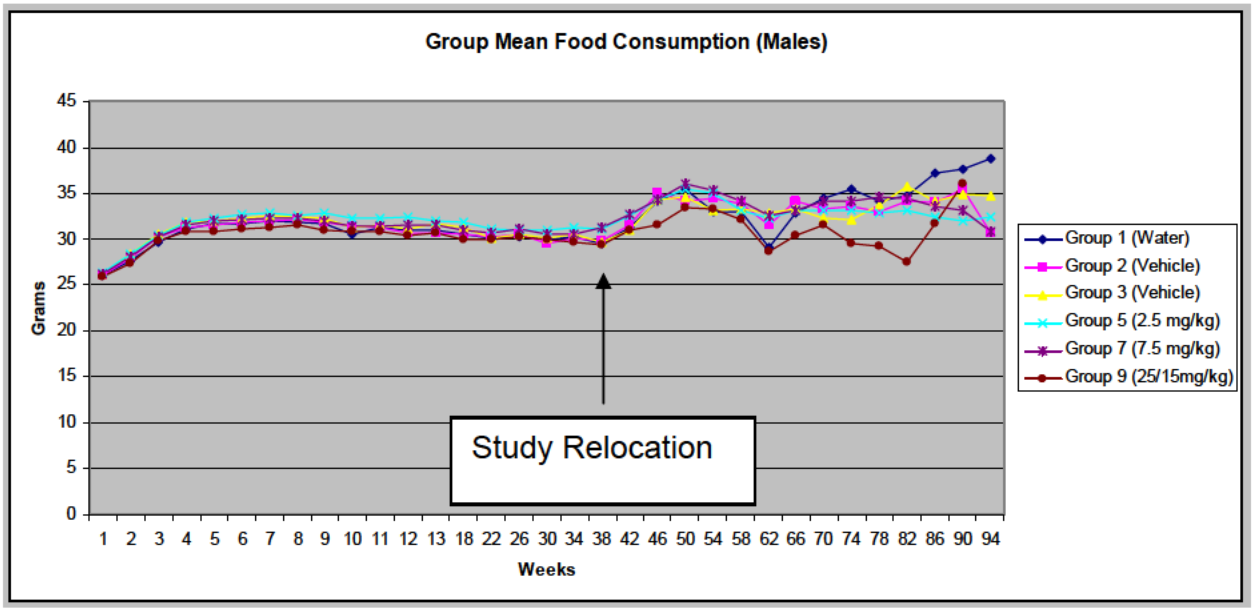
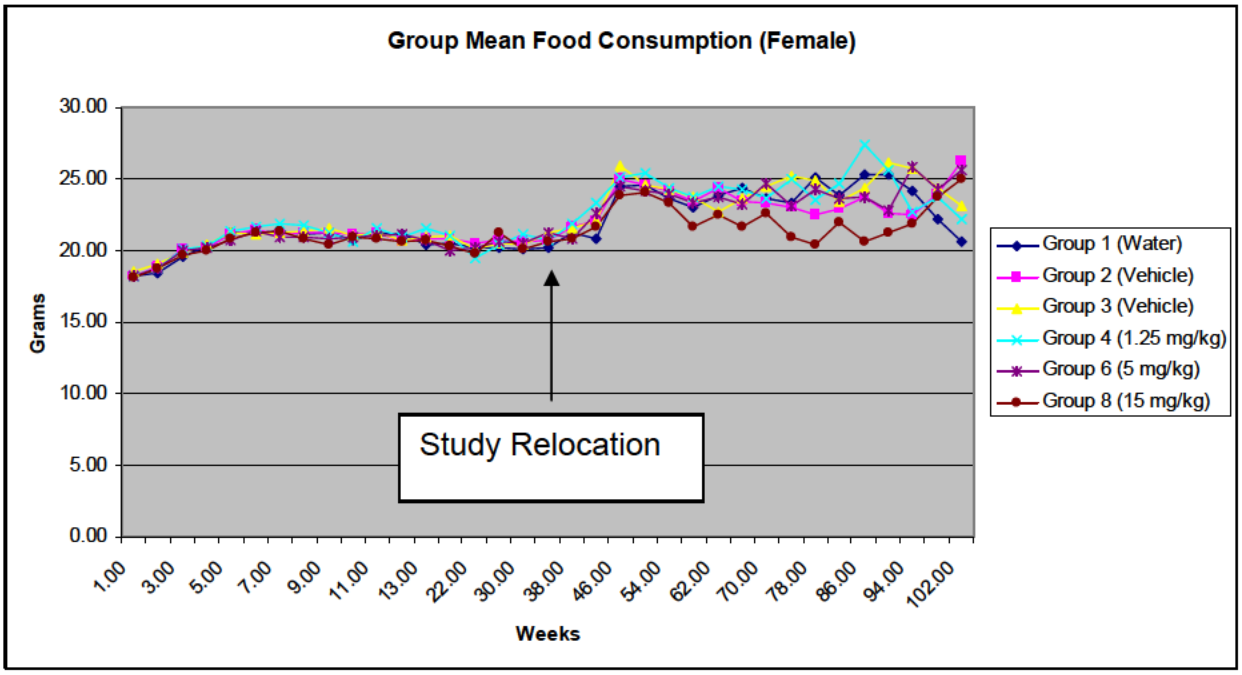


Figure 6: Female Group Mean Food Consumption



Ophthalmology:

There were no treatment-related effects on ophthalmological endpoints in this study.

Hematology:

Hematology was not conducted

Overall, the predominant cause of death for all dose groups was pituitary and mammary tumors; however, there were no differences between treatment groups in the assigned causes of death among animals that died or were euthanized for humane reasons prior to scheduled termination.

Table 10: Probable Cause of Death Among Preterm Decedents (All Groups):

	MALES						FEMALES					
Dose (mg/kg/day)	**0**	**0**	**0**	**2.5**	**7.5**	**25/15**	**0**	**0**	**0**	**1.5**	**5**	**15**
# Examined	40	34	29	35	36	45	40	44	40	41	42	43
Pituitary Tumor	**18**	**10**	**10**	**15**	**14**	**12**	**23**	**32**	**21**	**25**	**27**	**29**
Mammary Gland Tumor	**0**	**0**	**0**	**0**	**0**	**0**	**11**	**6**	**14**	**10**	**7**	**4**
Undetermined	11	13	6	12	13	18	1	1	0	1	4	4
Lymphoreticular Tumor	1	1	2	0	1	1	1	1	0	1	0	0
Skin Tumor	**2**	**3**	**3**	**2**	**0**	**2**	**0**	**0**	**0**	**1**	**3**	**2**
Brain Tumor	0	0	1	0	0	0	0	0	0	1	0	1

Adrenal Tumor	1	0	1	0	0	0	0	0	0	0	0	0
Pancreatic Tumor	0	0	0	0	0	1	0	0	0	0	0	0
Ovarian Tumor	0	0	0	0	0	0	0	0	1	0	0	0
Uterine Tumor	0	0	0	0	0	0	0	1	0	0	0	0
Intestinal Tumor	0	0	0	1	0	0	0	0	0	0	0	0
Vascular Tumor	0	0	0	0	0	0	1	0	0	1	1	0
Nerve Sheath Tumor (Schwannoma)	2	0	1	1	1	2	1	1	2	0	0	0
Head Tumor	0	0	0	0	1	0	0	1	0	1	0	0
Severe Skin Lesions/Pododermatitis	0	1	0	0	2	0	0	0	0	0	0	0
Nerve Damage	1	0	0	0	0	0	0	0	1	0	0	0
Severe Nephropathy /Renal Injury	0	1	0	2	1	2	1	0	0	0	0	0
Urogenital Infection	0	1	2	0	1	1	0	0	0	0	0	0
Liver Necrosis	0	1	0	0	0	0	0	0	0	0	0	0
Gastrointestinal Lesions	0	0	0	0	0	2	0	0	0	0	0	0
Pancreatic Atrophy	0	0	0	0	0	1	0	0	0	0	0	0
Cardiomyopathy	1	0	0	0	0	0	0	0	0	0	0	0
Botryomycosis	0	0	1	0	0	0	0	0	0	0	0	0
Septicemia	0	0	0	0	0	0	0	0	0	0	0	1
Thrombosis	0	0	0	0	0	1	0	0	0	0	0	0
Brain Necrosis	1	0	0	0	0	0	0	0	0	0	0	0
Pituitary Necrosis	0	0	0	0	0	0	1	0	0	0	0	0
Polyarteritis Nodosa (Arteritis/Periarteritis)	0	0	0	0	1	0	0	0	0	0	0	0
Accidental (Gavage Related)	2	3	2	2	1	2	0	1	1	0	0	2

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Gross Pathology

There were few gross findings associated with bosutinib treatment. Most treatment-related gross observations (**Table 11**) occurred in the organs of the GI tract, but were not considered a cause of death in the animals, as reflected in **Table 10** above.

Table 11: Gross Necropsy Observations (all animals)

	MALES						FEMALES					
Dose (mg/kg/day)	**0**	**0**	**0**	**2.5**	**7.5**	**25/15**	**0**	**0**	**0**	**1.5**	**5**	**15**
Number Examined	60	60	60	60	60	60	60	60	60	60	60	60
Cecum												
Discolored	0	0	0	0	1	2	0	0	0	0	0	1
Distended	4	1	0	2	1	1	1	0	0	0	1	2
Colon												
Distended	*	*	*	*	*	*	0	0	0	0	1	1
Nodule	*	*	*	*	*	*	0	0	0	0	0	1
Duodenum												
Distended	0	0	0	0	0	1	0	0	0	0	0	1
Abnormal Contents	0	0	0	0	0	2	*	*	*	*	*	*
Mass	0	0	0	1	0	0	*	*	*	*	*	*
Extremity												
Swollen	2	1	2	1	5	5	1	0	0	0	0	3
General Comments												
Staining on Fur	5	9	6	7	8	4	8	7	7	6	12	11
Gut Lymph Tissue												
Discolored	*	*	*	*	*	*	0	0	0	0	0	1
Heart												
Enlarged	0	0	0	1	0	2	*	*	*	*	*	*
Mass	0	0	0	1	0	1	0	0	0	1	0	0
Distended	0	0	0	1	0	0	*	*	*	*	*	*
Ileum												
Distended	3	0	0	0	0	2	0	0	0	0	0	2
Abnormal Contents	0	0	0	0	0	3	0	0	0	0	0	1
Adhesion	*	*	*	*	*	*	0	1	0	0	0	0
Jejunum												
Distended	3	0	0	1	1	2	0	0	0	0	0	2
Abnormal Contents	0	0	0	0	1	3	0	0	0	0	0	1

Diverticulum	*	*	*	*	*	*	0	0	0	0	1	0
Kidneys												
Dilated Pelvis	0	2	1	4	5	2	0	3	1	0	1	2
Irregular Surface	0	1	0	1	2	4	0	0	0	0	0	4
Discolored	0	1	2	0	3	2	1	0	0	1	1	2
Mesenteric LN												
Discolored	0	0	0	0	4	14	0	0	0	0	1	8
Enlarged	0	0	0	0	2	4	1	0	0	0	1	2
Cysts	0	0	0	0	1	0	*	*	*	*	*	*
Rectum/Low Colon												
Abnormal Contents	0	0	0	0	0	2	*	*	*	*	*	*
Stomach												
Nodule	0	0	0	0	1	1	*	*	*	*	*	*
Abnormal Contents	0	0	0	0	0	4	*	*	*	*	*	*
Irregular Surface	0	0	0	0	0	1	*	*	*	*	*	*
Perforation	0	0	0	0	1	0	0	0	0	0	0	1
Abnormal surface	*	*	*	*	*	*	0	0	0	0	0	1
Thymus												
Discolored	0	0	0	1	0	0	0	0	0	0	0	1
Small	0	0	0	0	0	1						
Thyroid												
Enlarged	0	0	0	1	1	0	2	2	1	0	2	3
Mass	0	0	0	1	0	1	1	0	2	0	2	0
Urinary Bladder												
Distended	0	1	0	0	1	3	0	2	0	0	0	0
Calculus	1	0	0	0	1	2	*	*	*	*	*	*
Thickened	0	0	0	0	0	1	*	*	*	*	*	*
Abnormal contents	*	*	*	*	*	*	0	0	0	0	0	1

*Not specified

Histopathology

Peer Review: None

Neoplastic findings: All animals

In **Table 12** below, the frequencies of tumor types listed in boldface type exceeded those of concurrent control; however, upon statistical analysis, none was considered statistically significant, either when analyzed alone or in combination.

Table 12 Summary of All Neoplastic Findings – All Organs and Groups

	MALES						FEMALES					
Number Examined	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**	**60**
Doses	**0**	**0**	**0**	**2.5**	**7.5**	**25/15**	**0**	**0**	**0**	**1.5**	**5**	**15**
Adipose Tissue												
B-Hemangioma	0	0	0	0	**1**	0	0	0	0	0	0	0
Adrenals												
B-Cortex: Adenoma	0	0	1	1	2	0	1	2	2	**3**	2	0
M-Cortex: Carcinoma	1	0	0	0	0	0	0	1	0	2	0	0
B-Pheochromocytoma	5	5	6	6	3	3	1	0	0	3	0	0
M-Pheochromocytoma	1	1	0	0	0	0	0	0	0	0	0	0
M-Medulla Ganglioneuroma	0	0	1	0	0	0	0	0	0	0	0	0
Brain												
M-Astrocytoma	0	0	1	0	0	0	1	1	0	0	0	0
M-Oligodendroglioma	0	0	1	0	0	0	0	0	0	1	0	0
B-Granular Cell Tumor	0	0	0	0	0	0	0	0	0	0	**1**	**1**
Colon												
B-Leiomyoma	0	0	0	0	0	0	0	0	0	0	0	**1**
Duodenum												
M-Adenocarcinoma	0	0	0	**1**	0	0	0	0	0	0	0	0
Extremity												
B-Hemangioma	0	0	1	0	0	0	0	0	0	0	0	0
Head												
M-Squamous Cell Carcinoma	0	0	1	0	1	1	0	0	0	1	0	0
M-Fibrosarcoma	0	0	0	0	0	0	0	2	0	0	0	0
M-Schwannoma	0	0	1	0	0	0	0	0	0	0	0	0
Heart												
B-Endocardial Schwannoma	0	2	0	0	0	0	0	0	0	0	0	0

M-Endocardial Schwannoma	1	0	3	0	0	0	0	1	0	1	1	0
M-Myxoma	0	1	0	0	0	0	0	0	0	0	0	0
Kidneys												
B-Hemangioma	0	0	0	**1**	0	0	0	0	0	0	0	0
B-Lipoma	0	1	0	0	0	0	0	1	1	0	0	1
M-Hemangiosarcoma	0	0	0	0	0	1	0	0	0	0	0	0
M-Liposarcoma	0	0	0	0	0	0	0	0	0	0	0	1
M-Mesenchymal Tumor	0	0	0	0	0	0	0	0	0	0	0	1
M-Transitional Cell Carcinoma	0	0	0	0	0	0	0	0	1	0	0	0
Liver												
B-Hepatocellular Adenoma	0	1	0	0	0	1	1	0	0	0	0	0
M-Hepatocellular Carcinoma	0	3	1	0	1	0	0	0	0	0	0	0
Lungs												
B-Bronchiolo/Alveolar Adenoma	0	1	0	1	0	0	0	1	1	1	0	0
Lymph Node Other												
M-Hemangiosarcoma	0	1	0	0	0	0	0	0	0	0	0	0
Lymph/Retic Sys												
M-Malignant Lymphoma	0	1	0	0	0	1	2	2	0	2	0	0
M-Granulocytic Leukemia	1	0	0	0	0	0	0	0	0	0	0	0
M-Histiocytic Sarcoma	3	2	2	1	1	0	0	0	1	0	0	0
Mammary												
B-Fibroadenoma	1	0	2	1	0	0	37	38	35	33	28	11
B-Adenoma	1	0	0	0	0	0	0	1	0	0	0	1
M-Adenocarcinoma	0	0	0	0	0	0	17	11	22	17	20	10
Mammary Protocol												
B-Fibroadenoma	0	0	0	0	0	0	5	0	2	**6**	5	2
B-Adenoma	0	0	0	0	0	0	0	1	0	2	0	0
M-Adenocarcinoma	0	0	0	0	0	0	1	1	0	1	0	1
Mediastinal Tiss												
B-Paraganglioma	0	0	0	0	1	0	0	0	0	0	0	0
M-Schwannoma	1	0	0	0	0	0	0	0	0	0	0	0
Mesentery/Perito												
B-Fibroma	0	0	0	0	0	0	0	1	0	0	0	0
B-Benign Mesothelioma	1	0	0	0	0	0	0	0	0	0	0	0
M-Hemangiosarcoma	0	0	0	0	0	0	1	0	0	0	0	0

Muscle (Other)												
M-Hemangiosarcoma	0	0	0	0	0	0	0	0	0	0	**1**	0
Ovary												
B-Sertoliform/Stromal Tumor	*	*	*	*	*	*	1	0	0	0	0	0
B-Luteoma	*	*	*	*	*	*	0	0	1	0	0	0
M-Leiomyosarcoma	*	*	*	*	*	*	0	0	1	0	0	0
Oviducts												
M-Adenocarcinoma	*	*	*	*	*	*	0	0	0	0	0	**1**
Pancreas												
B-Islet Cell Adenoma	3	6	1	1	1	0	1	1	1	1	2	0
M-Islet Cell Carcinoma	5	5	2	5	5	4	2	2	4	3	0	2
Parathyroid												
B-Adenoma	0	0	1	0	0	0	0	0	0	0	0	0
Pituitary												
B-Pars Distalis-Adenoma	30	30	33	31	41	33	47	39	43	47	**49**	45
B-Pars Intermedia: Adenoma	1	1	1	0	2	1	0	2	0	2	0	1
M-Pars Distalis: Carcinoma	0	1	1	0	0	0	2	5	0	2	2	2
Prostate												
B-Adenoma	0	0	0	0	0	1	*	*	*	*	*	*
M-Adenocarcinoma	0	0	1	0	0	0	*	*	*	*	*	*
M-Schwannoma	1	0	0	0	0	0	*	*	*	*	*	*
M-Fibrosarcoma	0	0	1	0	0	0	*	*	*	*	*	*
Seminal Vesicles												
M-Carcinosarcoma	1	0	0	0	1	0	*	*	*	*	*	*
Skin (Other)												
B-Keratoacanthoma	3	11	5	0	4	2	1	0	0	0	0	1
B-Squamous Cell Papilloma	0	1	3	1	0	3	0	0	0	1	0	0
B-Benign Basal Cell Tumor	0	0	1	0	0	0	0	0	0	0	0	0
B-Benign Trichoepithelioma	1	0	0	0	0	0	0	0	0	0	0	0
B-Fibroma	4	2	2	9	3	0	6	1	1	1	5	1
B-Lipoma	0	3	2	1	0	0	1	0	0	2	2	0
B-Hemangioma	0	0	0	0	**1**	0	0	0	0	0	0	0
M-Squamous Cell Carcinoma	1	1	1	1	1	0	0	0	0	0	0	1
M-Fibrous Histiocytoma	0	0	1	0	0	1	0	1	0	0	2	2
M-Fibrosarcoma	0	3	1	3	0	0	1	2	1	1	0	1

M-Liposarcoma	0	0	1	0	0	1	0	0	0	1	0	0
M-Leiomyosarcoma	0	0	1	0	0	0	0	0	0	0	0	0
M-Malignant Schwannoma	2	0	0	1	1	2	0	0	0	0	0	0
M-Hemangiosarcoma	0	0	0	0	0	0	0	0	0	**1**	0	0
Skin Protocol												
B-Keratoacanthoma	0	0	0	0	**1**	0	0	0	0	0	0	0
Spleen												
M-Hemangiosarcoma	0	0	0	**1**	0	0	0	0	0	0	**1**	0
Testes												
B-Leydig Cell Adenoma	5	1	4	2	2	1	*	*	*	*	*	*
M-Mesothelioma	1	0	0	0	0	0	*	*	*	*	*	*
Thymus												
B-Benign Thymoma	0	0	0	0	0	0	0	1	0	0	0	1
Thyroid												
B-C-Cell Adenoma	5	5	5	4	4	2	4	4	4	5	4	1
M-C-Cell Carcinoma	0	0	0	0	0	1	1	1	2	0	1	0
B-Follicular Cell Adenoma	2	2	3	2	2	3	0	0	0	2	1	1
M-Follicular Cell Carcinoma	1	1	1	0	2	**3**	0	1	1	0	1	0
Urinary Bladder												
B-Transitional Cell Papilloma	0	0	1	0	0	0	0	0	0	0	0	1
B-Granular Cell Tumor	0	0	1	0	1	0	0	0	0	0	0	0
Uterus												
B-Hemangioma	*	*	*	*	*	*	0	1	0	0	0	0
B-Granular Cell Tumor	*	*	*	*	*	*	0	1	0	1	0	2
B-Endometrial Stromal Polyp	*	*	*	*	*	*	2	2	2	1	1	**3**
M-Malignant Schwannoma	*	*	*	*	*	*	0	1	1	0	0	0
Vagina												
B-Benign Granular Cell Tumor	*	*	*	*	*	*	2	1	0	2	2	**3**
B-Stromal Polyp	*	*	*	*	*	*	0	0	0	1	0	0
M-Squamous Cell Carcinoma	*	*	*	*	*	*	0	0	0	0	0	1
Vascular Tissue												
C-Hemangioma	0	0	1	1	**2**	0	0	2	0	0	0	0
C-Hemangiosarcoma	0	1	0	1	0	1	1	0	0	1	**2**	0

B = benign; M = malignant; C = Multicentric; LN = lymph nodes; * not applicable

Neoplastic Findings: Terminal necropsy:

Of the tumors described in **Table 12** above, the subset of tumors observed in animals that survived to scheduled necropsy are listed in **Table 13**, below.

Table 13: Tumors Observed in Animals at Scheduled Termination

	MALES						FEMALES					
Number Examined	**20**	**26**	**31**	**25**	**24**	**15**	**20**	**16**	**20**	**19**	**18**	**17**
Doses	**0**	**0**	**0**	**2.5**	**7.5**	**25/15**	**0**	**0**	**0**	**1.5**	**5**	**15**
Adipose Tissue												
B-Hemangioma	0	0	0	0	0	0	0	0	0	1	1	0
Adrenals												
B-Cortex: Adenoma	0	0	0	0	1	0	0	1	1	1	0	0
M-Cortex: Carcinoma	0	0	0	0	0	0	0	0	0	1	0	0
B-Pheochromocytoma	4	2	4	3	1	2	0	0	0	3	0	0
M-Pheochromocytoma	1	0	0	0	0	0	0	0	0	0	0	0
Brain												
M-Astrocytoma	0	0	1	0	0	0	0	1	0	0	0	0
Colon												
B-Leiomyoma	0	0	0	0	0	0	0	0	0	0	0	1
Head												
M-Squamous Cell Carcinoma	0	0	0	0	0	1	0	0	0	0	0	0
M-Fibrosarcoma	0	0	0	0	0	0	0	1	0	0	0	0
Heart												
M-Endocardial Schwannoma	1	0	3	0	0	0	0	0	0	0	0	0
M-Myxoma	0	1	0	0	0	0	0	0	0	0	0	0
Kidneys												
B-Hemangioma	0	0	0	1	0	0	0	0	0	0	0	0
B-Lipoma	0	0	0	0	0	0	0	0	1	0	0	1
M-Mesenchymal Tumor	0	0	0	0	0	0	0	0	1	0	0	0
Liver												
B-Hepatocellular Adenoma	0	1	0	0	0	1	1	0	0	0	0	0
M-Hepatocellular Carcinoma	0	1	1	0	1	0	0	0	0	0	0	0
Lungs												
B-Bronchiolo/Alveolar Adenoma	0	1	0	0	0	0	0	1	1	0	0	0
Lymph Node Other												
M-Hemangiosarcoma	0	1	0	0	0	0	0	0	0	0	0	0
Lymph/Retic Sys												

M-Malignant Lymphoma	0	0	0	0	0	0	0	0	0	1	0	0
M-Histiocytic Sarcoma	2	2	0	0	0	0	0	0	0	0	0	0
Mammary												
B-Fibroadenoma	0	0	1	0	0	0	16	11	14	17	11	3
B-Adenoma	1	0	0	0	0	0	0	1	0	0	0	0
M-Adenocarcinoma	0	0	0	0	0	0	8	5	7	1	8	7
Mammary Protocol												
B-Fibroadenoma	0	0	0	0	0	0	2	0	1	3	2	0
B-Adenoma	0	0	0	0	0	0	0	1	0	2	0	0
M-Adenocarcinoma	0	0	0	0	0	0	0	0	0	0	0	1
Muscle (Other)												
M-Hemangiosarcoma	0	1	0	0	0	0	0	0	0	0	1	0
Ovary												
B-Sertoliform/Stromal Tumor	*	*	*	*	*	*	1	0	0	0	0	0
B-Luteoma	*	*	*	*	*	*	0	0	1	0	0	0
Oviducts												
M-Adenocarcinoma	*	*	*	*	*	*	0	0	0	0	0	0
Pancreas												
B-Islet Cell Adenoma	2	4	1	1	0	0	1	1	1	1	1	0
M-Islet Cell Carcinoma	1	3	2	4	1	2	1	0	1	3	0	2
Pituitary												
B-Pars Distalis-Adenoma	9	16	19	11	19	7	15	9	12	13	13	13
B-Pars Intermedia: Adenoma	0	1	1	0	1	1	0	2	0	0	0	0
M-Pars Distalis: Carcinoma	0	1	0	0	0	0	1	0	0	0	1	1
Prostate												
B-Adenoma	0	0	0	0	0	1	*	*	*	*	*	*
M-Adenocarcinoma	0	0	1	0	0	0	*	*	*	*	*	*
M-Schwannoma	1	0	0	0	0	0	*	*	*	*	*	*
M-Fibrosarcoma	0	0	1	0	0	0	*	*	*	*	*	*
Skin (Other)												
B-Keratoacanthoma	2	6	4	0	1	1	1	0	0	0	0	1
B-Squamous Cell Papilloma	0	0	1	0	0	1	0	0	0	0	0	0
B-Fibroma	1	2	0	3	1	0	5	0	1	1	2	1
B-Lipoma	0	3	2	0	0	0	1	0	0	1	0	0
B-Hemangioma	0	0	0	0	1	0	0	0	0	0	0	0
M-Squamous Cell Carcinoma	0	0	0	1	1	0	0	0	0	0	0	0

M-Fibrous Histiocytoma	0	0	0	0	0	1	0	0	0	0	1	1
M-Fibrosarcoma	0	1	1	2	0	0	1	2	1	0	0	0
M-Liposarcoma	0	0	1	0	0	0	0	0	0	0	0	0
M-Malignant Schwannoma	1	0	0	0	0	0	0	1	0	0	0	0
Skin Protocol												
B-Keratoacanthoma	0	0	0	0	1	0	0	0	0	0	0	0
Spleen												
M-Hemangiosarcoma	0	0	0	1	0	0	0	0	0	0	0	0
Testes												
B-Leydig Cell Adenoma	3	1	3	1	1	1	*	*	*	*	*	*
M-Mesothelioma	0	0	0	0	0	0	*	*	*	*	*	*
Thyroid												
B-C-Cell Adenoma	2	3	4	2	3	2	2	2	3	1	2	1
M-C-Cell Carcinoma	0	1	1	2	1	1	0	0	1	0	0	0
B-Follicular Cell Adenoma	0	1	1	2	1	1	0	0	0	1	0	0
M-Follicular Cell Carcinoma	1	1	1	0	2	2	0	0	0	0	1	0
Urinary Bladder												
B-Transitional Cell Papilloma	0	0	1	0	0	0	0	0	0	0	0	1
B-Granular Cell Tumor	0	0	1	0	1	0	0	0	0	0	0	0
Uterus												
B-Hemangioma	*	*	*	*	*	*	0	0	0	0	0	0
B-Granular Cell Tumor	*	*	*	*	*	*	0	1	0	0	0	2
B-Endometrial Stromal Polyp	*	*	*	*	*	*	1	0	0	1	0	2
M-Malignant Schwannoma	*	*	*	*	*	*	0	0	0	0	0	0
Vagina												
B-Benign Granular Cell Tumor	*	*	*	*	*	*	0	1	0	0	1	1
B-Stromal Polyp	*	*	*	*	*	*	0	0	0	1	0	0
M-Squamous Cell Carcinoma	*	*	*	*	*	*	0	0	0	0	0	0
Vascular Tissue												
C-Hemangioma	0	0	0	1	1	0	0	0	0	0	0	0
C-Hemangiosarcoma	0	1	0	1	0	0	0	0	0	0	1	0

B = benign; M = malignant; C = Multicentric; LN = lymph nodes; * not applicable

The summary incidence of non-neoplastic findings that exceed the frequency or severity (as appropriate) of concurrent controls are given in **Table 14**.

Non Neoplastic

Table 14: Non neoplastic Findings

	MALES						FEMALES					
Dose (mg/kg/Day)	**0**	**0**	**0**	**2.5**	**7.5**	**25/ 15**	**0**	**0**	**0**	**1.5**	**5**	**15**
Adrenals												
Hyperplasia/Hypertrophy: Zona Glomerulosa	0	0	0	0	0	2	0	0	0	1	0	0
Esophagus												
Mucosa: Squamous Cell Hyperplasia	0	0	0	0	0	0	0	1	0	0	0	0
Ileum												
Erosion/Ulceration/Regenerative Hyperplasia: Epithelial	0	0	0	0	0	4	0	0	0	0	0	4
Kidney												
Urothelium: Hyperplasia	4	5	2	2	4	5	13	9	12	10	11	12
Liver												
Bile Duct Hyperplasia/Fibrosis	7	3	11	8	7	8	4	2	6	4	6	5
Hypertrophy: Centrilobular/Periportal	2	1	1	1	1	5	1	0	1	1	0	0
Mammary												
Acinar Hyperplasia	0	0	0	0	0	0	0	1	0	0	1	0
Ovaries												
Sertoliform/Stromal Hyperplasia	*	*	*	*	*	*	2	7	4	4	3	7
Grade 1	*	*	*	*	*	*	0	1	0	0	0	0
Grade 2	*	*	*	*	*	*	1	3	4	1	2	2
Grade 3	*	*	*	*	*	*	0	3	0	2	1	3
Grade 4	*	*	*	*	*	*	1	0	0	1	0	2
Pancreas												
Islet Hyperplasia	3	3	2	1	1	3	3	2	1	4	2	2
Seminal Vesicles												
Hyperplasia, Epithelial	0	0	0	1	0	0	*	*	*	*	*	*
Stomach												
Forestomach: Epithelial Hyperplasia/Hyperkeratosis	3	3	3	4	5	4	0	2	2	2	2	8
Forestomach: Atypical Epithelial Hyperplasia	0	0	0	0	0	1	0	0	0	0	0	0

Submandibular Salivary Glands												
Mucinous Acinar Hypertrophy	0	0	0	0	0	0	0	0	1	1	0	1
Urinary Bladder												
Urothelial Hyperplasia	3	2	4	0	0	4	8	3	7	3	4	1

* = not applicable

Toxicokinetics

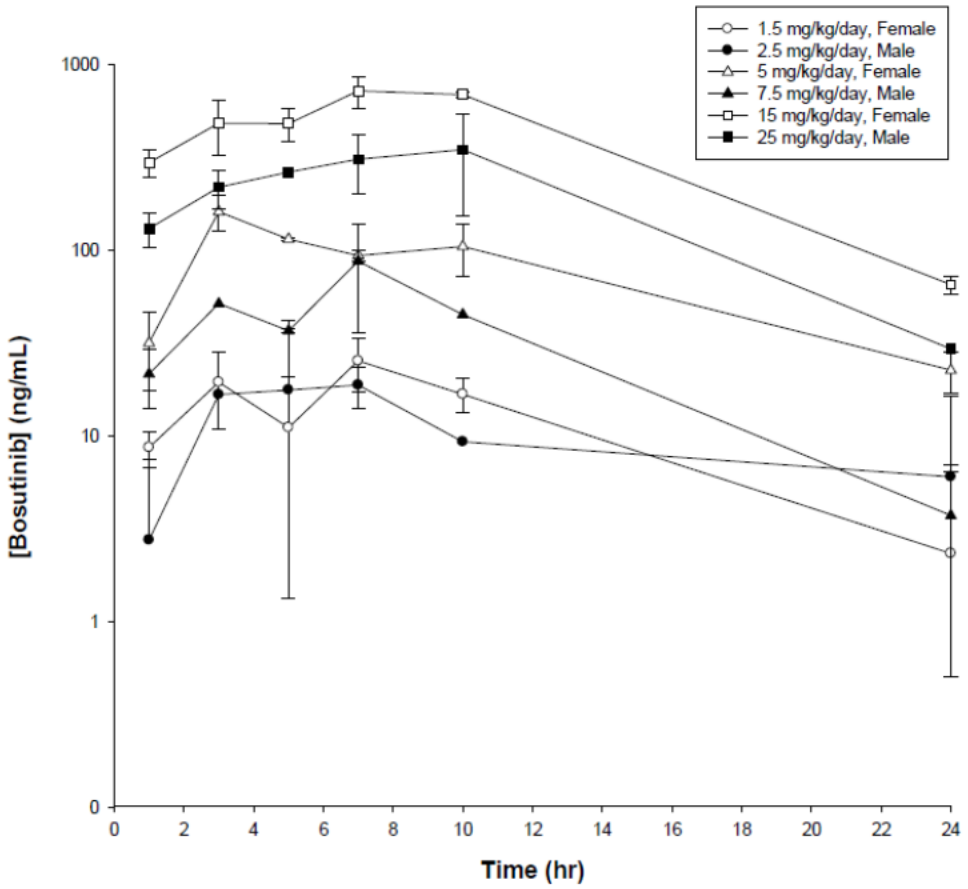
Sampling for toxicokinetic analysis of bosutinib and its two metabolites, M2 (oxydechlorinated bosntinib;WAY-198760) and M5 (N-desmethyl bonutinib;WAY-173607), was performed once, on Day 182 of study. ISR confirmed analytical reproducibility for bosutinib only, as the Sponsor reports that levels of M2 and M5 were too low to provide an accurate assessment of reproducibility.

Both AUC0-24 and Cmax increased 20-30-fold in males and females, respectively, over the 10-fold dose range on Day 182 (see **Sponsor Table 1-1 and Sponsor Figures 7.1-7.3**). High dose exposures in males and females represent an approximately 1.4-2.9-fold multiple (AUC_{0-24}). of the therapeutic exposure (3650 ng*hr/mL) achieved with the oral clinical dose of 500 mg//day.

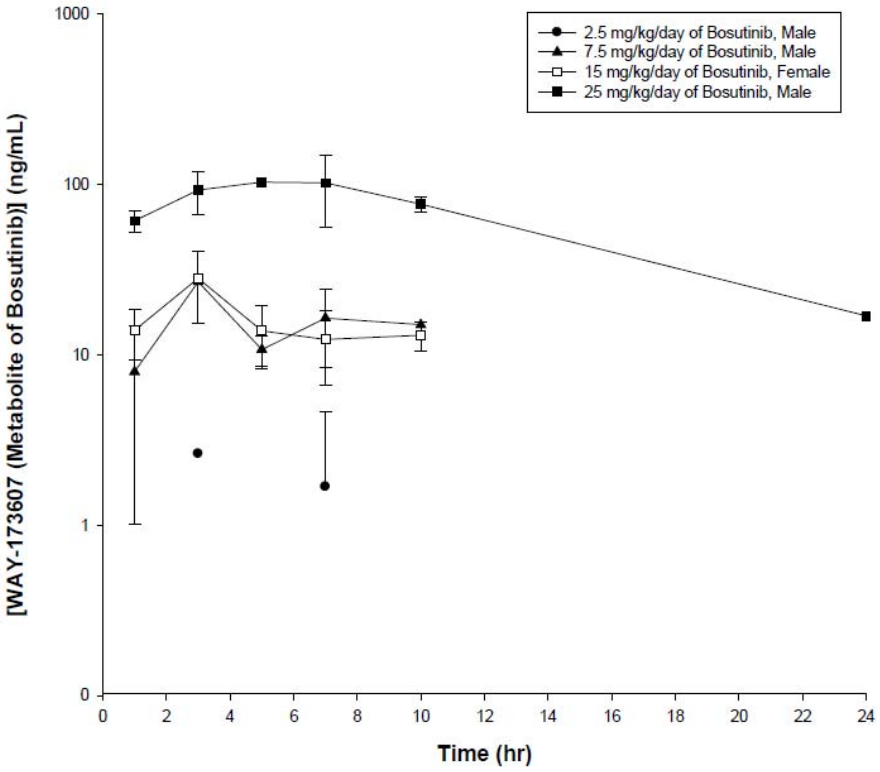
Table 1-1: Mean (± SE) Bosutinib Pharmacokinetic Parameters in Male and Female Rats Following Administration of Bosutinib for 182 Days - Day 182 Data (Protocol 09_0837)

Dosage (mg/kg/day)	Sex	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	AUC_{0-24} /Dosage
1.5	F	25.4 ± 4.8	7.0	298 ± 33	199 ± 22
2.5	M	18.8 ± 2.7	7.0	244 ± 76	97.6 ± 30.2
5	F	162 ± 20	3.0	1902 ± 203	380 ± 41
7.5	M	87.6 ± 29.9	7.0	840 ± 94	112 ± 12
15	F	724 ± 80	7.0	10570 ± 327	705 ± 22
25	M	349 ± 113	10.0	5127 ± 1038	205 ± 42

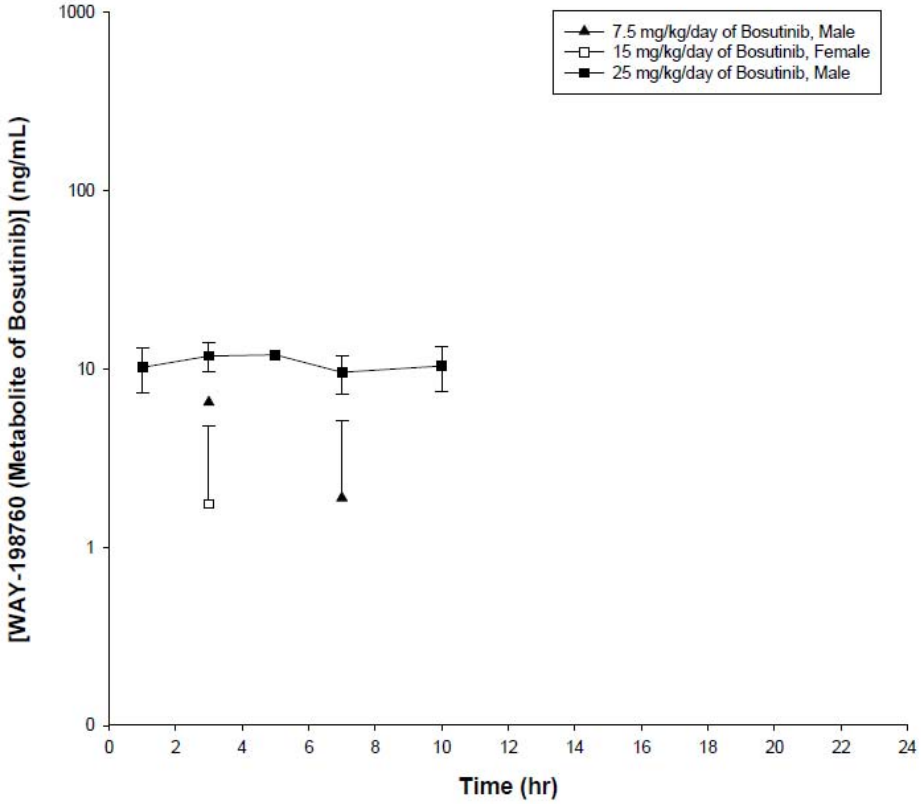
7.1 Mean (± SD) Plasma Concentrations of Bosutinib in Male and Female Rats After Once Daily, Oral (Gavage) Dosing of Bosutinib for 182 Days: Day 182 Data (Protocol 09_0837)



7.2 Mean (± SD) Plasma Concentrations of M5 (WAY-173607) in Male and Female Rats After Once Daily, Oral (Gavage) Dosing of Bosutinib for 182 Days: Day 182 Data (Protocol 09_0837)



7.3 Mean (± SD) Plasma Concentrations of M2 (WAY-198760) in Male and Female Rats After Once Daily, Oral (Gavage) Dosing of Bosutinib for 182 Days: Day 182 Data (Protocol 09_0837)



Dosing Solution Analysis

❖ Concentration and Homogeneity

Low and high-concentration bosutinib formulation samples were tested for homogeneity on Weeks 1 and 25 at the Sponsor site, then on weeks 52, 79 and 94 at (b) (4). Homogeneity assessment was performed by HPLC concentration assessment using samples collected from the top, middle and bottom of the formulation vessel. All assessments met the pre-specified acceptance criteria of ± 10% of nominal and were therefore considered homogenous.

Analysis of dose formulations was weekly during month, then by the Sponsor in weeks 7, 10, 11, 13, 16, 19, 22, 25, 28, 31, 34, and 36. Following transfer to (b) (4) concentrations were evaluated during Weeks 37, 41, 46, 50, 54, 59, 63, 67, 72, 76, 80, 85, 89, 93, and 98. Analysis was performed using a validated HPLC concentration method.

All measured solutions met the pre-specified acceptance criteria of ± 10% of nominal. **Sponsor-derived table 15** and **Table 16** provide mean analytical concentrations for weeks 26-36 (Sponsor-conducted portion of the study) and Weeks 37-98 (the (b) (4) conducted portion), respectively.

Table 15: Measured Formulation Concentrations (Weeks 26-36)

GROUP	NOMINAL CONCENTRATION (mg/mL)	ANALYTICAL CONCENTRATION (% of Nominal)
4	0.15	98.6
5	0.25	98.9
6	0.5	99.2
7	0.75	96.7
8	1.5	96.4
9	2.5	97.8

Table 16: Measured Formulation Concentrations (weeks 37-98)

GROUP	NOMINAL CONCENTRATION (mg/mL)	ANALYTICAL CONCENTRATION (% of Nominal)
4	0.15	97.0
5	0.25	98.4
6	0.5	97.7
7	0.75	99.1
8	1.5	98.7
9	2.5	100.1

Integrated Summary and Safety Evaluation

Daily oral administration of bosutinib for 2 years to male and female Sprague-Dawley rats at doses of up to 25/15 (males) or 15 (females) mg/kg/day resulted in a dose-related decrease in survival, body weight and food consumption; thus, the study achieved the MTD. Early mortality led to the premature termination of males in the highest dose group of 25/15 mg/kg/day, and all males by week 91.

There was no evidence of increased tumor formation in bosutinib-treated animals versus either the water (N = 60/sex) or the dual vehicle (N = 120/sex) groups. Due to excessive mortality in the high dose group, it is possible however, that animals died prior to tumor formation, thus, the excessive toxicity may have biased against the ability to detect tumors, particularly for high dose males. A few tumors achieved weak statistical significance by either trend or pairwise comparison; however, none met the criteria for statistical significance required to be deemed treatment-related. Moreover, when high dose groups were excluded in the analyses, there was no significant trend for any tumor type in this study. Taken together, these data indicate that, bosutinib is negative for carcinogenicity in this two-year rat study.

High dose exposures achieved in this study represent a 1.4-2.9-fold multiple of the clinical exposures. At tolerable dose levels (mid-dose of 7.5 mg/kg in males and 5 mg/kg/day in females), this represents an approximately 0.25-0.52X multiple of the clinical AUC in humans.

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/s/

SHAWNA L WEIS
07/26/2012

HALEH SABER
07/26/2012

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	203341
Supporting document/s:	N-000
Applicant's letter date:	November 17, 2011
CDER stamp date:	November 18, 2011
Product:	Bosutinib (Bosulif)
Indication:	Treatment of chronic, accelerated, or blast phase Ph+ chronic myelogenous leukemia (CML) in adult patients with resistance, or intolerance to prior therapy
Applicant:	Wyeth Pharmaceuticals, Inc.
Review Division:	Division of Hematology and Oncology Toxicology (DHOT), for Division of Hematology Products (DHP)
Reviewer:	Shwu-Luan Lee, Ph.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John Leighton, Ph.D. (DHOT) Ann Farrell, MD (DHP)
Project Manager:	Diane Hanner

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 203341 are owned by Wyeth or are data for which Wyeth has obtained a written right of reference.
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1 Executive Summary

1.1 Recommendations

There are no pharmacology/toxicology issues which preclude approval of bosutinib (Bosulif®) for the proposed indication.

1.1.1 Approvability

Recommending approval.

1.1.2 Additional Non Clinical Recommendations

No additional non-clinical studies are required for the proposed indication

1.1.3 Labeling

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

1.2 Brief Discussion of Nonclinical Findings

Bosutinib (SKI-606) inhibits Bcr-Abl and Src tyrosine kinases. In *in vitro* and/or *in vivo* systems bosutinib inhibited the cellular activities of several imatinib-resistant Bcr-Abl mutants including E255K, G250E, D276G and Y253F mutations. These mutations are commonly identified in CML patients who relapsed after or were resistant to imatinib treatment. Bosutinib exhibited much less effect against T315I mutation than the wild-type Abl.

Orally administered bosutinib was absorbed fairly rapidly (t_{max} of ~ 1.3-5.5 hr), with variable oral bioavailability (23% to 64%) in animal species. Bosutinib was highly bound to plasma proteins in all species tested (over 90%): mouse, rat, rabbit, dog, and human plasma. Metabolite M5 exhibited a similar protein binding. Following an oral dose of [^{14}C]bosutinib in Sprague-Dawley rats, radioactivity was distributed in most tissues and organs, except for brain, indicating a limited ability of bosutinib and/or its metabolites to cross the blood-brain barrier in rats. Radioactivity was found in the placenta, fetus, as well as in the milk of lactating rats. The level of radioactivity in milk was up to 8-fold higher than that in maternal plasma, suggesting excretion of bosutinib and/or its metabolites into the milk of lactating rats. Radioactivity was present in the plasma of suckling pups 24 to 48 hours after lactating rats received a single oral dose of radioactive bosutinib. The level of radioactivity in pup plasma at 24 and 48 hr post-dose was at least 8-fold higher than that in the maternal plasma. In a separate distribution study, tissues rich in melanin, such as uveal tract, showed higher and longer radioactivity retention, indicating that the drug and/or its metabolites have affinity for

binding to melanin. However, phototoxicity assessment was negative in pigmented Long Evans rats. After oral administration of [^{14}C]bosutinib to mice, rats and dogs, bosutinib was the predominant radiolabeled component in plasma. The major circulating metabolites were M5 (11%) in mice, M9 (up to 17%-24%) in rats, and M5 (up to 20%)/M6 (up to 10%) in dogs. Similarly, bosutinib was the major component in patients' plasma. Of note, the prominent circulating metabolites in humans are M2 and M5 (~19% and 25% of the AUC of parent drug, respectively). The metabolite M2 is a human-specific metabolite. In human liver microsomes, bosutinib was predominantly metabolized by CYP3A4. Metabolite M2 was mainly metabolized via glucuronation by UGT enzymes. The primary route of elimination of bosutinib was via the feces in animals. Under the conditions tested, bosutinib did not inhibit or induce a panel of CYP enzymes. Based on studies conducted in caco-2 cells, P-glycoprotein transporter (P-gp) may be involved in bosutinib transport. There was a concentration dependent inhibition of P-gp mediated digoxin (a prototype P-gp substrate) efllux. While no gender effects were found in PK parameters in dogs, higher systemic exposures to bosutinib were found in female rats after oral administration of bosutinib.

The safety pharmacology studies and general toxicology studies in rats and dogs identified GI tract, lymphoid tissues, adrenal, thyroid and mammary glands as the target organs/tissues. The major findings are as follows:

- Gastric-intestinal tract:
  The most prominent effect of bosutinib in rats and dogs was dose-dependent GI toxicities. The toxicities were observed following a single or multiple oral administration of the drug, and were considered the cause of mortalities. Rats were more susceptible to GI toxicities than dogs. GI clinical signs included: liquid and mucoid feces with red pigment/blood. GI histopathology findings of mucosal/ goblet cell hyperplasia, hemorrhage, erosion and hyperkeratosis were dose-dependent and with a steep dose-response relationship. Similar GI-related effects were noted in rabbits in the embryofetal developmental study.

- Hematopoietic/lymphoid system:
  The hematology findings were primarily related to bosutinib-induced inflammation and bleeding. These findings included increased white counts (except for lymphocyte), increased platelet count, slight reduction in red cell mass, and increased fibrinogen.

- Liver:
  Hepatobiliary findings were reported in rats treated at 70 mg/kg of bosutinib (2-week and 4-week studies) but were of low incidence or of low severity. These findings included centrilobular hyperplasia. There were no changes in liver enzymes.

- Cardiovascular system:
  Bosutinib inhibited hERG channel currents at an IC_{50} value of 0.3 μM and may be considered a moderate potency blocker. In a single-dose safety pharmacology in Beagle dogs, an oral bosutinib dose of 10 mg/kg did not induce cardiovascular toxicity. This dose resulted in an exposure in animals that was less than 2-fold the exposure in

patients at the recommended dose of 500 mg. In a separate safety pharmacology study in dogs, transient increases in blood pressure and a secondary reduction in heart rate were observed for 2 minutes after IV infusion of bosutinib. QTc prolongation was reported in patients treated with bosutinib.

Bosutinib was not mutagenic in bacterial Ames test or clastogenic in a chromosome aberration test in human peripheral blood lymphocytes (HPBL). Bosutinib did not increase micronucleus formation in mice after oral doses up to 2000 mg/kg. Metabolite M2 was negative in two *in vitro* genotoxicity studies, the Ames test and the chromosome aberration assay in human peripheral blood lymphocytes (HPBL).

Reproductive and developmental toxicities of bosutinib were investigated in rats and rabbits. Bosutinib was administered orally to pregnant rats during the period of organogenesis at doses of 1, 3 and 10 mg/kg/day. There was no maternal toxicity or adverse embryo-fetal developmental effects in rats treated with bosutinib up to 10 mg/kg/day (AUCs comparable to those reported in patients at the 500 mg/day dose). This study did not expose pregnant rats to enough bosutinib to fully evaluate adverse outcomes. In a fertility and early embryonic developmental study, decreased implantation and reduced number of viable embryos were observed at 30 mg/kg/day of bosutinib; approximately 1.4 times the human exposure at the clinical dose of 500 mg/day.

In a study conducted in rabbits, bosutinib was administered orally to pregnant animals during the period of organogenesis at doses of 3, 10 and 30 mg/kg/day. At the maternally-toxic dose of 30 mg/kg/day of bosutinib, there were fetal anomalies (fused sternebrae, and two fetuses had various visceral observations), and an approximate 6% decrease in fetal body weight. The exposure at 30 mg/kg/day resulted in exposures (total AUC) approximately 4 times those in humans at the 500 mg/day dose of bosutinib.

In a rat fertility study, drug-treated males were mated with untreated females, or untreated males were mated with drug-treated females. The dose of 70 mg/kg/day of bosutinib resulted in reduced fertility in males as demonstrated by 16% reduction in the number of pregnancies. There were no lesions in the male reproductive organs at this dose. This dose of 70 mg/kg/day resulted in exposure (total AUC) in male rats approximately equal to that in humans at the 500 mg/day dose of bosutinib. Fertility (number of pregnancies) was not affected when female rats were treated with bosutinib; although, decreased implantation and embryonic toxicities were evident at the dose of 30 mg/kg/day (see above). There were no effects on reproductive organs in general toxicology studies.

The result of a 2-year carcinogenicity study in rats is under review.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number (Optional)

918639-08-4 (monohydrate)
380843-75-4 (anhydrous form)

2.1.2 Generic Name

Bosutinib

2.1.3 Code Name

SKI-606
WAY-173606
PF-05208763

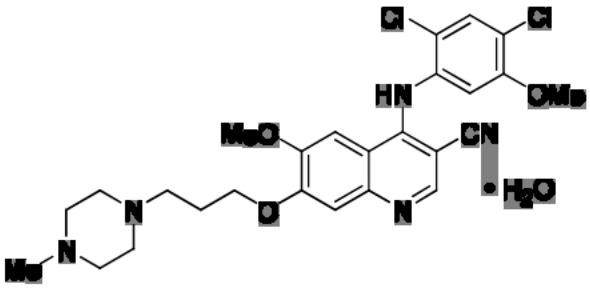
2.1.4 Chemical Name

3-Quinolinecarbonitrile, 4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-methyl-1-piperazinyl) propoxy]-, hydrate (1:1)

2.1.5 Molecular Formula/Molecular Weight

Molecular Formula:	$C_{26}H_{29}Cl_2N_5O_3 \cdot H_2O$
Molecular Weight:	548.46 (monohydrate)
	530.46 (anhydrous)

2.1.6 Structure



2.1.7 Pharmacologic class:

Kinase inhibitor

Mechanism of action: inhibitor of Src and Abl family of kinases

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

IND 68268

2.3 Clinical Formulation

2.3.1 Drug Formulation

Composition of film coated tablets, 100 mg and 500 mg: Table excerpted from the Applicant

Table 1 Clinical formulation

Ingredient	Quality Standard	% w/w (Uncoated Tablet)	Unit Dose *(mg/tablet)*	Unit Dose *(mg/tablet)*	Function
(b) (4)			**100 mg**	**500 mg**	
Bosutinib monohydrate	Pfizer [a]	(b) (4)	103.40 [b]	516.98 [c]	Active Ingredient
Microcrystalline cellulose	NF/Ph. Eur.		(b) (4)	(b) (4)	(b) (4)
Croscarmellose sodium	NF/Ph. Eur.				
Poloxamer	NF/Ph. Eur.				
Povidone	USP/Ph. Eur.				
(b) (4)					
Subtotal					
(b) (4)					
Total (Final Tablet)			**149.35**	**746.75**	

NA = Not applicable

a. Pfizer in-house specifications for Bosutinib Monohydrate are listed in the Bosutinib Monohydrate Specification RB-36100, which is provided in Section 3.2.S.4.1 Specification for Drug Substance.

b. Equivalent to 100 mg of bosutinib per tablet based on an assay value of 100% for bosutinib monohydrate.

c. Equivalent to 500 mg of bosutinib per tablet based on an assay value of 100% for bosutinib monohydrate.

The actual input of the active ingredient for both strengths is adjusted based on its actual assay value of the bosutinib monohydrate used. A corresponding adjustment is made in the amount of (b) (4) (b) (4)

(b) (4)

2.3.2 Comments on Novel Excipients

No novel excipients.

2.3.3 Comments on Impurities/Degradants of Concern

There are no standing issues with the impurities at this time.

The following impurities in the drug substance (DS) were identified:

- Organic impurities:
  - Specified impurities:

Table 2 Impurities (specified and unspecified)

(b) (4)

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(b) (4)

2.4 Proposed Clinical Population and Dosing Regimen

- Indication: for the treatment of chronic, accelerated, or blast phase Ph+ CML in patients with resistance or intolerance to prior therapy
- Dosing regimen: 500 mg, once daily

2.5 Regulatory Background

The Applicant submitted the NDA on November 17, 2011. There is no previous submission.

3 Studies Submitted

3.1 Studies Reviewed

Study Number	Study Title
Primary Pharmacology	
RPT-52664	Characterization of SKI-606 *in vitro*: Enzymatic and cellular activities
RPT-60167	SKI-606 activity against downstream proteins in CML
RPT-70367	Enzymatic activities of SKI-606: Further Studies
RPT-78153	Activity of WAY-173606 in Philadelphia Chromosome positive acute lymphoblastic leukemia cells
RPT-58432	Studies with SKI-606 in CML: *In vitro* and *in vivo* studies
	Class effects of tyrosine kinase inhibitors in the treatment of chronic myeloid leukemia. Giles *et al.*, Leukemia 23:1698-1707, 2009
RPT-52665	Tumor xenograft studies with SKI-606
	In vitro and *in vivo* activity of bosutinib, a novel Src- Abl inhibitor, against imatinib-resistant Bcr-Abl+ neoplastic cells. Puttini *et al.*, Cancer Res. 66:11314-11322, 2006.
	Activity of bosutinib, dasatinib, and nilotinib against 18 imatinib-resistant BCR/ABL mutants. Redaelli *et al.*, J Clin Oncol 27: 469-471, 2009
	SKI-606, a 4-anilino-3-quinolinecarbonitrile dual inhibitor of Src and Abl kinases, is a potent antiproliferative agent against chronic myelogenous leukemia cells in culture and causes regression of K562 xenografts in nude mice. Golas *et al.*, Cancer Res 63:375- 381, 2003
RPT-77627	*In Vitro* Activities of WAY-173606 (Bosutinib) Metabolites M2 (Oxydechlorinated Bosutinib), M5 (N-desmethyl Bosutinib) and M6 (Bosutinib N-oxide)
Secondary Pharmacology	
RPT-52666	Nova screen data for SKI-606
#75400039-2	*In Vitro* Pharmacology: 2010 Pfizer (b) (4) Full Safety Profile (CFSP) Study of PF-05898965-00
#75400039-3	*In Vitro* Pharmacology: 2010 Pfizer (b) (4) Full Safety Profile (CFSP) Study of PF-05312061-00
Safety Pharmacology	
RPT-51764	SKI-606: Single dose oral (gavage) central nervous system safety pharmacology study in female rats
RPT-51763	SKI-606: Single dose oral (gavage) respiratory safety pharmacology study in female rats
RPT-54968	SKI-606: Effects on cloned hERG channels expressed in mammalian cells
RPT-61333	SKI-606: Effects on cloned hERG channels expressed in mammalian cells
RPT-51769	SKI-606: Single dose crossover oral (gavage) cardiovascular safety pharmacology study in dogs
RPT-50437	WAY-173606: An ascending intravenous dose cardiovascular safety pharmacology study in male dogs
PK/ADME	
RPT-51795	SKI-606 (WAY-173606): Pharmacokinetic study in female mice following a 5 mg/kg single intravenous and a 50 mg/kg single oral administration
RPT-53291	SKI-606: Pharmacokinetics following a single oral 50 mg/kg dose to fasted and fed female nude mouse
RPT-51749	SKI-606 (WAY-173606): Single dose intravenous and oral pharmacokinetic study in male and female Rats

RPT-51748	SKI-606: Single intravenous (bolus) and oral (gavage) dose pharmacokinetic study in female dogs
RPT-71680	SKI-606: Transport and inhibition of P-glycoprotein activity in Caco-2 cell monolayers
#WAY-173606_18AUG10_195303	WAY-173606 (PF-05208763): In vitro assessment of hepatic uptake in human hepatocyte suspensions
RPT-54959	SKI-606: Quantitative whole body-autoradiography and tissue distribution of rats following oral administration of [^{14}C]-SKI-606
RPT-54418	SKI-606: In vitro protein binding of [^{14}C]-SKI-606 in mouse, rat, rabbit, dog and human plasma
PF-05312061/11May10/122621	Protein binding of PF-05312061 in mouse, rat, rabbit, dog, and human plasma
#WAY-173606_02AUG10_113243	Blood to plasma concentration ratio of PF-0520863 (WAY-173606) in rat, dog, and human whole blood
RPT-53088	SKI-606: Metabolism of [^{14}C]SKI-606 in male and female Sprague Dawley rats following a single oral (50 mg/kg) administration
RPT-53089	SKI-606: Metabolism and excretion of [^{14}C]SKI-606 in male dogs following a single oral (5 mg/kg) administration
RPT-53085	SKI-606: *In vitro* metabolism in rat, dog and human hepatocytes and liver microsomes of CD-1 mice, Sprague Dawley rats, Beagle dogs and humans
RPT-77073	SKI-606: Metabolite profiles in male CD-1 mice following a single oral 50 mg/kg dose of [^{14}C]SKI-606
RPT-77097	SKI-606: placental transfer of [^{14}C]SKI-606 following a single oral (10 mg/kg) dose to gravid rats
RPT-77578	SKI-606: Transfer of radioactivity into breast milk of lactating rats and plasma of nursing pups following a single oral (10 mg/kg)dose of [^{14}C]SKI-606
RPT-53085	SKI-606: *In vitro* metabolism in rat, dog and human hepatocytes and liver microsomes of CD-1 mice, Sprague Dawley rats, Beagle dogs and humans
RPT-53086	SKI-606 (WAY-173606): P450 isozyme identification study using cDNA expressed P450 isozymes and chemical inhibition studies in human liver microsomes
RPT-63186	SKI-606: Role of flavin-containing monooxygense enzymes in the *in vitro* metabolism in human liver and kidney
General toxicology	
Repeat-dose toxicity	
RPT-63644/RPT-64288 (TK)	SKI-606: six-month oral (gavage) toxicity study
RPT-65542/RPT-66535 (TK)	SKI-606: 9-month oral (gavage) toxicity study in FED dogs with a 28 day recovery
RPT-74244	WAY-198760 (SKI-606 M2 metabolite): 14-days oral toxicity study in rats
RPT-64729	SKI-606: 14-days oral (gavage) impurity qualification study in rats
Genotoxicity	
RPT-49003	WAY-173606: Bacterial Reverse Mutation Test with *Salmonella typhimurium* and *Escherichia coli*
RPT-50332	WAY-173606: *In vitro* mammalian chromosome aberration test in human peripheral blood lymphocytes
RPT-52501	SKI-606: Single dose oral (garage) bone marrow micronucleus study in male mice
RPT-46058	WAY-173606 (Batch 11): *Salmonella typhimurium* reverse mutation screening assay (Ames Test)
RPT-73086	SKI-606: Bacteria reverse mutation test of M2 metabolite with *Salmonella typhimurium* and *Escherichia coli*

RPT-73087	SKI-606: *In vitro* mammalian chromosome aberration test of M2 metabolite in human peripheral blood lymphocytes
Reproductive and developmental toxicology	
RPT-63257	SKI-606: Oral (gavage) fertility study in rats
RPT-63107	SKI-606: Oral (gavage) developmental toxicity study in rats
RPT-62710/RPT-64285 (TK)	SKI-606: Oral (gavage) developmental toxicity dose ranging study in mated rabbits
RPT-65533	SKI-606: Oral (gavage) developmental toxicity study in mated rabbits
Phototoxicity	
RPT-74360	Bosutinib: multiple (2-day) dose phototoxicity study to determine the effects of oral (gavage) administration on eyes and skin in pigmented rats

3.2 Studies Not Reviewed

SP3810_TK	Safety pharmacology-Echocardiographic assessment of imatinib (Gleevec) and PF-05208763 (Bosutinib, SKI-606) in rats
PF-05208763_27SEP10_183648	Identification of PF-05208763 metabolite M3 in rat plasma
PF-05898965_13OCT10_201157	PF-05898965 (WAY-198760)- M2 metabolite of PF-05208763 (WAY-173606): In vitro UGT reaction phenotyping
RPT-70327	SKI-606: metabolic profiles in human plasma and urine following single or multiple oral administrations using samples from clinical protocols 3160A1-100-US and 3160A1-103-EU
RPT-53087	SKI-606: Single oral (50 mg/kg) dose [^{14}C]SKI-606 mass balance study in male rats
General toxicology	
Single dose toxicity	
RPT-52016	SKI-606: Acute intraperitoneal toxicity study in mice
RPT-52017	SKI-606: Acute oral toxicity study in mice
RPT-52013	SKI-606: Acute oral toxicity study in rats
RPT-52015	SKI-606: Acute intraperitoneal toxicity study in rats
Repeat-dose toxicity	
RPT-49310/RPT-50258 (TK)	WAY-173606: multiple (7 days) dose oral (gavage) ranging study in rats
RPT-52772/RPT-52934 (TK)	SKI-606: Twenty-eight day oral (gavage) toxicity study in rats
RPT-57924	SKI-606: Twenty-eight day oral (gavage) toxicity study with a 8-day recovery in rats
RPT-50039/RPT-53053 (TK)	WAY-173606: multiple (10 days) dose oral (gavage) ranging study in female dogs
RPT-60569	WAY-173606: multiple (2 weeks) dose oral (gavage) tolerability study in male dogs
RPT-52074/RPT-53029 (TK)	SKI-606: Twenty-eight day oral (gavage) toxicity study with a 8-day recovery in dogs

3.3 Previous Reviews Referenced

Safety review of IND 68268 (by Dr. Leigh Verbois).

4 Pharmacology

4.1 Primary Pharmacology

Background:
Src protein family, a family of non-receptor tyrosine kinases, is associated with signal transduction pathways governing various pivotal biological (e.g., cell-cell adhesion) and pathological (increased cell motility, invasiveness and metastatic potential of cancer cells) events (Pellicena and Miller, Front Biosci 7: d256-267, 2002). The proteins in the Src family consist of an N-terminal membrane associated domain, SH2, SH3 catalytic domains with a carboxyl terminal regulatory domain (Martin, Nature Reviews Molecular Cell Biology 2: 467-475, 2001; Schwartzberg, Oncogene, 17:1463-1468, 1998). Activation of a Src family protein (by opening the protein structure), may be due to one or more of the following events: binding of heterologous proteins (such as Csk or its homolog, Chk) to the SH2/SH3 docking motifs (Bjorge *et al.*, Oncogene 19: 5620-5635, 2000; Bougeret *et al.*, J Biol Chem 276: 33711-33720, 2001), dephosphorylation of tyrosine 530 (Y530 in the C-terminus) (Thomas and Brugge, Ann Rev Cell Develo Bio 13: 513-609, 1997; Courtneidge Biochem Soc Trans 30: 11-17, 2002), and/or phosphorylation of tyrosine 418 (Y418) in the activation loop. Src upregulation and dysregulation of Y530 (e.g., decreased levels of Csk or Chk, enzymes that phosphorylate Y530, in cancer cells) and/or Y418 (elevated autophosphorylation) may be associated with tumorigenesis. Furthermore, Src may also play roles in progression of malignancies via mechanisms such as: plasminogen activation, phosphorylating components of adherens junctions (such as E-cadherin and β-catenin), enhancing invasiveness, and promoting vascular permeability.

The molecular hallmark of CML is the constitutively active tyrosine kinase Bcr-Abl. Different mechanisms of resistance to therapy have been demonstrated, which include mutations in the Abl kinase domain. For instance, Bcr-Abl protein with T315I mutation develops after treatment with tyrosine kinase inhibitors and is known to be resistant to a variety of agents. Bosutinib (SKI-606) inhibits mainly the wild-type protein, with little inhibitory activity for the T315I mutant cells.

In vitro Studies:
The anti-cancer activities of SKI-606 included the following: inhibition of Abl and Src kinases, inhibition of phosphorylation of down-stream target proteins, reduction of Src Y418 auto-phosphorylation, and reduction in the ability of tumor cells to promote the formation of osteoclasts. These investigations were reported in Studies: #RPT-52664, #RPT-60167, #RPT-70367, and #RPT-78153.

Mechanism of action:
It is proposed that bosutinib may exert its anti-CML activity via its dual inhibition toward tyrosine kinases Abl and Src. The enzyme assay data were based on the studies conducted by Invitrogen in their Z-Lyte assay format, where bosutinib (aka WAY-

173606 or SKI-606) at 0.2 μM, ATP: 100 μM was tested against approximately 222 kinases. Enzyme activities of Abl and Src were assessed by ELISA (streptavidin-coated plates) or a Lance (FRET) assay; cell-based activities were assessed by cell proliferation, anti-phosphorylation activities.

- Enzyme assay:

o Investigation by Invitrogen (#RPT-70367):

SKI-606 inhibited Src and Abl activity at IC_{50} values of 3.5 and 1 nM, respectively. SKI-606 also inhibited certain imatinib-resistant Abl mutants, ephrin receptors, Ste20 kinases, mutant EGFRs , c-FMS, as well as certain germinal center kinase related (such as GCKR, KHS1, MAP4K5) activity. The last set of enzymes was reported associating with CrkL and JNK. Both JNK and KHS1 are speculated to impair Bcr-Abl transforming activity. Other kinases inhibited by SKI-606 included: Tec family enzymes, Axl family receptors, GAK, and 2 calmodulin-dependent kinases (data not shown in the table). SKI-606 exerted little or no inhibitory activity towards c-Kit and platelet-derived growth factor receptor (PDGFR). The table below is excerpted from the Applicant's submission. (Pharmacology written summary, Module 2):

Table 4 Inhibition of c-Abl, Src and imatinib-resistant mutants (IC_{50} values)

Kinase	IC_{50} (μM) or % Inhibition
Src	0.0035
c-Abl	0.001
T315I Abl	0.3
c-Kit	6313
PDGFR (alpha)	Inactive @ 10 uM
E255K	98% @ 0.2 uM
G250E	97% @ 0.2 uM
Y253F	99% @ 0.2 uM
M351T	98% inhibition @ 1 uM
Q252H	100% inhibition @ 1 uM

o Effects on Src and Abl (#RPT-52664):

An enzyme assay was performed on a panel of kinases and the IC_{50} values are tabulated below (table from the Applicant). SKI-606 inhibited Src and c-Abl catalyzed phosphorylation of a target peptide with IC_{50} values at 1 nM (via ELISA) - 3.5 nM (via FRET; data not shown in the table below).

	IC_{50} (μM)
p38	0.95
CAMKII	6.25
PKA	5.03
PKC-a	1.47
$P70^{S6K}$	6.09
Tpl2	>40
Pdk	>66
KDR	7.00
IKK	>20
Cdk4	>50
raf/mek	0.50
Src	0.001
c-Abl	0.001
T315I Abl	0.3

IC_{50} = Concentration at which there is 50% inhibition.

o Human CML cell lines

Study #RPT-52664

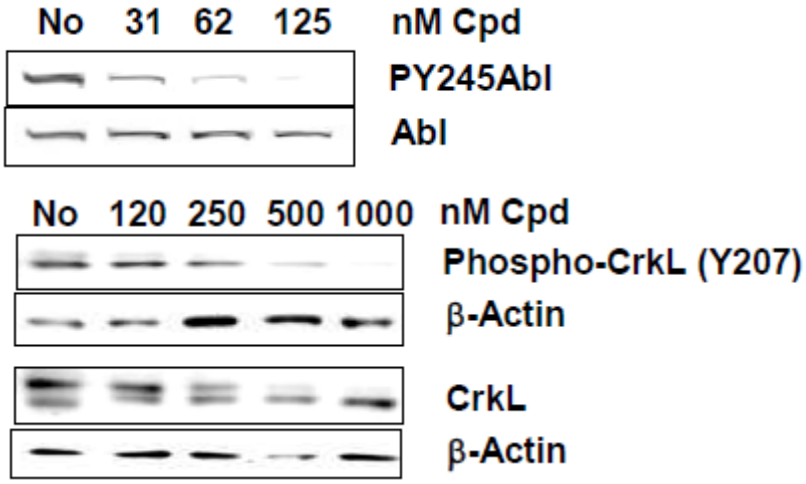
Bosutinib inhibited proliferation of KU812 and K562 CML lines at IC_{50} of 5 nm and 20-30 nM, respectively. Bosutinib inhibited the tyrosine phosphorylation (PY) of Bcr-Abl, Lyn (a Src family kinase), as well as transcription factor Stat5 in a concentration-dependent fashion. Concentration-dependent inhibition of phosphorylation of tyrosine residue on Lyn (Y397) was also noted (figures from the Applicant).

Figure 1 Inhibition of phosphorylation of Bcr-Abl and Src proteins

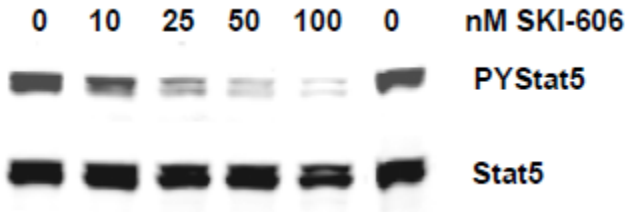
(B) Inhibition of Bcr-Abl phosphorylation by SKI-606. Bcr-Abl immunoprecipitates from extracts of KU812 cells treated with SKI-606 for 4 hours were analyzed for levels of phosphotyrosine. (C) Inhibition of Lyn autophosphorylation in KU812 cells treated with SKI-606 for 24 h. (D) Inhibition of Stat5 phosphorylation on Y697 after exposure to SKI-606 for 4 hours.

In two separate studies (#RPT-58432 and #RPT-60167), the inhibitory effect of SKI-606 on CML cells was demonstrated. Human CML K562 and KU812 cells were incubated with SKI-606 and the phosphorylation status of pivotal tyrosine residues of Abl and CrkL, i.e., Y245 and Y207, respectively, as well as Stat5 Y694, was evaluated. The results are shown in the figures below (figures from the Applicant):

SKI-606 inhibits phosphorylation of Abl Y245 and CrkL Y207 in K562 CML cells



Inhibition of Stat5 Phosphorylation on Y694

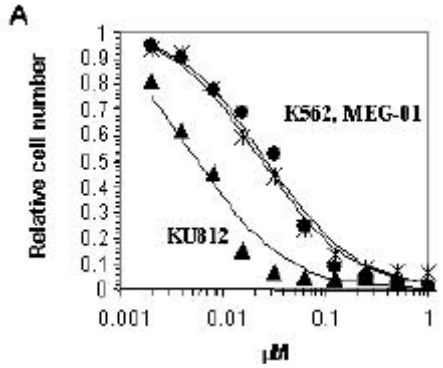


Phosphorylation of Stat5 Y694, Abl Y245 and CrkL Y207 was reduced with IC_{50} values < 20 nM, < 50 nM and < 200nM, respectively (#RPT-58432). Phosphorylation of Abl Y245 was nearly ablated at 125 nM SKI-606. On the other hand, CrkL phosphorylation was less sensitive to SKI-606 treatment than Y245 of Abl or Y694 of Stat5. The reasons for these differences are not known.

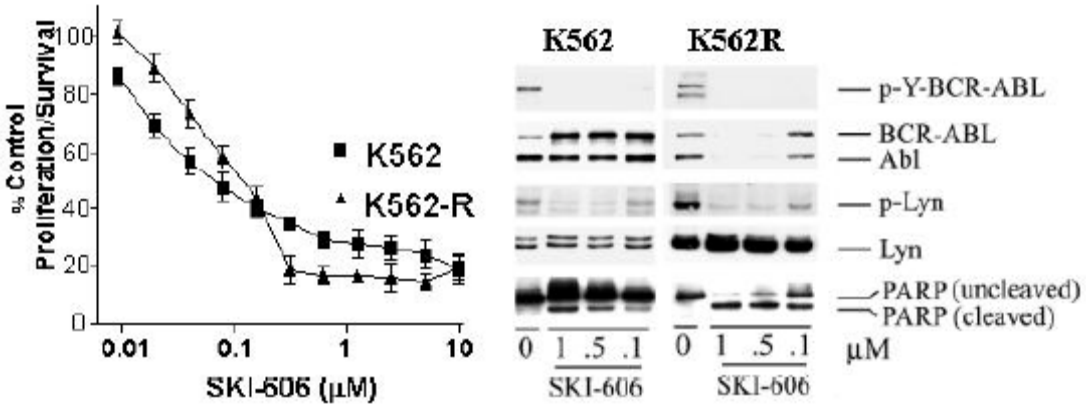
- **Inhibition of cell proliferation**

The suppression of cellular proliferation of CML cell lines, expression as IC_{50} values.
Study#RPT-52664
The figure below (from the Applicant) illustrates antiproliferative activity of SKI-606 against three CML lines, KU812, K562, and MEG-01, 3 days after incubation with SKI-606. The IC_{50} values were 5 nM for KU812 and 20-30 nM for K562 CML line, respectively.

Figure 2 Inhibition of cell proliferation in CML cell lines



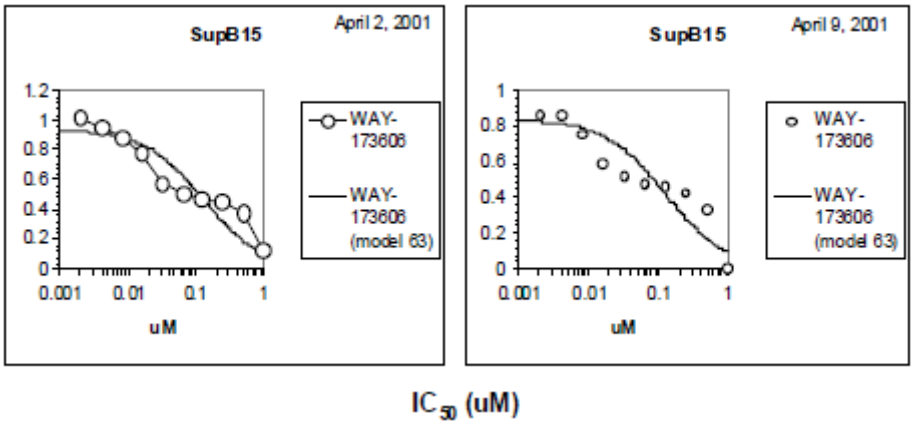
In the same study report, SKI-606 was also shown to inhibit the cell proliferation of an imatinib resistant K562 CML line; K562-R has down-regulated Bcr-Abl and up-regulated Lyn (a Src family tyrosine kinase). (Figures from the Applicant)



The K562R line was generated by propagating a K562 CML line in the presence of increasing concentrations of imatinib. The K562 and K562-R line were treated with SKI-606 for two days after which viable cell number was determined (right). The K562R line does not respond to 10 μM imatinib (left). Bcr-Abl and PY Bcr-Abl were examined in extracts from cells treated for 24 hours, while Lyn inhibition was examined in cells treated for 10 minutes. PARP cleavage was examined in cells treated for 24 hours.

<u>Study#RPT-78153:</u>

The anti-proliferative activity of bosutinib was evaluated in Bcr-Abl fusion protein $p190^{Bcr\text{-}Abl}$ expressing SupB15 cells, a Ph+ cell line obtained from a childhood acute lymphoblastic leukemia (ALL) patient. In comparison to Bcr-Abl fusion protein $p210^{Bcr\text{-}Abl}$ in CML, $p190^{Bcr\text{-}Abl}$ is a shorter form. Based on published articles, Bcr-Abl positive ALL tends to be less responsive to tyrosine kinase inhibitor treatment in the clinic (Reichert *et al.*, Blood 97: 1399-1403, 2001). SupB15 cells were treated with a range of bosutinib concentrations for 72 hours. The figure below demonstrates results of two experiments where IC_{50} values were similar, i.e., at 0.13 μM.



***In vivo* Studies:**

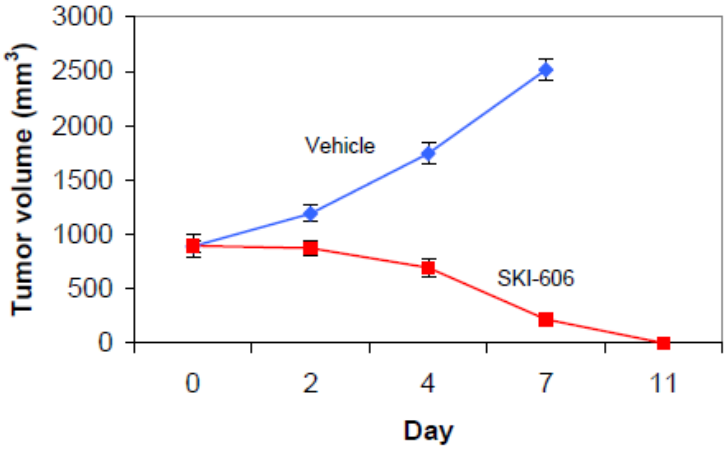
Murine xenograft models were used in the in vivo studies. These investigations were reported in Studies: #RPT-52665, and #RPT-58432.

K562 CML xenograft tumors in nude mice:

Study#RPT-52665:

Under the study condition, oral gavage of SKI-606 at 50 mg/kg for 5 days resulted in tumor regression in 50% of the animals, while dosing at 150 mg/kg completely eliminated xenografted K562 CML tumor in all mice. The compound was effective in eliminating large (~1 g) tumors when administered at 100 mg/kg.

Figure 3 Anti-tumor activity: CML cells in a murine xenograft model



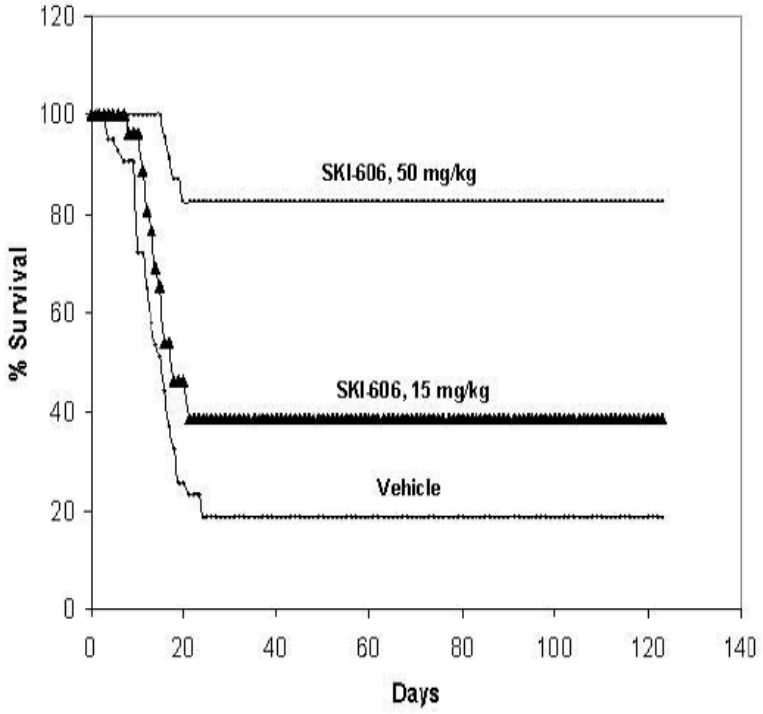
Regression of K562 tumor xenografts upon treatment with SKI-606. K562 tumors were staged to ~ 850 mg after which compound was delivered by oral gavage at 100 mg/kg for 5 days. Animals in the treated group were tumor free for > 40 days.

Study#RPT-58432:

A minimal effective dose (MED) in this study was 15 mg/kg.. The MED was defined as a greater than 2-fold increase in the regression frequency observed at this dose relative to vehicle-treated animals over 120 days (see figure below, from the Applicant). Systemic

exposure associated with the MED of 15 mg/kg (2054 ng·h/mL)** was lower than the exposure (total AUC) achieved at the proposed clinical dose of 500 mg daily for CML (3650 ng·h/mL).

**Of note, a value deduced from a single PK study (#RPT-56291) in female nude mice (see Section 5.1 "Pharmacokinetics/ADME), where an average AUC of 6848 ng·h/mL was obtained following one single oral dose of bosutinib at 50 mg/kg.



Special investigations:

- CML treatment resistance: Abl mutants

Mutation on Abl protein has been speculated as one of the mechanisms underlying drug-resistance to tyrosine kinase inhibitor (TKI) agents in treatment of CML. Puttini and coworkers proposed that SKI-606 may be effective in the treatment of certain resistant Abl mutants. The proposal is illustrated in figures excerpted from their work (Cancer Research 66: 11314-11322, 2006).

Figure 4 Bosutinib and Abl protein

Figure 5 SKI-606: anti-tumor activity in wild type and mutant Abl

In vivo effects of SKI-606 against Ba/F3 cells harboring Bcr-Abl WT (A) or kinase point mutations D276G (B), Y253F (C), T315I (D) injected in nude mice. Groups of four animals were used. Treatment was started when tumors became measurable (mean tumor weight range, 131-375 mg). Tumor growth of untreated controls (solid line) or SKI-606-treated animals (dashed line). SKI-606 was given at 150 mg/kg once daily for 11 days (5 days weekly).

Effects of SKI-606 on tumor-free survival of nude mice injected with Ba/F3 cells harboring different Bcr-Abl mutations. Treatment was started the day after tumor cells injection on six animals per group. Results are presented as Kaplan-Meier plots of tumor-free survival (left) and tumor growth (right ; points, mean; bars, SE) of Bcr-Abl WT (A), E255K (B), Y253F (C), D276G (D) xenografts given vehicle (solid line) or SKI-606 (dashed line). SKI-606 was given at 150 mg/kg once daily (A and B) or 75 mg/kg twice daily (C and D) from day 1 to 11.

It is apparent that while SKI-606 reduced tumor burdens and prolonged the survival of wild type tumors and tumors harboring several mutants, it did not exert as much inhibition on E255K and T315I mutants.

Further investigations indicated that the location of mutations at the Abl protein exhibited different sensitivity to SKI-606 inhibition. The table below is excerpted from the submission (Pharmacology written summary, Module 2, based on Redaeli et al.., J Clin Oncol 27: 469-471, 2009). Based on Redaeli and co-workers, except for mutants T315I and V299L, bosutinib demonstrated antiproliferative effects on 16 of 18 mutated forms of Bcr-Abl expressed in Ba/F3 transfected cells. The relative activities of bosutinib, based on the IC_{50} values, are tabulated below.

- Comparison of tyrosine kinase inhibitors (TKIs):

Puttini and coworkers also compared the inhibitory effect of SKI-606 and imatinib on cellular activity of transfected Ba/F3 myeloid cells.

These authors also compared the anti-proliferative activity of bosutinib with imatinib in Ba/F3 cells transfected with wild-type and mutant Bcr-Abl genes, Tel-PDGFR (a fusion protein of TEL and PDGFR) and 2 mutant c-Kit genes, as well as four Bcr-Abl mutant genes: D276G, Y253F, E255K and T315I.

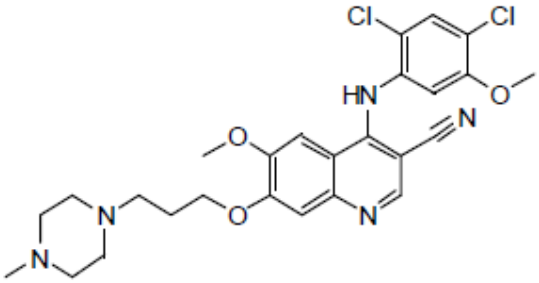
More results are tabulated as follows:
Anti-proliferative activity of bosutinib and imatinib in CML cell lines (Puttini *et al.*, 2006 and Golas *et al.*, Cancer Res 23: 375-381, 2003)

- Pharmacological effects of major metabolites: M2, M5 and M6

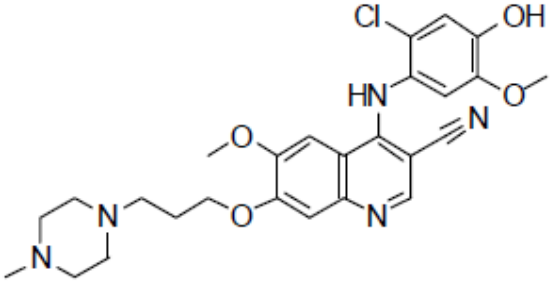
(Study #77627; Module 4)

The enzymatic and cellular activities of metabolite M2, oxydechlorinated bosutinib (WAY-198760), M5, N-desmethyl bosutinib (WAY-173607) and M6, bosutinib N-oxide (WAY-266857) of bosutinib (WAY-173606) in the Src enzyme assay (either the ELISA or the homogeneous (LANCE) assay) and the Src-dependent cell proliferation assay were determined relative to bosutinib (WAY-173606).

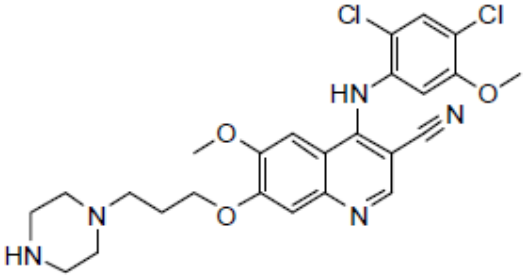
Figure 7 Structure of bosutinib and three major metabolites



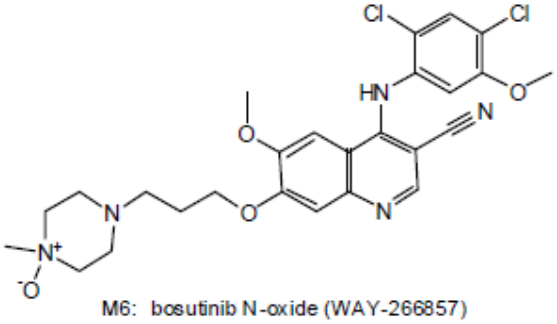
Bosutinib (SKI-606; WAY-173606)



M2: oxydechlorinated bosutinib (WAY-198760)



M5: N-desmethyl bosutinib (WAY-173607)



M6: bosutinib N-oxide (WAY-266857)

The result is tabulated as following (table from the Applicant):

Table 7 Enzymatic and cellular activity; comparison of bosutinib and three metabolites

(IC_{50} values - nM)

	WAY-173606	WAY-173607	WAY-198760	WAY-266857
Enzyme (ELISA)	1.3	1.1		
Enzyme (Lance)	3.5		11.1	3.7
Src (Cell)	100	1700	4100	5000

In comparison to bosutinib, the metabolites exhibited less cell-based activity, despite a comparable enzymatic activity.

4.2 Secondary Pharmacology

- Wide ligand profile (Study #RPT-52666)

In 62 assays, SKI-606 (10 μM) was tested against a panel of receptors, peptides and ion channels. These targets included receptors for neurotransmitters, steroid hormones, growth factor and hormone receptors, prostaglandins, brain/gut peptides and calcium, sodium and potassium ion channels. The result indicated (data not shown) that SKI-606 is active against some neurologically relevant channel proteins at relatively high concentrations, with Ki values in the range of 10^{-10} and 10^{-9} M. The compound

inhibited alpha 1 (80%) and 2 (60%) non-selective adrenergic receptors; histamine H2 receptor (89%); non-selective muscarinic receptor (central) (64%); sodium site 2 ion channel (66%); serotonin transporter (71%); Sigma non-selective receptor (76%); and neurokinin A receptor (63%). Since the activities against these targets were noted at relatively high concentrations which are not likely to be achieved in a clinical setting upon oral administration, the possibility of off-target effects mediated through these targets is small. There were no apparent neurological toxicities noted in the non-clinical studies or in patients in the clinical trials following oral treatment of SKI-606.

- SKI-606 metabolite activities (Study #75400039-2 and #75400039-3):

Bosutinib metabolites, M2 (PF-05898965) and M5 (PF-05312061), were tested against a panel of receptors, peptides, ion channels, transporters, and enzymes in a (b) (4) wide ligand profile screen at concentrations up to 10 μM.

Metabolite M2: (#75400039-3)
Greater than or equal to 50% Inhibition by metabolite M2 (10 μM) were observed for: the A2A, adrenergic α1, D2S, H3, M1, M3, NK2, and 5-HT1B receptors, and dopamine (DA) and 5-HT transporters. The K_i/IC_{50} values of these receptors, ion channels, and transporters were ≥ 900 nM (i.e., ~19-fold the total maximal plasma concentration of M2 in humans (47.7 nM, or 24.4 ng/mL)).

Metabolite M5: (#75400039-2)
Greater than or equal to 50% Inhibition by metabolite M5 (10 μM) included: the A_{2A}, adrenergic α1, D1, D2S, M1, M2, M3, NK2, 5-HT1B, 5-HT2A, and δ_1 receptors, sodium (site 2) channels, and dopamine (DA) and choline (CHT1) transporters. The K_i/IC_{50} values of these receptors, ion channels, and transporters were ≥ 140 nM (i.e., ~27-fold the unbound* maximal plasma concentration of M5 in humans (5.2 nM, or 2.6 ng/mL)).

*In humans, the ratio of unbound-to-total AUC of M5 is approximately 1:18 (Clinical Protocol 3160A4-1106-US). Thus the Ki/IC_{50} values were approximately 1.5 fold the total maximal plasma concentration of M5 in humans.

Given the low levels of metabolites M2 and M5 relative to their affinity for the targets, secondary (off-target) pharmacology is unlikely at clinically relevant concentrations.

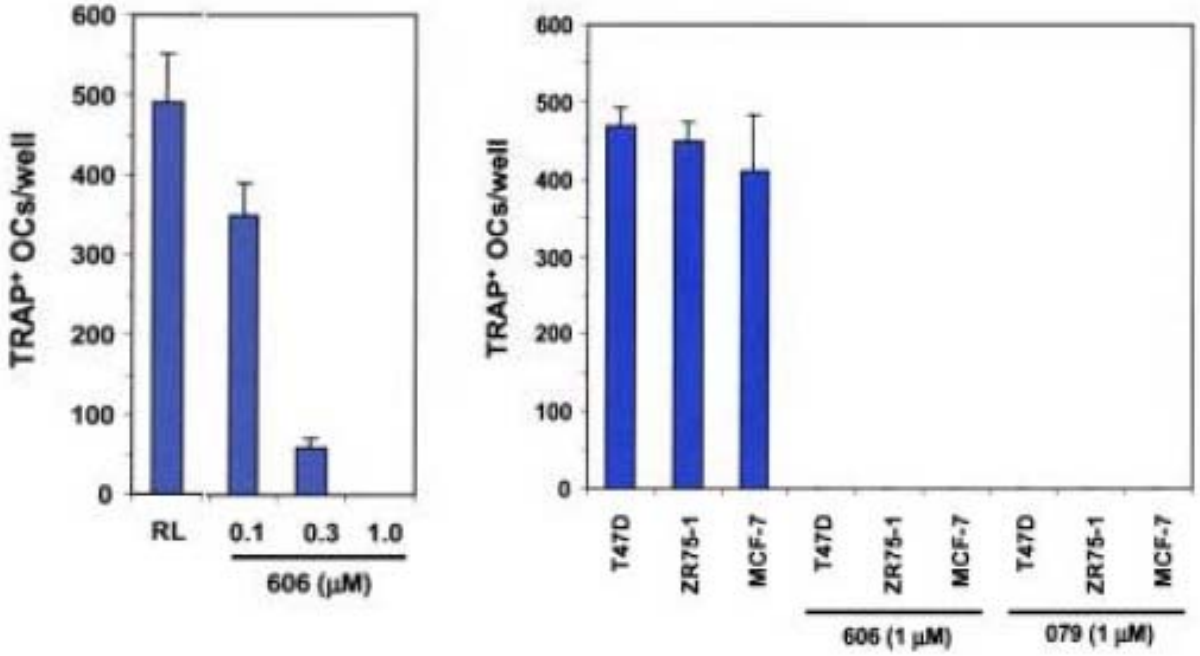
- Inhibition of osteoclast formation:

Bone metastasis occurs in some cancers, e.g. in breast cancer. Bone-metastatic tumor cells secrete cytokines and growth factors that stimulate the bone-resorbing activity of osteoclasts. Src acts on several signal transduction pathways in osteoclast precursors and osteoclasts, including TNFα- dependent activation of NFκB, CSF-1, and vitamin D pathways. The inhibitory effect of SKI-606 towards Src and osteoclasts was demonstrated in this submission.

RAW cells (104 cells/well in a 24-well dish) were exposed to SKI-606 at 0.1, 0.3 and 1.0 uM final concentration for 3 hours. The cells were then stimulated with RANKL (100 ng/ml). On day 5, cells were stained for TRAP and the total number of TRAP+

multinucleated cells was counted. (right) RAW cells (3000 cells/well in a 24-well dish) were co-cultured with T47D, zR75-1 and MCF7 breast tumor cells (300 cells/well) in the presence or absence of SKI-606 (1 uM). TRAP+-multinucleated cells were scored on day 5 as above.

Inhibition of osteoclast formation by SKI-606. (left) Inhibition of RANKL-stimulated formation of osteoclasts from RAW cells.



4.3 Safety Pharmacology

Neurological and respiratory effects:
Study #RPT-51764/51763: SKI-606: Single dose crossover oral (gavage) central nervous system/respiratory safety pharmacology study in female rats (Wyeth Protocol 03_1542/03_1543)

Three groups of rats (n=8/group) were rotated in these two studies, i.e., the vehicle control, 100 mg/kg and 300 mg/kg. Study #RPT-51763 was conducted 2 weeks before RPT-51764. The review of these studies is reported together.

Key study findings:
Single oral doses of SKI-606 up to 600 mg/kg to female rats did not exhibit adverse effects on function of CNS or respiratory system under the condition of the study.

A single oral (gavage) dose of SKI-606 (Lot# MP030203; purity 100%) was administered to female rats (Crl:CD®(SD) IGS BR rats, n=8/group; dosing volume 10 mL/kg) at dosage of 0 (vehicle control: 0.5% methylcellulose (4000 cps) (w/v), 2% polysorbate 80, NF (w/v), 0.06% acetic acid (w/v) and purified water), 100, 300, and 600

mg/kg. Animals were assessed for integrated central nervous system function once prior to dosing and approximately 4, 8, and 24 hours after dosing. Assessments included a functional observational battery (FOB), measurement of hindlimb foot splay, measurement of grip strength, and recording of rectal temperature. Changes in these parameters were analyzed as a function of time (i.e., hours after a single dose) and treatment group. Evidence of an overall group effect, or evidence of a time by treatment group interaction, indicates that groups differed in their behavior change over the course of the testing day. This measure provides between-groups comparison of the magnitude of behavioral change seen relative to animals' own pre-test values.

Respiratory function (respiratory rate, tidal volume, minute volume) was evaluated at predose and approximately 4, 8, and 24 hours post-dosing. Additionally, animals were also observed for mortality and treatment-related clinical signs.

Results:

- Clinical signs: mainly at ≥300 mg/kg; the number of rats affected is indicated.

Dose (mg/kg)	100	300	600
N	8	8	8
Discolored feces		3	
Dyspnea	1		
High carriage*		2	1*
Salivation	1	1	

***Animals walking on their toes in order to be mobile.**

The incidences were low and of minimal severity, and thus not considered adverse.

- CNS effect assessments:

Impaired gait (increased incidence, ≥300 mg/kg) and decreased pupil size (at ≥100 mg/kg) were considered SKI-606 related. However, because the severity was slight and they did not appear to impair the overall health of the animals, these effects were not considered to be adverse.

- Respiratory system assessments:

No treatment-related effects.

Cardiovascular effects:

In vitro studies:

The following two in vitro safety pharmacology studies (hERG assay) are reviewed (both studies are GLP compliant). The comment on these two studies is combined, based on similar study design and endpoints.

Study #RPT-54968: SKI-606: Effects on cloned hERG channels expressed in mammalian cells (Protocol 04_0833)

Study #RPT-61333: SKI-606: Effects on cloned hERG channels expressed in mammalian cells (Protocol 05_2323)

Key study findings: SKI-606 inhibited hERG current with an IC_{50} of 0.3 μM, indicating a pro-arrhythmic potential of SKI-606.

The potential effects of bosutinib (SKI-606) on the rapidly activating, delayed rectifier cardiac potassium ion current (IKr) were examined in hERG transfected HEK-293 (human embryonic kidney) cells (at 35 ± 2 ºC). N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES) buffered physiologic salt solution (HB-PS) was used as the negative control article and terfenadine (Sigma, 60 nM which blocks hERG current by approximately 80%) served as the positive control. In each individual study four concentrations of SKI-606 were used to construct a concentration-response relationship. The results are summarized in the following table (table from the Applicant, Module 2: 2.6.2 Pharmacology written summary, Table 6).

An apparent dilution error occurred during the preparation of the testing formulation in the first assay. However, the concentrations were corrected (see table below), adequate concentration-effect distribution was obtained, and the assay was accepted.

Table 8 Inhibition of hERG current

Test Group Nominal Concentration	---Actual Concentration---		Number of cells	-----Inhibition of hERG Current----- Mean	S-D	SEM
		First Assay				
Vehicle Control[a]	0		3	0.3%[b]	0.5%	0.3
Positive Control[c]	60 nM		3	75.4%[d]	6.3%	3.6
Bosutinib						
0.1 µM	0.05 µM	27 ng/mL	3	8.7%	2.5%	1.4
0.3 µM	0.112 µM	59 ng/mL	3	29.7%	2.9%	1.7
1 µM	0.604 µM	320 ng/mL	3	62.5%	1.1%	0.6
10 µM	8.69 µM	4610 ng/mL	3	95.8%	2.1%	1.2
		Second Assay				
Vehicle Control[a]	0		3	0.2%[b]	0.3%	0.2%
Positive Control[c]	60 nM		2	82.5	4.2	2.9
Bosutinib						
0.1 µM	0.088 µM	47 ng/mL	3	9.1%	0.9%	0.5%
0.3 µM	0.243 µM	129 ng/mL	4	20.8%	5.2%	2.6%
1 µM	1.095 µM	581 ng/mL	3	62.6%	3.3%	1.9%
10 µM	8.187 µM	4343 ng/mL	3	94.2%	0.8%	0.5%

DMSO = Dimethylsulfoxide; HEPES = N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid); hERG = Human ether-a-go-go-related gene; S-D = Standard deviation; SEM = Standard error of the mean.

[a] HEPES-buffered physiological saline (HB-PS contains 137 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose) and 0.1% DSMO.

[b] Testing facility historical data (mean ± S-D) for block of hERG by vehicle (0.1% DMSO in HB-PS) is 1.0% ± 2.2%, N=112.

[c] Terfenadine (MW = 471.7 g/mol), a drug known to block the hERG potassium ion current.

[d] Testing facility historical data for block of hERG by terfenadine is 82.5% ± 7.6%, N=252.

The calculated IC_{50} was 0.3 µM (159 ng/mL, molecular weight of bosutinib: 530.45 g/mol) and 0.7 µM (371 ng/mL) for the first and second assay, respectively.

<u>hERG assays for metabolite M2 and metabolite M5:</u>
According to the Applicant, both M2 and M5 produced a concentration-dependent inhibition of the hERG current; the derived IC_{50} values were 27.9 µM (14285 ng/mL, molecular weight of M2: 512 g/mol) and 8.7 µM (4494 ng/mL, molecular weight of M5:

516.5 g/mol) for M2 and M5, respectively. Based on the IC_{50} values, both metabolites M2 and M5 are not likely to be pro-arrhythmic.

In vivo Studies:
Study #RPT-51769: SKI-606: Single dose crossover oral (gavage) cardiovascular safety pharmacology study in dogs (Wyeth Protocol 03_1599)

Key study findings:
- A delayed onset of increases in heart rate (~15%, 14 bpm) occurred 17.5-24 hr following administration of 10 mg/kg bosutinib.
- There were no remarkable effects on blood pressure or ECG parameters.

A single oral (gavage) dose of SKI-606 (Lot# MP030203) was administered to Beagle dogs (n=4/sex; dosing volume 5 mL/kg) according to a Latin square crossover dosing paradigm at dosage of 0 (vehicle control: 0.5% methylcellulose (4000 cps) (w/v), 2% polysorbate 80, NF (w/v), 0.06% acetic acid (w/v) and purified (Type I) water), 2, 5, and 10 mg/kg. Each dose was administered to a pair of dogs (n=1/sex) and each dog received 4 administrations*; a 7-day washout period was in between two consecutive administrations. The telemetry data were collected for 30-second periods every 15 minutes, for 24 hours prior to and following dosing with vehicle control or SKI-606. Effects on heart rate, blood pressure, and ECG were evaluated using 4 male and 3 female dogs.
**Reviewer's note: Animal 8 (female) was removed from the study on Day 8 due to a failed telemetry device. No data from this animal were used in the statistical analysis.*

Post-dose ECGs were measured at approximately 3 (approximate t_{max}), 5, 10 ($t_{1/2}$), and 23 hours after dose administration. The additional pre-dose ECGs corresponded to 24 hours prior to each of these post-dose sampling times.

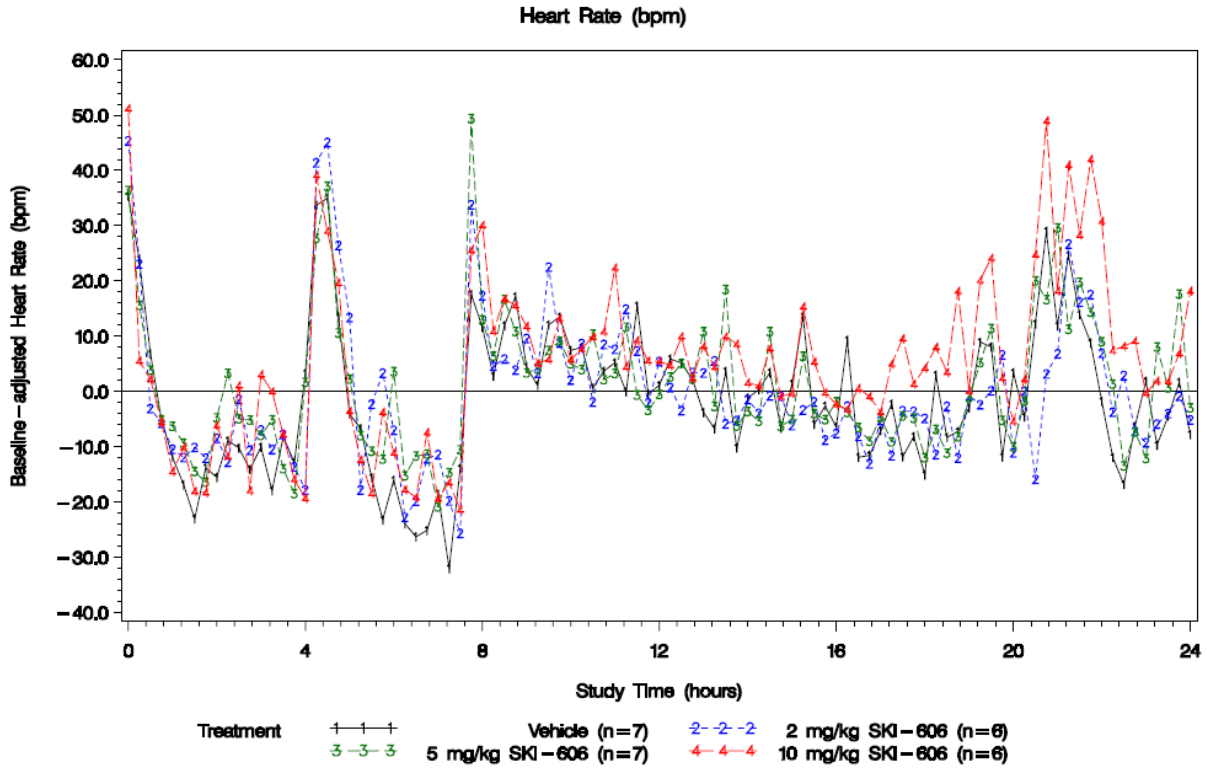
Results:
There was no treatment related mortality. GI clinical signs such as emesis occurred periodically in certain dogs According to report, the Study Director considered these episodes of emesis could have affected the actual dosage received and therefore these animals were removed from the statistical analysis . Incidentally all animals had soft feces that were not considered adverse.

- Heart rate and blood pressure:

Increased heart rate (~15%, i.e., 14 bpm) was noted beginning 17:5 hours to 24 hours postdose. The increase in heart rate in the current study is opposite to the finding of transient decreases in heart rate (5-17% from the baseline at 15 mg/kg) when bosutinib was administered intravenously to the dog (see below, Sudy #RPT-50437) No remarkable findings were seen in the blood pressure in the current study (data not shown).

The figure is from the Applicant:

Figure 8 Effect of SKI-606 on heart rate in telemetered dogs



- ECG

A slight increase in QTc interval (approximately 7-9 msec higher in mean QTc compared to vehicle control, 2 or 5 mg/kg bosutinib) was noted following administration of 10 mg/kg bosutinib. Five (5) of the 104 postdose QT interval observations fell outside of the 95% confidence bounds predicted for the relationship between predose heart rate and QT interval. See table below (from the Applicant).

Table 9 Effect of SKI-606 on QT intervals

Animal Number	Dosage (mg/kg)	Date	Time	Heart Rate (bpm)	QT Interval (msec)	95% Confidence Bounds (msec)
3878121	5	23 Sep 2003	1315	78	188	189.3 to 235.5
3411061	0	1 Oct 2003	915	114	216	167.2 to 213.4
3411061	2	17 Sep 2003	915	111	224	169.0 to 215.3
3411061	5	8 Oct 2003	915	99	224	176.4 to 222.6
3411061	10	24 Sep 2003	915	114	216	167.2 to 213.4

The change was of a small magnitude compared to the normal range in QT interval expected at a heart rate of 100 bpm (QT interval range: 178-217 msec based on predose data). The average predose range of difference in QTc among these four dosage group was around 1-8 msec. Thus, the 7-9 msec prolongation in QTc interval is not considered adverse. No remarkable findings in other ECG wave duration or morphology.

Study #RPT-50437: WAY-173606: An ascending intravenous dose cardiovascular safety pharmacology study in male dogs (Wyeth Protocol 03_0858)

Key study findings:

- Decreases in heart rate occurred during and following IV infusion of SKI-606 at 3, 7 and 15 mg/kg. The changes correlated with increases in mean arterial blood pressure. The changes in heart rate and blood pressure recovered after 120 minutes post-dosing.
- There were no remarkable effects on ECG parameters.

Ascending dosages of SKI-606 (WAY-173606, Lot #173606-14 DSC), at 9, 7, and 15 mg/kg, were administered as a 15-minute intravenous infusion to three male Beagle dogs, followed by a minimum one week washout before ascending to the next dosage. During the predose period, a 16-minute vehicle-control infusion (5% dextrose injection and 0.1N HCl) immediately preceded administration of test article. Parameters collected were arterial blood pressures (systolic, diastolic, and mean), heart rate, and lead II electrocardiogram (ECG). The telemetry data were collected for 20-second periods every minute, prior to test article infusion for approximately 30 minutes and for 2 hours after the test article infusion. Telemetry data were collected continuously during the 15 minute WAY-173606 infusion. Immediately prior to receiving the 15-minute intravenous infusion of the test article, each animal was dosed intravenously with vehicle for at least 16 minutes. The period of dosing with vehicle was regarded as providing the "baseline" response of the animal under the experimental conditions. Due to the limited number of animals in this study, no statistical tests were performed to compare mean response using p-values (i.e., no statistical hypothesis tests were performed). Other observations included mortality, clinical signs (once daily), and body weight (within 7 days prior to dosing, for dose calculation).

Results:

There was no treatment related mortality. Somnolence and diarrhea occurred in two dogs at the high dose (15 mg/kg).

- Heart rate and blood pressure:

At all three doses, during the 15 minutes of test article infusion heart rate was lower than baseline in all three animals. The average decreases among the three dogs were 12.0%, 11.0%, 16.1% at 3 mg/kg, 7 mg/kg, and 15 mg/kg, respectively. The table below is the summary of the largest decreases observed during the 15 minute infusion and the dose administered when the largest decrease occurred in each dogs:

Animal Number	Dose (mg/kg)	Baseline-adjusted heart rate and % decrease from the baseline
Dog #1	15	-30.5 bpm; 26.4%
Dog #2	All doses	-3.7 to -6.8 bpm; 4.4% -7.5%
Dog #3	3	-22.3 bpm; 16%
Dog #3	7	-20.2 bpm; 17.3%

Decreased heart rates persisted longer after the end of infusion. From 2 to 120 minutes after the end of the WAY-173606 infusion, heart rate was decreased (5.4 % to 17.3 %), relative to baseline at all three dose levels, but showed a gradual return to baseline values. For both 3 and 7 mg/kg, baseline-adjusted heart rate returned to close to the baseline 2 minutes following the end of dosing (i.e., baseline-adjusted heart rate was close to 0). However, baseline adjusted heart rate was still greatly below the baseline in Dog #1 (23.4 bpm; 20.2% below baseline) and Dog #3 (21.5 bpm; 18.3%) two minutes

following the end of 15 mg/kg WAT173606 infusion. From 75 to 120 minutes after the end of treatment, baseline-adjusted heart rate tended to move back towards baseline levels. The average baseline-adjusted heart rates and % decreases are summarized as follows:

Dose (mg/kg)	Baseline-adjusted heart rate and % decrease from the baseline	
	2 to 75 minutes post-dosing	75 to 120 minutes post-dosing
3	-5.3 to -36.3 bpm; 6.2% to 26%	+6.1 bpm (4.9%) to -27.8 bpm (20%)
7	-9.3 to -25.2 bpm; 9.6% to 21.7%	+7.5 bpm (7.7%) to -13.4 bpm (11.5%)
15	-10.2 to -30.5 bpm; 12.4% to 25.9%	+2.1 bpm (1.8%) to -21.9 bpm (18.6%)

Decreases in heart rate may be secondary to increases in blood pressure. During the 15-minute infusion of WAY-173606 there were only relatively small changes from baseline in blood pressure. During the 2 minutes after the infusion ended, the mean blood pressure at 3 mg/kg, 7 mg/kg, and 15 mg/kg showed increases of 5.4 %, 15.3 %, and 12.8 %, respectively. From 2 to 120 minutes after the infusion ended, mean blood pressure values had returned to near baseline values in all dosage groups. More details are described in the following tables.

- During 15 minute infusion and 2 minutes after infusion:

Dose (mg/kg)	Baseline-adjusted mAP and % decrease (-)/increase (+) from the baseline	
	During 15 minute infusion	2 minutes post-dosing (increases only)
3	-6.5 to +1.7 mmHg; -4.5% to +1.7%	4.9 to 7.6 mmHg; 3.5% to 7.5%
7	0.0 to +7.4 mmHg; 0.0% to 7.4%	15.1 to 21.4 mmHg; 15.1% to 17%
15	-1.4 to +9.5 mmHg; -1.0% to +9.6%	11.7 to 20 mmHg; 9% to 20.2%

mAP: mean arterial blood pressure

- 2-75 minutes and 75-120 minutes after infusion: recovering mAP

Dose (mg/kg)	Baseline-adjusted mAP and % decrease (-)/increase (+) from the baseline	
	2 to 75 minutes post-dosing	75 to 120 minutes post-dosing
3	+0.8 mmHg (0.8%) to -12.1 mmHg (8.4%)	+2.8 mmHg (2%) to -8.1 mmHg (5.6%)
7	+0.9 mmHg (0.9%) to -11.9 mmHg (9.2%)	+7.8 mmHg (6.2%) to -5.8 mmHg (4.5%)
15	+7.9 mmHg (5.7%) to -6.6 mmHg (5.1%)	+0.8 mmHg (0.6%) to -12.1 mmHg (12.2%)

mAP: mean arterial blood pressure

- ECG parameters:

There were no remarkable changes in the ECG wave size and morphology. Results from the mixed model analysis of postdose QT interval data indicate that the relationship between QT interval and heart rate is similar for the 3, 7, and 15 mg/kg dosages. Although a slightly longer PR, QT and QTc interval values occurred at 15 mg/kg, higher values prior to the administration of 15 mg/kg were noted in comparison with those at 3 and 7 mg/kg. The magnitude of the change following dosing compared to predose values for PR and QTc are similar at each dose level. The average increase in QTc interval compared with predose value was 8, 5 and 8 msec for 3, 7, and 15 mg/kg, respectively. All of the postdose QT and QTc values fell within the 95% confidence bounds for this animal population.

<u>Echocardiography study in rats:</u>

The Study below is not reviewed (Study#SP3810). This is a study comparing the cardiovascular effects of bosutinib and imatinib as assessed by echocardiography in Sprague Dawley rats. The rats were treated with daily oral doses of 50 mg/kg for each

agent for 8 weeks. According to the Applicant, increases in heart weight and structural changes consistent with hypertrophy were observed in imatinib treated rats, but not bosutinib treated rats. The systemic exposure to bosutinib on Day 56 was 560 ng/mL for C_{max} and 5350 ng·hr/mL for AUC, respectively.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Brief summary:
PK/ADME properties of bosutinib were evaluated in mice, rats, dogs and rabbits. Orally administered bosutinib was absorbed fairly rapidly (t_{max} of ~ 1.3-5.5 hr), with variable oral bioavailability in non-clinical species (23% to 64%). Following an oral dose of [^{14}C]bosutinib in Sprague-Dawley rats, radioactivity was distributed in most tissues and organs, except for brain, indicating a limited ability of bosutinib and/or its metabolites to cross the blood-brain barrier in rats. On the other hand, radioactivity was found in the placenta, fetus, as well as in the milk. Bosutinib was highly bound to plasma proteins in all species tested: mouse, rat, rabbit, dog, and human plasma with mean unbound fractions (fu) of 0.059, 0.061, 0.028, 0.041, and 0.063 in the respective species. Metabolite M5 exhibited a similar protein binding and comparable mean fu values. Multiple-dose oral administration of bosutinib resulted in a minimal accumulation of the drug or drug-derived compounds. While no gender effects were found in dogs, higher systemic exposures to oral bosutinib were seen in female rats. After oral administration of [^{14}C]bosutinib to mice, rats and dogs, bosutinib was the predominant radiolabeled component in plasma. The major circulating metabolite were M5 (11%), M9 (approximately 17%-24%) and M5 (up to 20%)/M6 (up to 10%) in mice, rats, and dogs, respectively. Similarly, bosutinib appeared to be the major component in patients' plasma. Of note, the prominent circulating metabolites in humans are M2 and M5 (~19% and 25% of the AUC of parent drug, respectively). The following statement is based on Applicant's pharmacokinetic written summary (Module 2). The metabolite M2 is a human-specific metabolite. In human liver microsomes, it was found that bosutinib was predominantly metabolized by CYP3A4. In humans, co-administration of bosutinib with ketoconazole (a CYP3A and P-gp inhibitor) increased the plasma AUC by approximately 8-fold, wile co-administration of bosutinib with rifampin (a CYP3A and P-gp inducer) decreased the plasma AUC of bosutinib by approximately 94%. Metabolite M2 was mainly metabolized via glucuronation by UGT enzymes. After oral administration of [^{14}C]bosutinib to rats, dogs, and humans, the primary route of elimination of drug-derived radioactivity was via the feces. Under the conditions tested, bosutinib did not inhibit or induce a panel of CYP enzymes, suggesting low potential for drug-drug interactions through this mechanism. On the other hand, bosutinib may have the potential to affect the pharmacokinetics of drugs that are substrates of P-gp such as digoxin (pharmacokinetic written summary, Module 2).

Methods of analysis:
Not reviewed.

In brief, the determination of pharmacokinetic parameters and the quantitation of bosutinib in mouse, rat, and dog plasma and quantitation of metabolites M2 and M5 in rat plasma were performed by high-performance liquid chromatography/tandem mass spectrometer (HPLC/MS/MS) methods. Tissue distribution in rats, metabolism in mice, rats, dogs, and humans, and excretion in rats, dogs, and humans were conducted using [^{14}C]bosutinib. Other methods used included quantitative whole body autoradiography (QWBA), liquid scintillation counting (LSC) and HPLC, LC/MS for determination of the distribution, accumulation, retention and metabolite profiles of bosutinib in biological specimens.

Absorption:
- Single dose PK studies in mice, rats and dogs:

The PK profiles in laboratory animals were determined following a single oral and intravenous dose. The following studies are reviewed and summarized:

Table 10 Summary of Single dose PK studies

<table>
<tr><th>Study Number</th><th>Species</th><th>Salient findings</th></tr>
<tr><td>RPT-51795</td><td>Mice (nude)</td><td>* Oral: 50 mg/kg. IV: 5 mg/kg. PK profiles:
<table>
<tr><th>Administration Route</th><th>C_{max}[a] (ng/mL)</th><th>t_{max} (hr)</th><th>$AUC_{0-\infty}$ (ng•hr/mL)</th><th>$t_{1/2}$ (hr)</th><th>CL_T (L/hr/kg)</th><th>Vd_{ss} (L/kg)</th><th>F (%)</th></tr>
<tr><td>IV</td><td>1049</td><td>NA</td><td>2220</td><td>4.8</td><td>2.25</td><td>11.5</td><td>NA</td></tr>
<tr><td>PO</td><td>1509</td><td>4.0</td><td>11677</td><td>4.2</td><td>NA</td><td>NA</td><td>52.6</td></tr>
</table>
a: Concentration at 5 minutes after IV dosing. NA: Not applicable
F: Bioavailability</td></tr>
<tr><td>RPT-53297</td><td>Female mice (nude)</td><td>* Oral: 50 mg/kg; fast versus fed mice; AUC values were slightly higher in fasted condition. Average AUC: 6848 ng·hr/mL.
<table>
<tr><th>Dose (mg/kg)</th><th>Feeding Regimen</th><th>C_{max} (ng/mL)</th><th>T_{max} (hrs)</th><th>AUC_{0-24} (ng·hr/mL)</th><th>$AUC_{0-\infty}$ (ng·hr/mL)</th><th>$t_{1/2}$ (hours)</th></tr>
<tr><td>50</td><td>Fasted</td><td>1177</td><td>2 – 4</td><td>7947</td><td>7986</td><td>2.9</td></tr>
<tr><td>50</td><td>Fed</td><td>1132</td><td>2</td><td>5649</td><td>5710</td><td>3.5</td></tr>
</table></td></tr>
<tr><td>RPT-51749</td><td>Rats</td><td>Oral: 50 mg/kg. IV: 5 mg/kg. PK profiles:
<table>
<tr><th>Route</th><th>Sex</th><th>C_{max}</th><th>AUC</th><th>T_{max} (h)</th><th>$T_{1/2}$ (h)</th><th>CL</th><th>F (%)</th><th>Vd_{ss}</th></tr>
<tr><td>Oral</td><td>M</td><td>224</td><td>1507</td><td>3</td><td>3.7</td><td>---</td><td>23</td><td>---</td></tr>
<tr><td></td><td>F</td><td>834</td><td>7099</td><td>5.5</td><td>5.4</td><td>---</td><td>59.5</td><td>---</td></tr>
<tr><td>IV</td><td>M</td><td>646</td><td>655</td><td>---</td><td>2.5</td><td>7.68</td><td>---</td><td>15.2</td></tr>
<tr><td></td><td>F</td><td>723</td><td>1194</td><td>---</td><td>4.3</td><td>4.22</td><td>---</td><td>17.1</td></tr>
</table>
C_{max} (ng/mL); AUC (ng·hr/mL); CL (L/hr/kg, clearance); Vdss (L/kg, volume of distribution at the steady state); F (bioavailability)
Bolded prints: significantly different from males</td></tr>
</table>

<table>
<tr><td>RPT-51748</td><td>Dogs (females)</td><td>Oral: 5 mg/kg (fed or fast); IV: 2 mg/kg; PK profile; AUC was significantly higher in fed condition
<table>
<tr><td>Route</td><td>C_{max}</td><td>AUC</td><td>T_{max} (h)</td><td>$T_{1/2}$ (h)</td><td>CL</td><td>F (%)</td><td>Vd_{ss}</td></tr>
<tr><td>Oral (Fed)</td><td>230</td><td>3835</td><td>2.3</td><td>17.7</td><td></td><td>64</td><td></td></tr>
<tr><td>Oral (Fast)</td><td>206</td><td>3091</td><td>1.3</td><td>13.5</td><td></td><td>49.6</td><td></td></tr>
<tr><td>IV</td><td>605</td><td>2449</td><td></td><td>13.5</td><td>0.914</td><td></td><td>13.5</td></tr>
</table>
C_{max} (ng/mL); AUC (ng·hr/mL); T_{max} (h); CL (L/hr/kg, clearance); Vdss (L/kg, volume of distribution at the steady state); F (bioavailability)
Bolded prints: significantly different from fasted condition</td></tr>
</table>

*** Data from the Applicant**

Reviewer's note: the AUC value was statistically significantly higher in fed condition following a single oral dose of bosutinib in dogs, while the exposure was slightly higher in fasted condition in mice. In patients, food increased the AUC by ~1.7-fold compared to the fasted state.

- Permeability and transporters:
  - Studies in Caco-2 cells: the interaction with P-glycoprotein (P-gp) transporters (#RPT-71680):

  Efflux of SKI-606 crossing Caco-2 cell monolayers was concentration dependent (1-100 μM) and saturated at a concentration of 100 μM, suggesting that P-gp may be involved in SKI-606 transport. Under the testing conditions, it was also demonstrated that SKI-606 may be considered to be a weak P-gp substrate. In the study, SKI-606 showed a concentration dependent inhibition on P-gp mediated digoxin (a prototype P-gp substrate) efflux with an IC_{50} value of 2 μM and resulted in 95% inhibition of P-gp activity at 50 μM. Based on the C_{max} value of 206 ng/mL (0.4 μM), following an oral dose of 600 mg in patients, it would give rise to a ratio of C_{max}/IC_{50} of 0.2
  - Other transporters (#WAY-173606_18Aug10_195303):

  In a study using human hepatocytes, it was demonstrated that hepatic uptake transporters such as organic anion transporting polypeptides (OATP) 1B1 and 1B3 are unlikely to play a major role in the uptake of SKI-606 into liver cells. Studies in humans indicated renal organic anion transporters (OAT) 1 and 3, as wellas organic cation transporter (OCT) 2, are unlikely involved in the renal clearance of SKI-606.

<u>Distribution:</u>

- Study#RPT-54959: SKI-606: Quantitative whole body-autoradiography and tissue distribution of rats following oral administration of [^{14}C}-SKI-606 (Module 4)

A single oral (gavage) dose of [^{14}C]-SKI-606 (50 mg/kg; 10mL/kg dose volume) (specific activity= 10 μCi/mg) was administered to male Sprague Dawley (SD) rats (albino) (n=8) and male Long-Evans (LE) rats (pigmented) (n=8). Distribution of radioactivity was analyzed by quantitative whole body autoradiography (WBA) and quantitative tissue dissection (QTD) (n=2/time point). The distribution of radioactivity in selected melanin-containing tissues, including the skin and uveal tract, was detected in the LE rats (n=1/time point). The radioactivity in the plasma and tissues of each were evaluated at 1, 4, 8, 24, 48, 96, 168 and 672 hr after dosing. Plasma radioactivity concentrations were determined by liquid scintillation counting. The selected tissues were evaluated by

whole body autoradiography (WBA) using phosphorimaging or by QTD using liquid scintillation counting of solubilized or combusted whole tissues and tissue homogenates. BLQ (below the limit of quantitation) was <0.162 μg equivalents [^{14}C]-SKI-606.

Results:
Concentrations of radioactivity (μg equivalents/g) in Sprague-Dawley rats, as determined by WBA or excision (i.e., tissues), reached C_{max} by either 4 or 8 hours postdose with the exception of the small intestine and stomach, which reached C_{max} at 1 hour postdose and the harderian gland (11.6) and testis (0.375) which reached C_{max} at 24 hours postdose. GI tracts (especially the small intestine) were the tissues with the highest C_{max} values and highest tissue to plasma (T/P) ratio at one hour postdose; while harderian gland was the highest T/P ratio at 96 hours postdose and highest radioactivity exposure (AUC_{0-672}). The T/P ratio was greater than 10 for most tissues. The extensive tissue distribution was consistent with the large volume of distribution for bosutinib observed in rats (data not shown). Radioactivity concentrations in the brain (cerebrum, cerebellum, medulla and olfactory lobe) were below the limit of quantitation (BLQ) at all time points, indicating that the bosutinib and related compounds do not cross the blood-brain barrier or the transfer is minimal.

The elimination of radioactivity was almost complete by Day 7 postdose, i.e., C_{168}/C_{max} ratios <1 in most tissues of the SD rats, with the exception of harderian gland (0.412) and testis (0.976). There was no appreciable difference in the distribution and elimination of radioactivity from the skin of SD and LE rats. However, radioactivity was present in much higher concentrations (C_{max} values 11-fold higher) and for a longer time (C_{672}/C_{max} was <0.1 in SD rats and was 0.848 in LE rats) in the uveal tract of LE rats, suggesting binding to melanin.

- Protein binding (Study #RPT-54418)

The binding of [^{14}C]-SKI-606 to protein in plasma of male CD-1 mice, Sprague-Dawley rats, New Zealand white rabbits, Beagle dogs and human volunteers was assessed by an ultracentrifugation method at nominal total plasma concentrations of 100, 1000 and 10000 ng/mL, at approximately 37°C.

The percent of unbound [^{14}C]-SKI-606 was calculated as follows:

% Unbound = C_f/C_p x 100; where:
C_f = concentration of [^{14}C]-SKI-606 in the protein-free plasma fraction and
C_P = total concentration of [^{14}C]-SKI-606 in the plasma sample.

The percent of bound [^{14}C]-SKI-606 was calculated as follows:

% Bound = 100 - % unbound

The table below (from the Applicant) is the summary of mean (± SD) [^{14}C]-SKI-606 % bound to mouse, rat, rabbit, dog and human plasma protein at the tested drug concentrations. In this range of concentrations, i.e., within estimated therapeutic and toxicological concentrations, the % bound of SKI-606 to plasma protein was similar in all

the species, and the ranking of the [^{14}C]-SKI-606 % protein bound was generally of the order: mouse ≈ rat ≈ human < dog < rabbit.

Table 11 Plasma protein bindings of [^{14}C]SKI-606

Species	Nominal Plasma [^{14}C]-SKI-606 Concentration (ng/mL) 100	1000	10000
Mouse	92.6 ± 0.1	94.7 ± 0.1	95.0 ± 0.1
Rat	93.0 ± 0.4	94.4 ± 0.6	94.3 ± 0.1
Rabbit	97.6 ± 0.1	97.4 ± 0.0	96.7 ± 0.1
Dog	96.1 ± 0.1	96.1 ± 0.1	95.4 ± 0.0
Human	93.8 ± 0.1	93.9 ± 0.4	93.3 ± 0.3

The fraction (%) of unbound for the species tested is (table from the Applicant) as following: (

Species	Nominal Plasma [^{14}C]-SKI-606 Concentration (Ng/mL) 100	1000	10000
Mouse	7.4 ± 0.1	5.3 ± 0.1	5.0 ± 0.1
Rat	7.0 ± 0.4	5.6 ± 0.6	5.7 ± 0.1
Rabbit	2.4 ± 0.1	2.6 ± 0.0	3.3 ± 0.1
Dog	3.9 ± 0.1	3.9 ± 0.1	4.6 ± 0.0
Human	6.2 ± 0.1	6.1 ± 0.4	6.7 ± 0.3

Mean fraction of unbound (fu) [^{14}C]-SKI-606 is 0.059, 0.061, 0.028, 0.041 and 0.063 for mouse, rat, rabbit, dog and human plasma, respectively. The mean fu ranked in the order of: rabbit < dog < rat ≈ mouse ≈ human. Using Transil assay binding kits, it was reported that bosutinib was highly bound to human serum albumin (HSA) (95.4%) and moderately to α1-acid glycoprotein (AGP) (71.4%).

- Protein binding of metabolites M2 and M5 (Study #RPT-PF-05312061/11May10/122621)

The binding of metabolite M5 to protein in plasma of CD-1 mice, Sprague-Dawley rats, New Zealand white rabbits, Beagle dogs and humans was assessed by an equilibrium dialysis method at nominal total plasma concentrations of 50 and 500 ng/mL, at approximately 37°C. Like bosutinib, M5 was also highly protein bound, with mean fraction unbound (fu) of 0.049, 0.062, 0.023, 0.021 and 0.055 for mice, rats, rabbits, dogs and humans, respectively. The fu values followed a similar order as those for bosutinib in the species tested: dog ≈ rabbit < mouse ≈ rat ≈ human. There was no marked change in the percentage of unbound M5 at a plasma concentration of 500 ng/mL compared with those observed at 50 ng/mL in any of the evaluated species.

According to the Applicant, M2 was not stable in buffer or plasma under the incubation conditions for equilibrium dialysis (37°C for 6 hours). Thus, plasma protein binding for M2 was not conducted.

- Red blood cell partitioning (*in vitro*: Study #WAY-173606_02Aug10_113243) (*in vivo*: #RPT-77073; #RPT-53088; #EPT-53089)

The extent of partitioning of bosutinib into red blood cells was evaluated in rat, dog, and human whole blood at a nominal concentration of 1 μM (530 ng/mL) bosutinib. The whole blood samples were incubated with the drug at 37°C for approximately 3 hours. Aliquots of incubated blood samples were collected at 60 and 180 minutes. The contents of PF-05208763 in whole blood (C_b) and plasma (C_p) were analyzed by LC-MS/MS. The mean blood to plasma concentration ratios (C_b/C_p) of PF-05208763 (WAY-173606; SKI-606), were 1.6, 0.9 and 1.2 in rat, dog and human, respectively, indicating that SKI-606 does not preferentially distributed into red blood cells. The in vitro data were supported by in vivo data (see below: metabolism and excretion sections). The blood-to-plasma ratios for radioactivity were: 0.95-0.26 in mice, 0.97-1.31 in rats and 0.82-0.97 in dogs. Thus both *in vitro* and *in vivo* data indicated that the distribution of SKI-606 was relatively equal into blood cell and plasma compartments for mice, rats, dogs and humans.

- Distribution in gravid and lactating rats: the distribution in placenta, fetus and milk (Study #RPT-77097; #RPT-77578)

The distribution of [^{14}C]-SKI-606 derived radioactivity to maternal and fetal tissues was investigated after administration of a single oral dose of 10 mg/kg to gravid rats (GD 19). [^{14}C]-SKI-606 derived radioactivity concentrations in the maternal blood and plasma and in placenta, amniotic fluid and fetus were evaluated at 2, 4,8, 24, 48 and 72 hr after dosing. Peak maternal plasma concentration was observed at 4 hr post-dose. Blood-to-plasma radioactivity ratios were 1-1.3 at all time points. Peak concentrations of [^{14}C]-SKI-606 derived radioactivity in placenta and fetus were reached at 8 hr post-dose, while peak concentration in amniotic fluid was attained at 24 hr post-dose. The tissue-to-plasma ratios for these tissues increased over the 72 hr collection period, suggesting slower elimination of radioactivity from fetal tissue than from the maternal tissues. The ratios of AUC_{0-72} for radioequivalents in the placenta, amniotic fluid, and fetus to plasma were 21, 2.2, and 2.9, respectively. The study indicated that there was distribution of SKI-606 derived radioactivity into the placenta with a smaller degree to the amniotic fluid and fetuses.

The excretion of SKI-606 into breast milk was evaluated after a single oral [^{14}C]-SKI-606 dose of 10 mg/kg to lactating SD rats. [^{14}C]-SKI-606 derived radioactivity concentrations in milk and plasma were evaluated at 0.5, 2, 6, and 24 hours after dosing. The transfer of [^{14}C]-SKI-606-derived radioactivity to nursing pups was studied in 9 lactating rats and their litters by collecting blood from 3 dams/time point and 3 pups/dam at 0.5, 1, 2, 3, 4, 6, 8, 24, and 48 hr post-dose. Plasma samples were analyzed for total radioactivity concentrations. The results indicated that at the same sampling time point, the levels of radioactivity in milk were several fold (3-8 fold) higher than in maternal plasma, indicating a faster excretion of [^{14}C]-SKI-606 and/or its metabolites into the milk of lactating rats. The AUC_{0-24} ratio of radioequivalents for milk-to-plasma was 7.8. Comparing pup versus maternal plasma radioactivity levels, it was found that pup plasma concentrations at 24 and 48 hr post-dose were at least 8-fold higher than maternal plasma concentrations. Radioactivity in pups increased over time; the radioactivity in pups was lower than that in maternal plasma at 0.5 to 8 hr post-dose. The AUC_{0-48} ratio of radioequivalents for pup-to-dam plasma was 2.6. This result

suggested that the radioactivity in nursing pups occurred as a result of exposure to maternal milk.

Metabolism

The *In vivo* studies to investigate metabolism and excretion of SKI-606 in mice, rats and dogs were performed by administration of a single oral dose of [^{14}C]-SKI-606, then blood and plasma samples were obtained at various time points within the duration of 24 hour post-dose. Urine and feces were collected 0-24 hour following dosing. The dose used was 50 mg/kg in male mice and rats and 5 mg/kg in dogs, respectively. Samples were analyzed for radioactivity concentrations and for metabolite profiles using HPLC with radioactivity flow detection. Major metabolites were characterized by LC/MS.

- Bosutinib metabolites: products of *in vivo* metabolism
  M1 - *N*-Desmethyl, oxydechlorinated bosutinib;
  M2 = Oxydechlorinated bosutinib;
  M3 = Dioxydechlorinated bosutinib; M12 = Regioisomer of M3;
  M4 = *O*-Desmethyl bosutinib;
  M5 = *N*-Desmethyl bosutinib;
  M6 = Bosutinib *N*-oxide;
  M8 = *O*-Dealkyl bosutinib
  M9 = Uncharacterized metabolite
  M10 = Uncharacterized metabolite
  M11 = Oxydechlorinated bosutinib sulfate;
  M13 = oxydechlorinated bosutinib glucuronide
  M14 = Quinoline ring *O*-desmethyl bosutinib

- In vitro studies
  - Results of studies conducted in liver microsomes and hepatocytes (Study #RPT-53085; #PF-05208763_27Sep10_183648; not reviewed) (Tables from the Applicant) The *in vitro* metabolism of [^{14}C]bosutinib was investigated by incubation of the drug with liver microsomes and hepatocytes.

Table 12 in vitro metabolism of bosutinib in liver microsomes

	Liver Microsomes					Hepatocytes		
Enzyme System:	Mouse	M Rat	F Rat	Dog	Human	Rat	Dog	Human
[^{14}C]Bosutinib Concentration (μM):	100	100	100	50	50	5 or 50	5 or 50	5 or 50
Metabolite								
Bosutinib (parent)	X	X	X	X	X	X	X	X
N-Desmethyl, oxydechlorinated bosutinib (M1)		X			X			
Oxydechlorinated bosutinib (M2)	X	X	X		X			X
Dioxydechlorinated bosutinib (M3)		X			X			
O-Desmethyl bosutinib (M4)		X	X		X			
N-Desmethyl bosutinib (M5)	X	X	X	X	X	X	X	X
Bosutinib *N*-oxide (M6)	X	X	X	X	X	X	X	X
O-Dealkyl bosutinib *O*-glucuronide (M7)	X	X	X	X	X	X	X	X

X: present

- Cytochrome P450 enzymes involved in metabolism of bosutinib in human liver misrosomes (Study #RPT-53086; not reviewed)

The table below described the CYP enzymes that may be involved in the formation of metabolites M2, M5 and M6:

Table 13 CYP isozymes involved in metabolism of bosutinib

	cDNA Study		
	Rates of NADPH-Dependent Formation of Major Bosutinib Metabolites (nmoles/nmole of P450/min)[a]		
CYP Enzyme[b]	M2 Formation	M5 Formation	M6 Formation
CYP1A2	–	–	–
CYP2A6	–	–	–
CYP2B6	–	–	–
CYP2C8	–	–	–
CYP2C9	–	–	–
CYP2C19	–	–	–
CYP2D6	–	–	–
CYP2E1	–	–	–
CYP3A4[c]	5.5	7.5	1.8
CYP3A5	–	–	–

As indicated, isozyme CYP3A4 was the major CYP enzyme involved in the formation of major metabolites of bosutinib.

- Other enzymes involved in metabolism of bosutinib (Study #RPT-63186 and #PF-05898965_13Oct10_201157; not reviewed)
  - Flavin-containing manooxygenase (FMO) enzymes in human liver and kidney: The result suggests that FMO (i.e., mainly FMO1 and 3) is the major enzyme involved in the formation of M6 in human kidney microsomes (data not shown).
  - UGT enzymes:
    The study suggested that multiple UGT enzymes were able to catalyze the formation of M13 (human metabolite M2 glucuronide); these enzymes were located in both intestine and liver I humans (data not shown).

- *In vivo* studies (Study #RPT-77073, #RPT-53088, #RPT-53089, #RPT-70327)

The tables below are excerpted from Applicant's "Pharmacokinetics tabulated Summary" (Module 2). The metabolite profiles in plasma, urine and feces in laboratory animals and humans are tabulated. In addition, the blood:plasma radioactivity ratios are also summarized.

- Plasma: Profiles at various time points post-dose; expressed as % of radioactivity (mean) in sample for laboratory animals and % of bosutinib in humans.

Table 14 Metabolite profiles in plasma and excreta

2 hour (1 hr for mice) post-dose:

Species	Gender	Bosutinib	M2	M4	M5	M6	M9	M10	M14
Mice	Male	89.4	NA	NA	10.6	ND	NA	NA	NA
Rats	Male	NA	NA	NA	NA	NA	NA	NA	NA
	Female	NA	NA	NA	NA	NA	NA	NA	NA
Dogs	Male	76.8	NA	NA	6.9	10.2			
Humans	Male (Day 1)		8.2		28	5.1			
	(Day 15)		7.7		19	4.1			

4 hour (3 hr for mice) post-dose:

Species	Gender	Bosutinib	M2	M4	M5	M6	M9	M10	M14
Mice	Female	85.2	NA	NA	9.0	5.8			

Rats	Male	55.9	NA	2.1	8.8	6.7		17.4	ND
	Female	79.6	NA	1.3	1.1	7.6		7.9	ND
Dogs	Male	66.3	NA	NA	20.3	9.0			
Humans	Male (Day 1)		10.5		32.1	5.8			
	(Day 15)		6.5		25.7	4.8			

8 hour (6 hr for mice) post-dose:

Species	Gender	Bosutinib	M2	M4	M5	M6	M9	M10	M14
Mice	Male	84.7			9.6	5.6			
Rats	Male	57.8		0.6	6.3	4.8	27.5	ND	
	Female	83.1		1.8	1.8	4.4	6.5	ND	
Dogs	Male	78.7	NA	NA	9.5	7.8			
Humans	Male (Day 1)		10.3		26.1	6.1			
	(Day 15)		7.6		13.8	4.6			

24 hour post-dose:

Species	Gender	Bosutinib	M2	M4	M5	M6	M9	M10	M14
Mice	Male	87.1	NA	NA	7.9	5.1	ND	ND	NA
Rats	Male	35.6	NA	ND	13	2.7	39.7	8.9	NA
	Female	77.8	NA	ND	3.8	7.5	6.0	9.0	NA
Dogs	Male	86.7	NA	ND	2.8	9.2	NA	NA	NA
Humans	Male (Day 1)	NA	7.6	ND	13.8	4.6	ND	ND	ND
	(Day 15)	NA	9.6	ND	17.3	4.0	ND	ND	ND

NA: not applicable; ND: not determined

- Urine: 0-24 hour sample

Species	Gender	% dosage	Bosutinib	M2	M4	M5	M6	M8	M9	M10	M14
Mice	Male	1.1	45.8	NA	1.9	22	21.1		NA	NA	NA
Rats (8-24 hr)	Male	0.52	8	11.2	9.5	16	32.2	8.4	NA	NA	NA
	Female	1	14.6	0.8	3.1	4.8	66.1	4.6	NA	NA	NA
Dogs (8-24 hr)	Male	0.33	50.3	NA	NA	17.9	25.6	NA	NA	NA	NA
Humans	Male (8-24 hr)[a]			0.32		0.08	0.08				
	(0-24 hr)[b]		72	7.5		ND					

- Feces: 0-24 hour sample

Species	Gender	% dosage	Bosutinib	M2	M4	M5	M6	M8	M9	M10	M14
Mice	Male	47.5	66.4	NA	8.1	14.3	ND	NA	NA	NA	1.6
Rats (8-24 hr)	Male	61.5	84.2	2.3	4.1	7.1	NA	NA	NA	NA	NA
	Female	36.5	82	0.9	8.6	8.0	NA	NA	NA	NA	NA
Dogs	Male	43.6	62.6	NA	NA	28.5	NA	NA	NA	NA	NA
Humans	Mlale (0-24 hr)[b]		40	1.6		22					

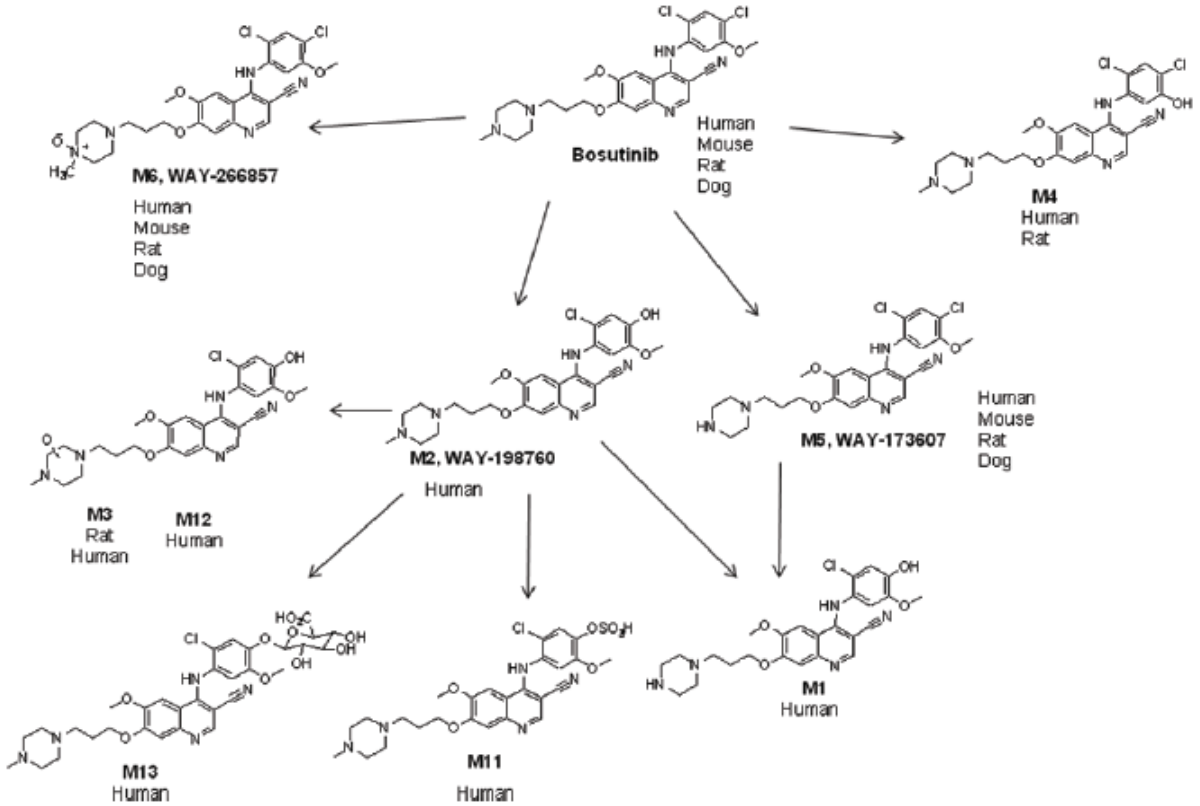
a: Study #RPT-70327; b: #RPT-79197

According to the Applicant, other metabolites of bosutinib observed by LC/MS in human plasma and/or urine include the following: M1, M3, M4, M11, and M12 in plasma, and M13 in plasma and urine.

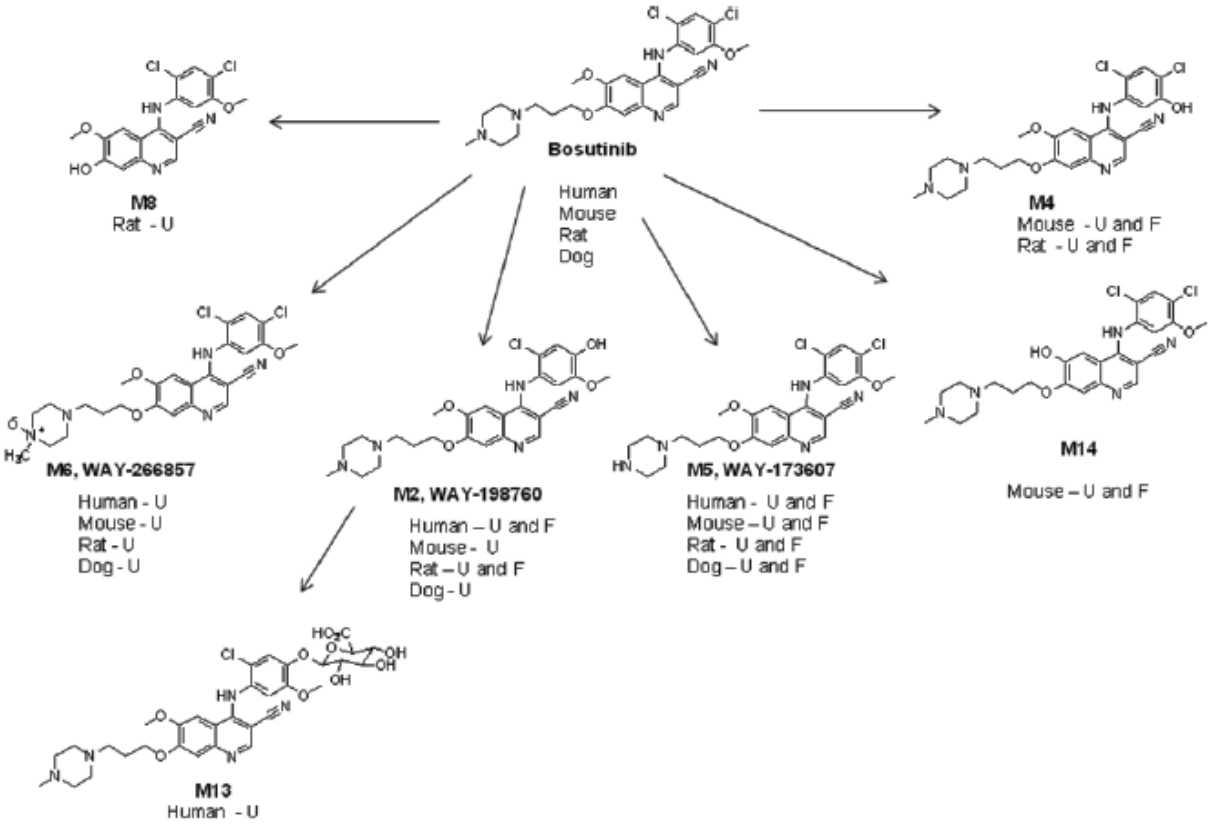
The results are also depicted in the figures:

Figure 9 Metabolite profiles in plasma and excreta

Plasma:



Excreta: urine (U) and feces (F)



- Metabolites in laboratory animals and humans:

Based on clinical trial data (CSR-76358; Study 3160A4-1106-US), the prominent metabolites for bosutinib in humans were oxydechlorinated bosutinib (M2) and *N*-desmethyl bosutinib (M5). The systemic exposure to M2 and M5 in healthy subjects given a single oral dose of 500 mg of bosutinib was 19% and 25% of the parent drug, respectively. The exposure comparisons for M2 and M5 in rats, dogs and humans are tabulated as follows (from the Applicant's submission):

Table 15 Comparison of metabolites in rats, dogs and humans

Species	----M2---- Observed Total AUC (ng•h/mL)	----M5---- Observed Total/Unbound AUC (ng•h/mL)	Ratio of AUC for M5 to Parent	Estimated Total[e]/Unbound[f] AUC at NOAEL (ng•h/mL)
Rats				
Male	929[a]	NA	0.34[c]	1295/80
Female	1488[a]	NA	NA	NA
Male Dogs			0.137[d]	
Day 1	NA	NA	NA	932/20
Day 273	NA	NA	NA	1132/24
Humans	502[b]	654[b]/36	NA	NA

AUC = Area under the concentration-versus-time curve; M2 = Oxydechlorinated bosutinib (WAY-198760 and PF-05898965); M5 = *N*-Desmethyl bosutinib (WAY-173607 and PF-05312061); NA = Not applicable; NOAEL = No-observed-adverse-effect level.

[a]RPT-74244.

[b]Values represent AUC(0-∞); CSR-76358, Protocol 3160A4-1106-US.

[c]Mean of ratios (AUC of M5:AUC of Parent) from Days 1, 28, and 56 (SP3810).

[d]Value obtained from the radiolabel study in male dogs (RPT-53089).

[e]Values calculated by multiplying the total AUC value of parent at the NOAEL by the ratio of AUC for M5 to parent (0.34, rat; 0.137, dog) in the corresponding species (total AUC values of parent at NOAEL were 3810 ng•h/mL in male rats in the 6-month study [RPT-64288], 6805 ng•h/mL in male dogs on Day 1 and 8265 ng•h/mL in male dogs on Day 273 in the 9-month study [RPT-66535]).

[f]Values calculated by multiplying the estimated total AUC value of M5 by the unbound fraction of M5 in plasma in the corresponding species (fu = 0.062, 0.021, and 0.055 in rats, dogs, and humans, respectively).

As noted, the exposures to M2 were not significant in rats and dogs following oral administration of bosutinib. Safety assessment of M2 was conducted in separate studies where synthesized metabolite M2 was used in general toxicology and genotoxicity studies (see below: Section 6.2 and Section 7.4, respectively). At up to 210 mg/kg/day when administered for 2 weeks, there were no remarkable M2-related toxicities in rats. Indicated in the table above, exposures to orally administered M2 (210 mg/kg) in rats were 2-3 fold higher than that in humans following a single 500 mg dose (~ 8.3 mg/kg for a 60 kg human) of bosutinib. Thus the PK data shows adequate exposure in the 2-week study in rats. The coverage of M5 in non-clinical studies was supported by the 6-month toxicology study in rats. Based on an investigative toxicology study (#SP3810; not reviewed), where male rats were administered 50 mg/kg bosutinib for 56 days, AUC values of M5 were 29-38% of parent (mean value of 34%). Thus the estimated AUC of M5 at NOAEL (30 mg/kg) of bosutinib in the 6-month toxicology study in rats (~1295 ng·h/mL: AUC of bosutinib: 3810 ng·h/mL x 0.34) was approximately 2-fold higher than the AUC of M5 in humans (i.e., 654 ng·h/mL).

Excretion:

The excretion of [^{14}C]bosutinib following oral doses of 50 mg/kg, 5 mg/kg and 500 mg in male SD rats (n=4), male Beagle dogs (n=4) and healthy male subjects (n=6), respectively, is summarized in tables below (from the Applicant):

Table 16 Excretion of [^{14}C]bosutinib in rats, dogs and humans

Rats (Study #RPT-53087):

Excretion Route:	% of Dosage (Mean ± SD) Urine	Feces	Cage Rinse
Time (h)			
0 – 8	0.03 ± 0.24	0.00 ± 0.00	ND
0 – 24	1.13 ± 0.38	81.56 ± 14.89	0.13 ± 0.06
0 – 48	1.22 ± 0.42	96.02 ± 2.08	0.16 ± 0.07
0 – 72	1.26 ± 0.43	97.19 ± 1.62	0.18 ± 0.07
0 – 96	1.28 ± 0.44	97.47 ± 1.57	0.18 ± 0.07
0 – 120	1.29 ± 0.44	97.60 ± 1.55	0.19 ± 0.07
Total Recovery (Urine+Feces+Cage Rinse, % of Dosage)[a]	99.08 ± 1.45		

ND: not determined

Dogs (Study #RPT-53089):

Excretion Route:	% of Dosage (Mean ± SD) Urine	Feces	Cage Rinse
Time (h)			
0 – 8	0.18 ± 0.14	ND	ND
0 – 24	0.43 ± 0.29	43.56 ± 30.92	1.32 ± 2.36
0 – 48	1.10 ± 0.42	63.56 ± 29.54	1.46 ± 2.55
0 – 72	1.22 ± 0.48	86.45 ± 4.87	1.52 ± 2.57
0 – 96	1.27 ± 0.50	89.52 ± 5.28	1.56 ± 2.62
0 – 120	1.28 ± 0.51	90.29 ± 5.45	1.58 ± 2.62
0 – 144	1.30 ± 0.51	90.82 ± 5.44	1.59 ± 2.63
0 – 168	1.31 ± 0.51	91.06 ± 5.44	1.60 ± 2.64
Total Recovery (Urine+Feces+Cage Rinse, % of Dosage)[a]	93.97 ± 2.98		

Humans (Study #RPT-79197):

Excretion Route:	% of Dosage (Mean ± SD) Urine	Feces
Time (h)		
0 - 4	0.491 ± 0.144	ND
0 - 8	0.909 ± 0.181	ND
0 - 12	1.566 ± 0.912	ND
0 - 24	2.177 ± 1.31	43.2 ± 11.1
0 - 48	2.639 ± 1.29	45.3 ± 7.47
0 - 72	2.914 ± 1.25	56.1 ± 14.4
0 - 96	3.068 ± 1.24	72.4 ± 17.1
0 - 120	3.126 ± 1.23	74.6 ± 18.0
0 - 144	3.127 ± 1.23	86.2 ± 8.55
0 - 168	3.191 ± 1.31	89.5 ± 8.53
0 - 192	3.273 ± 1.41	90.3 ± 8.90
0 - 216	3.291 ± 1.40	91.3 ± 8.39
Total Recovery (Urine+Feces, % of Dosage)[b]	94.6 ± 7.49	

As noted, the major route of elimination of radioactivity in rats, dogs and humans was the feces.

Pharmacokinetic drug interactions:

In brief, bosutinib up to 100 μM did not inhibit CYP1A2, 2A6 or 2C9 activity, and showed little inhibition on CYP2C8 activity at 200 μM. In a study using the approach of mechanism-based inhibition (i.e., positive result when the inhibition ≥ 20%) in the absence or presence of an NADPH regenerating system, bosutinib did not inhibit the activity of CYP2C9, 2C19, 2D6 or 3A4 (i.e., inhibition <20%). The mean steady state bosutinib C_{max} was 200 ng/mL (or 0.38 μM) (following a single oral dose of 500 mg); thus the ratios of C_{max}/Ki were less than 0.1 for CYP3A4, 2C19 and 2D6. In addition,. bosutinib, up to 9.5 μM, did not induce CYP enzymes at the mRNA level and enzyme activity levels. The concentration of 9.5 μM is ~25 fold of the mean steady state bosutinib C_{max} (see above);

Based on the study in Caco-2 monolayers, it was suggested that bosutinib may have the potential to affect the absorption and/or pharmacokinetics of drugs that are substrates of P-gp, such as digoxin.

5.2 Toxicokinetics

Table 17 Summary of toxicokinetics

Summary table of system exposures in the toxicology studies:
The following studies are reviewed in the General Toxicology section (Section 6), including repeat-dose toxicity (Section 6.2), genetic toxicity section (*in vivo* assay, Section 7.3), and reproductive and developmental toxicology (Section 9) studies. These studies are: in mice: Study #RPT-52501- SKI-606: Single dose oral (gavage) bone marrow micronucleus studying male mice; in rats, #RPT-63107- SKI-606: Oral (gavage) developmental toxicity study in rats, RPT-52772- 4-week oral (gavage) toxicity study in rats, and #RPT-63644- SKI-606: Six-month oral (gavage) toxicity study in rats; in rabbits: #RPT-62710SKI-606: Oral (gavage) developmental toxicity dose ranging study in mated rabbits, and #RPT-65533-Oral (gavage) developmental toxicity study in mated rabbits; in dogs: #RPT-52772- 4-week oral (gavage) toxicity study in dogs, and #RPT-65542- SKI-606- 9-month oral (gavage) toxicity study in FED dogs with a 28-day recovery. In addition, TK data of safety assessment of metabolite M2 (#RPT-74244: WAY-198760 (SKI-606 M2 metabolite): 14-day oral toxicity study in rats) are also included. See respective sections for reviews.
Reviewer's note: the exposure margin (i.e., ratio of animal to human exposure, C_{max} or AUC) = animal exposure (at dose [mg/kg]) divided by the human exposure (at 500 mg, not 8.3 mg/kg, i.e., not corrected by an average adult body weight of 60 kg)

Species (Duration)	Dose		Sex	C_{max} (ng/mL)		AUC (ng•h/mL)		Dose normalized C_{max}		Dose normalized AUC		Ratio of animal to human exposure	
	(mg/kg)	(mg/m^2)		Day 1	End of Study	Day 1	End of Study	Day 1	End of Study	Day 1	End of Study	C_{max}	AUC
Mice (single dose)	2000	6000	M	9811		172495		4.91		86.25		49.06	47.25
Rats§ (3-6 wks: F)	3	18	F		70.4		857		23.5		286	0.352	0.23
(7 wk: M)	30	180	M	154	156	1293	1686	2.2	5.2	18.47	56.2	0.78	1.22
Rats (GD 6-17)	1	6	F		17.3		204		17.3		204	0.0865	0.06
	3	18	F		70.4		857		23.5		286	0.352	0.23
	10	60	F		377		4463		37.7		446	1.885	1.22
Rat (28 day)	10	60	M F		31.0 126		318 1467		3.1 12.6		31.8 146.7	0.155 0.63	0.09 0.40
	30	180	M F	154 607	156 539	1293 5023	1686 5017	2.2 8.67	5.2 17.97	18.47 71.76	56.2 167.23	0.78 2.695	0.46 1.37

Species (Duration)	Dose		Sex	C_{max} (ng/mL)		AUC (ng•h/mL)		Dose normalized C_{max}		Dose normalized AUC		Ratio of animal to human exposure	
	(mg/kg)	(mg/m^2)		Day 1	End of Study	Day 1	End of Study	Day 1	End of Study	Day 1	End of Study	C_{max}	AUC
	70	420	M F		314 1500		4092 14090		4.49 21.43		58.46 201.29	1.57 7.5	1.12 3.86
Rat (6 month)	10	60	M F		84.5 394		807 4387		8.45 39.4		80.7 438.7	0.4225 1.97	0.22 1.20
	30	180	M F		346 1222		3810 15405		11.53 40.73		127 513.5	1.73 6.11	1.04 4.22
	100/ 70	600/42 0	M F		503 1474		7630 22692		7.19 21.06		109 324.17	2.515 7.37	2.09 6.22
Rabbits (GD 6-19) DRF	10	120	F		706		6235		70.6		623.5	3.53	1.71
	30	360	F		2325		20750		77.5		691.67	11.625	5.68
	60	720	F		3389		40293		56.48		671.55	16.945	11.04
Rabbits (GD 6-19)	3	36	F		129		1308		43		436	0.645	0.36
	10	120	F		505		5451		50.5		545.1	2.525	1.49
	30	360	F		1857		14002		61.9		466.73	9.285	3.84
Dog (4 week)	0.5	10	M F	13 16	16.7 20.8	149 154	229 232	26 32	33.4 41.6	298 308	458 464	0.0825 0.104	0.06 0.06
	1.5	30	M F	50.4 52.3	77.6 68.8	758 599	985 824	33.6 34.87	51.73 45.87	505.33 399.33	656.67 549.33	0.388 0.344	0.27 0.23
	5	100	M F	257 177	364 291	2954 2405	3888 3450	51.4 35.6	72.8 58.2	590.8 481	777.6 690	1.82 1.455	1.07 0.95
Dog (9 month)	1	20	M F	34.2 38.9	34.9 66.4	396 506	575 823	34.2 38.9	34.9 66.4	396 506	575 823	0.1745 0.332	0.16 0.23
	3	60	M F	142 154	133 160	1670 1686	2008 2372	47.33 51.33	44.33 53.33	556.67 562	669.33 790.67	0.665 0.8	0.55 0.65
	10	200	M F	652 419	583 513	6805 4891	8265 6657	65.2 41.9	58.3 51.3	680.5 489.1	826.5 665.7	2.915 2.565	2.26 1.82
Human* (15 day)	8.3**	312.5	M/F	----	200 (T) /12.6 (U)	----	3650 (T)/230 (U)	----		----		Not Applicable	Not Applicable
M2 metabolite													
Rat (14 day)	70	420	M F		36.8 110		354 631		0.53 1.57		5.1 9		

Species (Duration)	Dose		Sex	C_{max} (ng/mL)		AUC (ng•h/mL)		Dose normalized C_{max}		Dose normalized AUC		Ratio of animal to human exposure	
	(mg/kg)	(mg/m^2)		Day 1	End of Study	Day 1	End of Study	Day 1	End of Study	Day 1	End of Study	C_{max}	AUC
	210	1260	M F		93.8 120		929 1488		0.45 0.57		4.4 7.1	3.3 4.2	1.85 2.96
Human*** (single dose)	8.3	312.5			28.4		502					Not Applicable	Not Applicable

*** Clinical trial #3160A-100_US; *** Clinical trial #3160A4-1106_US**
**** At dose of 500 mg, based on a theoretical body weight of 60 kg (~1.6 m^2), the dose was 8.3 mg/kg (312.5 mg/m^2).**
§ TK parameters were not determined in Study #PRT-63257: SKI-606: Oral (gavage) fertility study in rats. Data shown in table for females at 3 mg/kg (3-6 wk) and males at 30 mg/kg(7 wk) were referred to the AUC values of female and male rats at the same dosage in Study #RPT-63107 and Study #RPT-52772, respectively.
The AUC values indicated were total AUC values in the respective species, unless specified, i.e., (T): Total, (U): Unbound
The TK parameters in the reproductive and developmental toxicology studies in rats and in rabbits were taken on GD 15.

6 General Toxicology

6.1 Single-Dose Toxicity

Not reviewed. Summary:

6.2 Repeat-Dose Toxicity

Rats:
Reviewer's note: the result of 4-week repeat dose toxicology study (Study# RPT-52772, GLP study) (TK study: #RPT-52934) was summarized by Dr. Leigh Verbois under IND 68268. The following summary is excerpted from Dr. Verbois' report:
SKI-606 was administered orally by gavage to Sprague Dawley rats at dosages of 0, 60, 180 or 420 mg/m^2/day (i.e., 0, 10, 30 and 70 mg/kg/day) for four weeks. Doses were selected based on mortality in a previous 7-day dose-range finding study in which severe dehydration, weight loss, and decreased food consumption resulted in early euthanasia in animals treated with 1800 mg/m^2/day (i.e., 300 mg/kg/day). Additionally, animals treated with 600 mg/m^2/day (100 mg/kg/day) exhibited similar yet less severe clinical signs. Upon necropsy, animals in the dose-range finding study that received ≥600 mg/m^2/day had evidence of gastrointestinal toxicity (distention of the GI, and slight to moderate hypertrophy/hyperplasia of the large intestine) and discoloration of the mesenteric lymph nodes.

Evaluations in the 28 day study consisted of mortality, clinical observations, body weight, food consumption, ophthalmoscopy, hematology, clinical chemistry, organ weights, and macro/microscopic examinations. Toxicokinetics were evaluated in satellite groups on Day 1 (70 mg/kg/day only, or 420 mg/m^2/day) and on Day 28 (all dose groups). In this study there were no compound related deaths. One vehicle control male and 1 HD female were found dead after blood collection and gavage, respectively. Hemorrhage and gavage error were evident in these animals respectively. Unlike the dose-range finding study, no dose dependent clinical signs, effects on body weight or food consumption were noted. At the end of the treatment period, incidences of abnormal findings in ophthalmoscopy were limited to single females that received 180 and 420 mg/m^2/day. Chromodacryorrhea, choroidal vascular anomaly, and corneal opacity with vascularization were each observed in single female that received 180 or 420 mg/m^2/day*. Additional drug dependent abnormalities in in-life assessments were dose dependent decreases in reticulocytes (20-50%, Days 6 and 27) and increases in fibrinogen (5-26%, Day 27) in both males and females and increases in T3 (~30- 50%, Days 6 and 27), increases in T4 (5-35%, Days 6 and 27) and decreases in TSH (35-66%, Day 6) in males**. Alterations in organ weight [Prostate (↓) and spleen (↑) in males, in the heart (↑) of males and females, and in the thyroid (↑) of females] were not correlated with drug dependent changes in in-life assessments and were small in magnitude (≤10%).

**Reviewer's note: the ocular findings in Week 4 were: Chromodacryorrhea (#31, 180 mg/m^2), choroidal vascular anomaly (#147, 420 mg/m^2) and corneal opacity with vascularization (#151, 420 mg/m^2). According to the veterinary ophthalmologist, these findings were considered incidental and not related to treatment with SKI-606.*
***Reviewer's note: the elevation of T3 and T4 levels on Days 6 and 27 in males were not dose-related, although a dose-dependent decrease of TSH was noted on Day 6. The reviewer considered the thyroid hormone findings unlikely to be toxicologically significant, based on: the finding was only in males, the reduction of TSH was transient and the elevation of T3/T4 was not dose-related; furthermore, there was no anatomical pathology evidence in the thyroid (although ↑thyroid weight in females).*

Drug-dependent macroscopic findings were limited to the liver, spleen, and eye (1 HD female). Findings were described as a focus in the liver and spleen and opaque areas in the eye. These findings occurred concomitantly with capsular fibrosis of the liver and spleen and centrilobular hypertrophy of the liver of one animal. Additional findings in the liver included bile duct hyperplasia in one high dose male. Additional dose-dependent findings included sinus erythrocytosis and hemosiderosis of the mesenteric lymph nodes, and an increased incidence of mixed cell inflammation with eosinophilic crystals in the lungs. A single histological measure of gastrointestinal toxicity was described as edema of the stomach in one male treated at 420 mg/m^2.

Toxicokinetic analysis indicated that exposure to SKI606 increased with increasing dosage in both males and females between Day 1 and Day 28 of oral administration, however the increase was greater than dose proportional in females (the dose-normalized AUC value at 70 mg/kg was significantly greater than that at 10 mg/kg). Exposure (AUC/dose) on Day 28 was significantly higher in females than in males (see table below). There was no evidence of accumulation, based on AUC exposures Day 1 and Day 28 at 30 mg/kg.

Table 18 Toxicokinetic parameters: 4-week study in rats

Day	Dose (mg/kg)	Dose (mg/m^2)	Gender	C_{max} (ng/mL)	AUC_{0-24} (ng·hr/mL)	AUC_{0-24}/dose	T_{max} (hr)
1	30	180	M	154	1293	43	3
	30	180	F	607	5023	168	3
28	10	60	M	31	318	32	5
	10	60	F	126	1467*	147*	5
	30	180	M	156	1686	56	5
	30	180	F	539	5017 *	167*	5
	70	420	M	314	4092	59	5
	70	420	F	1500	14090 *§	201*§	5

*** Statistically significant different (i.e., increased AUC values in females compared to those in males); §: AUC/dose at 70 mg/kg was statistically greater than that at 10 mg/kg.**

Study title: SKI-606: Six-month oral (gavage) toxicity study in rats

Study no.:	RPT-63644 (Protocol 05_1716)
Study report location:	Wyeth
Conducting laboratory and location:	Wyeth European Drug Safety & Metabolism Research Center, Catania, Italy
Date of study initiation:	September 6, 2005
GLP compliance:	The Applicant claimed GLP compliant: with signature pages attached, but without a QA page
QA statement:	Yes
Drug, lot #, and % purity:	Bosutinib (SKI-606), lot#RB5626, 95.6% (largest single impurity: 0.07%)

Key Study Findings

- Orally (gavage) administered SKI-606 at 10, 30 and 100→70 mg/kg/day in CD rats for 6 months induced dose-related mortality in males and females and GI-related clinical signs. The target organs were GI tract (mainly small intestine) and lymphoid tissues (mainly mesenteric lymph nodes, and spleen and thymus), The hematological and clinical chemistry findings correlated with inflammation in multiple organs.
- Dose reduction (from 100 mg/kg to 70 mg/kg) took place on Day 43, due to mortality and severe clinical signs. The cause of death was GI toxicities.
- The systemic exposures to SKI-606 were increased with increased doses in a dose-proportional fashion. Exposures to SKI-606 in females were greater than those in male rats (3 to 5 fold). The higher exposure explains the more severe toxicities (e.g., mortality) in females.

Methods

Doses:	0 (control), 10, 30, *100→70 mg/kg/day as Groups 1, 2, 3, and 4, respectively.
Frequency of dosing:	Once daily
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (4000 cps), 2% polysorbate 80 (NF), 0.06% glacial acetic acid and sterile water for injection
Species/Strain:	Rats (Crl:CD (SD))
Number/Sex/Group:	Main study Groups: 20; Toxicokinetic Groups: 9 (except for n=3 in the Control group); note: no recovery groups
Age:	~ 7 weeks
Weight:	143-241 g
Satellite groups:	Toxicokinetics groups
Unique study design:	Not remarkable

Deviation from study protocol: Not remarkable
***Beginning on Day 43, the dosage was decreased to 70 mg/kg/day for the remainder of the study.**

Observations and Results

Clinical signs:	At least twice daily for mortality, moribundity and gross abnormality during pretest (Day -8) and dosing period and once for animals scheduled for euthanasia. Detailed examinations were conducted twice pretest (on Days -8 and -1) in all animals; once weekly to Week 13, then monthly and the last week (in Groups 1-4, not TK animals).
Body weight:	Twice pretest (on Days -8 and -1) in all Groups (main study and TK groups); once weekly to Week 13, then once every 2 weeks thereafter, and the last week.
Food consumption:	In main study groups (Groups 1-4): Once in the week (Days -7 to -1) prior to treatment initiation, and once weekly to Week 13, then once every 2 weeks thereafter, and the last week.
Ophthalmology:	Once pretest and during Week 25, in Groups 1-4 only.
Hematology:	In main study groups (Groups 1-4): Weeks 13 (Days 90-91) and 26 (Days 176-177).
Clinical chemistry:	See “hematology”.
Urinalysis:	Not conducted.
Bone marrow smears:	From all animals in Groups 1-4; samples prepared but not evaluated.
Gross pathology:	At scheduled sacrifice in all animals.
Organ weights:	At scheduled sacrifice in all animals. The following organs were weighted: adrenal, brain, heart, kidneys, liver, ovaries, pituitary, prostate, testes, and thyroid with parathyroid.
Histopathology:	At scheduled sacrifice. All tissues collected from all animal. All fixed organs and tissues from the vehicle-control group, high dosage group, and found dead rats, as well as lung (females only), heart, pancreas, kidneys, adrenal cortex, duodenum, jejunum, ileum, mesenteric lymph nodes, and ovaries and macroscopic findings from the other groups, were processed and examined microscopically. In addition, cecum, spleen (females only), thyroid (females only), thymus (females only), and mammary gland (females only) from the mid dosage group were processed and examined microscopically. Lung (males only), liver, spleen (males only) from the remaining groups were processed but not examined microscopically. See inventory list for organs examined.
Toxicokinetics:	Blood samples, 0.5 and 1 mL, were collected on Day 180, at 1, 3, 5, 7, 10 and 24 hours after dosing (n=2/sex/ group/time point). The control group was only sampled at 5 hours after doing on Day 180. Concentrations of SKI-606 were analyzing via LC/MS/MS. Lower limit of quantification (LLOQ): 5 ng/mL using 0.2 mL of rat plasma. (Study #RPT-64288)

Mortality

There were treatment-related mortalities mainly at doses ≥ 30 mg/kg. Dosing (gavage) and procedure related deaths occurred in all groups, including the control. Mortality at the initial dose 100 mg/kg and the reduced dose 70 mg/kg was comparable. Deaths due to SKI-606 related toxicity (GI toxicities) were more in females, possibly due to higher systemic exposure. The Applicant summarized the incidence, days and cause of death in the table below:

Table 19 Mortality: 6-month study in rats

	Male				Female			
	Dosage (mg/kg/day)				Dosage (mg/kg/day)			
Finding	0	10	30	100/70[a]	0	10	30	100/70
Unscheduled deaths								
Days 1-42	1	1	0	1	0	0	0	3
Days 43-176	2	1	2	2	0	0	1	4
Total[b]	3	2	2	3	0	0	1	7
Cause of death:								
Gavage trauma	2	1	2	-	-	-	-	-
Trauma	1	-	-	-	-	-	-	-
Blood collection procedure	-	-	-	-	-	-	-	1
SKI-606-related gastro-intestinal toxicity	-	-	-	3	-	-	1	6
Undetermined, not SKI-606-related	-	1	-	-	-	-	-	-

a. Dosage of 100 mg/kg/day was lowered to 70 mg/kg/day at Day 43.
b. Toxicokinetics and toxicology groups combined.

The Group 2 male (10 mg/kg) died on Day 101. The cause of this death was not determined, but was not considered SKI-606-related because of the absence of SKI-606-related clinical signs, macroscopic or microscopic observation.

The description of in-life observations and pathological findings in SKI-606-induced deaths is included in relevant sections below.

Clinical Signs

The high dose group animals were initially administered at 100 mg/kg according to the protocol. On Day 43 the dose was reduced to 70 mg/kg/day, due to reduced body weight (~7-9%) and food consumption (~3-4%) and the following clinical signs observed (starting from Week 3): soft/liquid feces, salivation, red pigment around nose/mouth, rough hair coat, feces adhered to fur, yellow discoloration of perineal pelage, and alopecia in the neck/thorax.

The table below is the summary of clinical signs:

Table 20 Clinical signs: 6-month study in rats

Males: results of detailed observation in main study males were included in the table.

Group	1	2	3	4	1 (TK)	2 (TK)	3 (TK)	4 (TK)
Dose (mg/kg)	0	10	30	100→70	0	10	30	100→70
Number	20	20	20	20	3	9	9	9
Scheduled deaths	17	19	18	19	3	8	9	7
Found dead	3	1	2	1	0	1	0	2
Appearance								
Red pigment around eye; bilateral								1
Red pigment around nose/mouth				2				
Reddened area around mouth				1				
Salivation				1				
Feces								
No feces			1					
Liquid feces, slight				17				8
Soft feces				4				3
Hair								
Alopecia	6	9	3	1				
Rough hair coat				1				1

Females:

Group	1	2	3	4	1 (TK)	2 (TK)	3 (TK)	4 (TK)
Dose (mg/kg)	0	10	30	100→70	0	10	30	100→70
Number	20	20	20	20	3	9	9	9
Scheduled deaths	20	20	19	14	3	9	9	8
Found dead	0	0	1	6	0	0	0	1
Activity and behaviors								
Decreased motor activity, slight to marked			1	3				
Appearance								
Dyspnea				1				
Hunched appearance				3				1
Pale appearance			1	3				
Red pigment around eye; bilateral			1					
Red pigment around nose/mouth				17				8
Reddened area around mouth				11				6
Salivation				9				5
Swollen area				3				
Yellow discoloration of perineal pelage				13				4
Eyes								
Ptosis; unilateral right				1				
Red pigment around eye(s); unilateral right			1					1
Feces								
Decreased feces			1					
No feces		1	1	3				
Liquid feces, slight to marked				22			2	9
Soft feces				10				5
Hair								
Alopecia				16				14
Feces adhered to fur				6				3
Rough hair coat				14				7
Discolored urine; red			1	1				

Detailed observation in main study group females:

Group	1	2	3	4
Dose (mg/kg)	0	10	30	100→70
Number	20	20	20	20
Appearance				
Crusts; tail				4
Red pigment around nose/mouth				1
Thin appearance				3
Yellow discoloration of perineal pelage				15
Eye				
Injured; unilateral		1	1	1
Hair				
Alopecia	6	4	2	8
Feces adhered to fur				1

Findings in the pre-scheduled deaths:
The findings were mainly GI-toxicity and related signs: body weight loss, rough hair coat, decreased motor activity, hunched and pale appearance, liquid feces, feces adhered to fur, yellow discoloration of perineal pelage and pigment around mouth and nose.

Body Weights

Statistically significant reduction in group mean body weight was observed only in Group 4 through out the 6 month period.

Table 21 Group mean body weight and weight gain: 6-month study in rats

Body weight reduction in Group 4 male and female rats: % reduction from the control

	D14	D21	D28	D35	D42	D49	D56	D63	D70	D77
Males	5	6	6	9	10	10	9	10	11	11
Females	NS	NS	NS	7	10	8	5	6	6	8
	D84	D91	D105	D119	D133	D147	D161	D175	D182	
Males	12	12	13	15	16	16	17	19	18	
Females	8	10	8	NS	12	13	12	14	12	

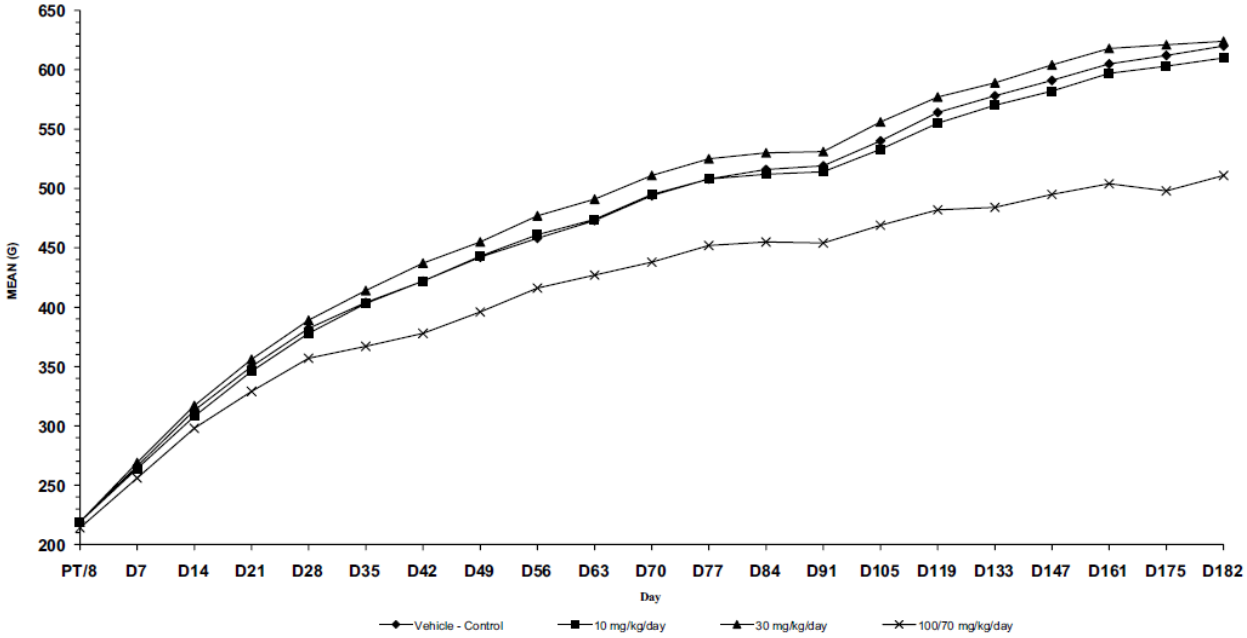
NS: changes not reach statistical significance.

Net body weight gains: % reduction from the control also included in the parentheses.

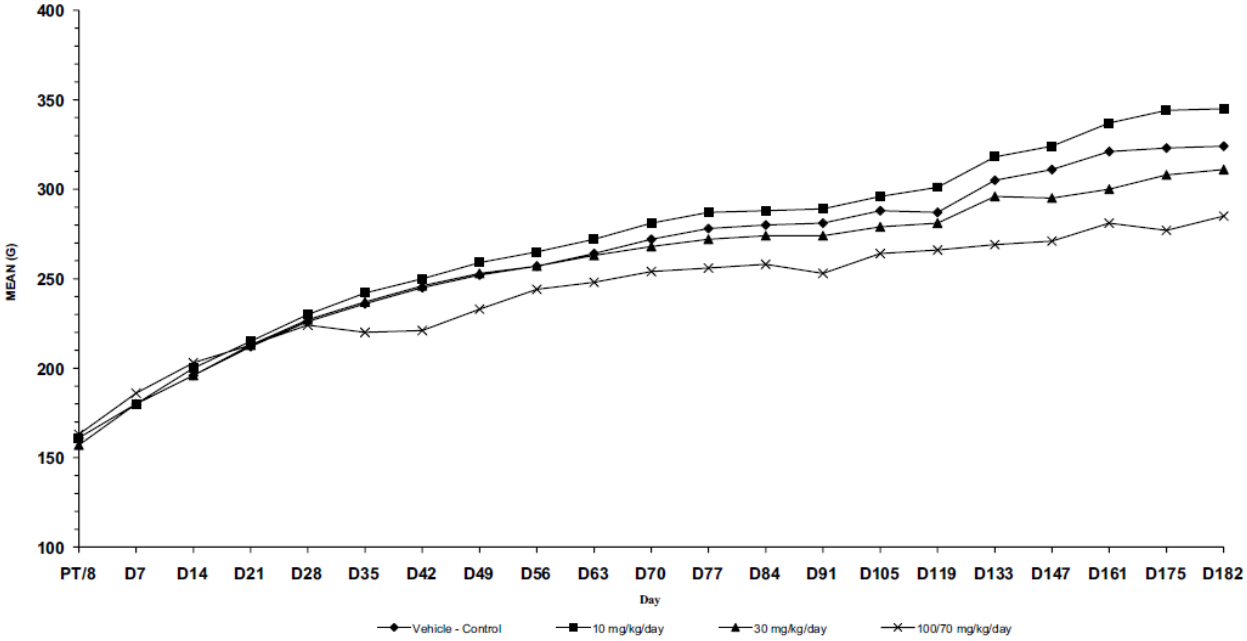
	Males		Females	
	Group 1 (control)	Group 4 (100→70)	Group 1 (control)	Group 4 (100→70)
Day-8 to Day91	301 g	241 g (↓ 20%)	120 g	90 g (↓ 25%)
Day-8 to Day182	401 g	298 g (↓ 26%)	163 g	121 g (↓ 26%)
Day91 to Day182	98 g	57 g (↓ 42%)	43 g	31 g (↓ 28%)

Figure 10 Group mean body weight: 6-month study in rats

Group mean body weights: figures from the Applicant
Males:



Females:



Feed Consumption

Only transient reduction in Week 19 (↓ 7-8%) in males was observed, the reduction recovered by Week 26.

Ophthalmoscopy

Not remarkable

ECG

Not conducted.

Hematology

Table 22 Hematology parameters: 6-month study in rats

Hematology parameters on Days 90 and 176 (% changes from the control), and gains (from Day 90 to Day 176, + greater gain, - less gain than gains found with the control):

	Males						Females					
Group	Group 3			Group 4			Group 3			Group 4		
Dose (mg/kg)	30			70			30			70		
Days	90	176	Gain	90	176	Gain	90	176	Gain	90	176	Gain
N	19	18	18	19	19	19	20	19	19	17	15	15
Retic (Ab) ↑					14	+	15	8		22	41	
RBC ↓	↑ 3			↑ 3		-		3			5	-
HGB ↓	↑ 4			↑ 3			4	6		7	10	-
Hct ↓	↑ 4		-	↑ 3		-	2	4		6	8	-
MCV ↓										4	3	+
MCH ↓							3	3		5	5	
MCHC ↓							2	1		1	2	
RDW ↑				6		+	4	5		11	19	+
Platelets ↑	7	12		19	20		16	20		49	42	
MPV ↑											4	
WBC ↑				32		+				61	133	+
Lymph (Ab) ↑						+						+
Neut (Ab) ↑	28	32§		104	190	+	33	102	+	513	894	+
Mono (Ab) ↑				72	92	+	33	51	+	238	284	+
Eos (Ab) ↑	67	56		49	88		59	61		191	188	
Baso (Ab) ↑										82	87	
LUC ↑										48	140	+
PT ↑				6	4			3			7	+
Fibrinogen ↑	5	18		15	19		30	36	+§	59	89	+§

§NS: Not statistically significant changes from the control.

The hematological findings were mainly at doses ≥ 30 mg/kg and primarily correlated with SKI 606-induce inflammation, with greater magnitude in changes in female rats than in males. Reduced red cell mass may be due to suppression of hematopoiesis secondary to profound inflammation, as well as to drug's effect on bone marrow, since hypocellularity was found in the high dose group, especially those died prematurely.

Clinical Chemistry

Table 23 Clinical chemistry parameters: 6-month study in rats

Clinical chemistry parameters on Days 90 and 176 (% changes from the control), and gains (from Day 90 to Day 176, + greater gain, - less gain than gains found with the control):

	Males						Females					
Group	Group 3			Group 4			Group 3			Group 4		
Dose (mg/kg)	30			70			30			70		
Days	90	176	Gain	90	176	Gain	90	176	Gain	90	176	Gain
N	19	18	18	19	19	19	20	19	19	17	15	15
ALP ↓		17		27	34							
Total Protein ↓				7	9				-	9	18	-
Albumin ↓				8	10			7	-	22	34	-
Globulin ↑		7	+	↓ 6			9	11		20	20	
A/G ↓			-		3	-	10	17	-	34	44	-
BUN ↑					16	+					11§	+§
Creatinine ↓										17	16	
B/CR ↑	↓ 9				17	+				33	31	
Cholesterol ↓		11		22	24		17	30	-	23	34	-
Triglyceride ↓	21			39	25	+		16§			19§	-
Calcium ↑		2			4	+		2		↓ 4		
Phosphorus ↓		9		23	25					25	16	+

§NS: Not statistically significant changes from the control.

The main findings occurred mainly at ≥ 30 mg/kg. Decreased albumin and increased globulin, and hence decreased A/G ratio, were in line with inflammation, as indicated in hematological findings above. Slight increases in BUN and decreased creatinine resulted in increased BUN/creatinine ratio. The GI toxicities and decreased body weight may suggest malnutrition in these animals, which may also be attributable to decreased total protein levels. However, there was no apparent reduction in food intake, it is not sure that these animals were with malnutrition.

Urinalysis

Not conducted.

Gross Pathology

The findings were mainly in the SKI-606 target organs, i.e., GI tract (small intestine: distended, thickening wall) and lymphoid organs (mesenteric lymph node: enlarged and discoloration). These findings are summarized in the following table (from the Applicant):

Table 24 Gross pathology: 6-month study in rats

Finding	Male Dosage (mg/kg/day) 0	10	30	100/70	Female Dosage (mg/kg/day) 0	10	30	100/70
Number Examined	17	19	18	19	20	20	19	14
Duodenum								
Distended	0	0	0	8	0	0	0	7
Thickened wall	0	0	0	11	0	0	0	7
Jejunum								
Distended	0	0	0	2	0	0	0	0
Thickened wall	0	0	0	10	0	0	0	4
Ileum								
Distended	0	0	0	4	0	0	0	2
Thickened wall	0	0	0	11	0	0	0	6
Mesenteric Lymph Nodes								
Enlarged	0	0	1	11	0	0	0	0
Discoloration	0	3	17	19	0	3	17	13
Thyroids								
Enlarged	0	0	2[a]	0	0	0	0	3

a. Not considered SKI-606-related as there were no microscopic findings and not dose responsive.

The findings in the pre-scheduled deaths (not included in the table) were similar. They were described as the following (excerpted from the Applicant; verified by the reviewer based on individual data): red mucosal foci and diffuse mucosal discoloration in the gastrointestinal tracts; abnormal contents in both small and large intestines, described in most cases as fluid or mucoid; distension of stomach and/or intestine; discoloration of the mesenteric lymph nodes; small spleen, and thymus. Small thymus was also found in few scheduled necropsy Group 4 animals (1 male and 2 females). Thickening wall was seen in colon of one Group 4 male sacrificed on schedule.

Organ Weights

Summary in the following table was excerpted from the Applicant. The reviewer occurs with the content. Data were expressed as % REF, using the vehicle control as the reference (REF, 100%).

Table 25 Organ weights: 6-month study in rats

		Male					Female				
	Dosage (mg/kg/day)	N[a]	G[b]	% REF[c]	% REF (TBW)[d,e]	% REF (BNW)[f]	N	G	% REF	% REF (TBW)	% REF (BNW)
Adrenals	0	17	0.054	REF	REF	REF	20	0.066	REF	REF	REF
	10	19	0.054	100	101	102	20	0.070	106	99	107
	30	18	0.059	110	109	110	19	0.067	101	105	104
	100/70	19	0.062	115[h]	141[i]	118[i]	14	0.088	133[i]	154[i]	138[i]
Heart	0	17	1.708	REF	REF	REF	20	1.176	REF	REF	REF
	10	19	1.829	107[i]	108[i]	109[i]	20	1.277	109[i]	103	110[i]
	30	18	2.030	119[i]	118[i]	119[i]	19	1.337	114[i]	119[i]	117[i]
	100/70	19	1.866	109[i]	134[i]	112[i]	14	1.466	125[i]	144[i]	130[i]
Liver	0	17	14.335	REF	REF	REF	20	8.000	REF	REF	REF
	10	19	14.476	101	102	103	20	8.854	111[i]	105[i]	112[i]
	30	18	15.756	110	109[i]	110	19	8.909	111[i]	116[i]	114[i]
	100/70	19	14.873	104	128[i]	106	14	10.878	136[i]	158[i]	142[i]
Ovaries	0	-	-	-	-	-	20	0.119	REF	REF	REF
	10	-	-	-	-	-	20	0.129	108	102	110[h]
	30	-	-	-	-	-	19	0.157	131[i]	137[i]	135[i]
	100/70	-	-	-	-	-	14	0.174	146[i]	165[i]	153[i]
Pituitary	0	17	0.011	REF	REF	REF	20	0.018	REF	REF	REF
	10	19	0.012	111[h]	112[i]	113[i]	20	0.019	110	103	111
	30	18	0.012	113[h]	112[i]	113[i]	19	0.015	87[i]	91[h]	89
	100/70	19	0.013	115[h]	142[i]	118[i]	14	0.014	76[i]	87[h]	79[i]
Testes	0	17	3.627	REF	REF	REF	-	-	-	-	-
	10	19	3.772	104	105	106	-	-	-	-	-
	30	18	3.843	106	105	106	-	-	-	-	-
	100/70	19	3.907	108[h]	132[i]	111[h]	-	-	-	-	-
Thyroids	0	17	0.023	REF	REF	REF	20	0.017	REF	REF	REF
	10	19	0.019	83[g]	85[g]	85	20	0.015	89	85[g]	91
	30	18	0.024	106	106	105	19	0.020	117[i]	122[i]	119[i]
	100/70	19	0.023	103	126[i]	105	14	0.028	165[i]	194[i]	173[i]
Brain[j]	0	17	2.216	REF	REF	-	20	2.029	REF	REF	-
	10	19	2.178	98	99	-	20	2.005	99	93	-
	30	18	2.217	100	99	-	19	1.982	98	102	-
	100/70	19	2.164	98	119[i]	-	14	1.947	96[h]	110[i]	-

a. Number of animals.
b. Mean absolute weight in grams.
c. % of reference group (controls).
d. Terminal body weight.
e. % of body weight values for adrenals, ovaries, pituitary and thyroids are multiplied by 100.
f. Brain weight.
g. Mean absolute or relative weights statistically significant (Pairwise $p \leq 0.05$).
h. Mean absolute or relative weights statistically significant (Trend $p \leq 0.05$).
i. Mean absolute or relative weights statistically significant (Trend and Pairwise $p \leq 0.05$).
j. Data for brain and terminal body weights included in the table for reference purposes to facilitate interpretation of organ to body and organ to brain weight ratios.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings: target organs including GI tract, lymphoid tissues, adrenal, thyroid and mammary glands (females)

Table 26 Histopathological findings: 6-month study in rats

Scheduled necropsy:
GI tract: small intestine

	Male Dosage (mg/kg/day)				Female Dosage (mg/kg/day)			
Finding	0	10	30	100/70	0	10	30	100/70
Duodenum[a]	17	19	18	19	20	20	19	14
Mucosal hyperplasia	0	0	1	16	0	0	6	13
Slight	0	0	1	14	0	0	6	12
Mild	0	0	0	2	0	0	0	1
Average Severity	(0.0)	(0.0)	(0.1)	(0.9)	(0.0)	(0.0)	(0.3)	(1.0)
Lumenal dilatation	0	0	0	9	0	0	0	10
Slight	0	0	0	6	0	0	0	9
Mild	0	0	0	3	0	0	0	1
Average Severity	(0.0)	(0.0)	(0.0)	(0.6)	(0.0)	(0.0)	(0.0)	(0.8)
Hemorrhage	0	0	0	11	0	0	0	2
Slight	0	0	0	11	0	0	0	2
Average Severity	(0.0)	(0.0)	(0.0)	(0.6)	(0.0)	(0.0)	(0.0)	(0.1)
Jejunum[a]	17	19	18	19	20	20	19	14
Mucosal hyperplasia	0	0	0	11	0	0	0	1
Slight	0	0	0	11	0	0	0	1
Average Severity	(0.0)	(0.0)	(0.0)	(0.6)	(0.0)	(0.0)	(0.0)	(0.1)
Lumenal dilatation	0	0	0	1	0	0	0	0
Slight	0	0	0	1	0	0	0	0
Average Severity	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)	(0.0)	(0.0)	(0.0)
Hemorrhage	0	0	0	1	0	0	0	0

Slight	0	0	0	1	0	0	0	0
Average Severity	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)	(0.0)	(0.0)	(0.0)
Ileum[a]	17	19	18	19	20	20	19	14
Goblet cell hypertrophy/ hyperplasia	4	16	15	19	1	17	17	12
Slight	4	16	15	16	1	17	17	11
Mild	0	0	0	3	0	0	0	1
Average Severity	(0.2)	(0.8)	(0.8)	(1.2)	(0.1)	(0.9)	(0.9)	(0.9)
Lumenal dilatation	0	0	0	6	0	0	0	3
Slight	0	0	0	6	0	0	0	3
Average Severity	(0.0)	(0.0)	(0.0)	(0.3)	(0.0)	(0.0)	(0.0)	(0.2)
Hemorrhage	0	0	2	7	0	0	0	0
Slight	0	0	2	7	0	0	0	0
Average Severity	(0.0)	(0.0)	(0.1)	(0.4)	(0.0)	(0.0)	(0.0)	(0.0)

a. Number examined

( ). Average severity (number affected/total number examined; 0 = no microscopic finding, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe).

Lymphoid tissues:

	Male Dosage (mg/kg/day)				Female Dosage (mg/kg/day)			
Finding	**0**	**10**	**30**	**100/70**	**0**	**10**	**30**	**100/70**
Mesenteric Limph Nodes[a]	17	19	18	19	20	20	19	14
Sinus erythrocytosis	3	10	17	19	1	7	19	14
Slight	3	6	2	0	1	4	1	2
Mild	0	3	3	4	0	2	8	4
Moderate	0	0	4	3	0	1	7	6
Marked	0	1	8	12	0	0	3	2
Average Severity	(0.2)	(0.8)	(2.9)	(3.4)	(0.1)	(0.6)	(2.6)	(2.6)
Pigment (Hemosiderin)	0	7	18	19	1	4	18	14
Slight	0	4	3	1	1	3	2	4
Mild	0	3	3	3	0	1	0	5
Moderate	0	0	4	3	0	0	5	3
Marked	0	0	7	10	0	0	10	1
Severe	0	0	1	2	0	0	1	1
Average Severity	(0.0)	(0.5)	(3.0)	(3.5)	(0.1)	(0.3)	(3.3)	(2.3)

Spleen[a]	17	0	0	19	20	0	19	14
Lymphoid atrophy (marginal zone)	0	0	0	0	0	0	0	9
Slight	0	0	0	0	0	0	0	9
Average Severity	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.6)
Thymus[a]	17	0	0	19	20	0	19	14
Lymphoid atrophy	0	0	0	2	3	0	0	9
Slight	0	0	0	2	1	0	0	4
Mild	0	0	0	0	2	0	0	3
Marked	0	0	0	0	0	0	0	1
Severe	0	0	0	0	0	0	0	1
Average Severity	(0.0)	(0.0)	(0.0)	(0.1)	(0.3)	(0.0)	(0.0)	(1.4)

Adrenal gland:

	Male				Female			
	Dosage (mg/kg/day)				Dosage (mg/kg/day)			
Finding	**0**	**10**	**30**	**100/70**	**0**	**10**	**30**	**100/70**
Adrenal Cortex[a]	17	19	18	19	20	0	19	14
Vacuolation	5	2	7	18	0	0	0	11
Slight	5	2	6	5	0	0	0	8
Mild	0	0	1	11	0	0	0	2
Moderate	0	0	0	2	0	0	0	1
Average Severity	(0.3)	(0.1)	(0.4)	(1.7)	(0.0)	(0.0)	(0.0)	(1.1)
Hypertrophy	0	0	0	0	0	0	0	4
Slight	0	0	0	0	0	0	0	3
Mild	0	0	0	0	0	0	0	1
Average Severity	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.4)

Thyroid:

	Male				Female			
	Dosage (mg/kg/day)				Dosage (mg/kg/day)			
Finding	**0**	**10**	**30**	**100/70**	**0**	**10**	**30**	**100/70**
Thyroids[a]	17	0	2	19	20	0	19	14
Increased colloid	0	0	0	0	0	0	0	7
Mild	0	0	0	0	0	0	0	5
Moderate	0	0	0	0	0	0	0	2
Average Severity	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(1.1)

Mammary gland:

Finding	Male Dosage (mg/kg/day) 0	10	30	100/70	Female Dosage (mg/kg/day) 0	10	30	100/70
Mammary gland[a]	14	0	0	19	20	0	18	14
Atrophy	0	0	0	0	1	0	1	6
Slight	0	0	0	0	1	0	1	2
Mild	0	0	0	0	0	0	0	3
Moderate	0	0	0	0	0	0	0	1
Average Severity	(0.0)	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)	(0.1)	(0.8)

Slight to moderate atrophy occurred at 100/70 mg/kg/day. The atrophy consisted of reduced size and increased basophilia of mammary acini and ductules with increased prominence of interstitial collagen. Mammary gland atrophy was considered to be secondary to debilitation and decreased terminal body weight.

Reviewer's note:
In addition to the histopathological findings in the target organs, which are supported by corresponding changes in organ weight and macroscopic findings, inflammation was observed in multiple organs, such as heart, kidney, liver, lung, pancreas, and vagina. Hypocellularity in bone marrow was found in 1/14 group 4 female.

Histopathologic findings in pre-schedule deaths:
Primary SKI-606-related microscopic effects in the pre-scheduled deaths in group 4 females were similar to the findings described, but more profound and with more severity: changes in the intestinal tract (hemorrhage, erosion and edema of the cecum and colon; hemorrhage, erosion, mixed cell inflammation and mucosal hyperplasia of the duodenum, jejunum, and ileum; sinus erythrocytosis and hemosiderin pigment in the mesenteric lymph node). The remaining SKI-606-related changes were also similar, including: lymphoid atrophy of the spleen and thymus; hypocellularity in the bone marrow; atrophy of the mammary gland and uterus; hemorrhage, hypertrophy and vacuolation of the adrenal cortex. These changes were likely secondary to deteriorated body condition resulting from gastrointestinal toxicity. Although GI lesions were not apparent in pre-scheduled deaths in Group 4 males, microscopic changes in these rats consisted of otherwise similar findings: moderate to marked lymphoid atrophy of the spleen and thymus, moderate atrophy of the prostate and moderate decreased content in the seminal vesicles. These findings were suggestive of debilitation.

Special Evaluation

None.

Toxicokinetics

Table 27 Toxicokinetic parameters: 6-month study in rats

TK parameters on Day 180 are summarized in the following table (from the Applicant, verified by the reviewer):

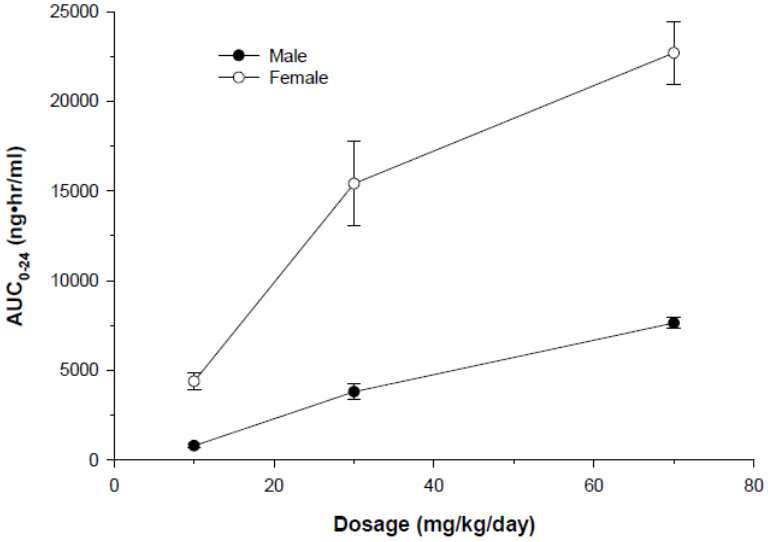
Dosage (mg/kg/day)	Sex	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	AUC/Dose
10	Male	84.5 ± 9.1	5.0	807 ± 125	80.7 ± 12.5
10	Female	394 ± 130	3.0	4387 ± 470	439 ± 47[a]
30	Male	346 ± 82	3.0	3810 ± 423	127 ± 14
30	Female	1222 ± 395	3.0	15405 ± 2363	514 ± 79[a]
100/70[b]	Male	503 ± 18	10.0	7630 ± 313	109 ± 4
100/70[b]	Female	1474 ± 68	5.0	22692 ± 1737	324 ± 25[a]

a. Significantly different than corresponding value in males (same dose).
b. Animals received 100 mg/kg/day for 42 days before being dosed with 70 mg/kg/day for the remainder of the study (used 70 mg/kg for dose normalization).

C_{max} and AUC values were increased with dose, with an approximately dose-proportional fashion. Since the blood samples for TK analysis were collected only on Day 180, and not on Day 1, without the data on an earlier time point, it is hard to comment on whether accumulation occurred following repeat administration of SKI-606. Based on the TK data in the 4-week study (Study# RPT-52772), there was no evidence of accumulation of SKI-606 following 4 –week administration.

Figure 11 Exposures in male and female rats

As indicated, exposures in female rats were 3-5 fold higher than in males:



Stability and Homogeneity

The stability and homogeneity was within the range of SOP. Based on the concentration, stability and uniformity analyses, all formulations were acceptable for use.

Dogs:

Reviewer's note: the result of 4-week repeat dose toxicology study (Study# RPT-52772, GLP study) was summarized by Dr. Leigh Verbois under IND 68268. The following summary is excerpted from Dr. Verbois' report:

Beagle dogs were administered 0, 0.5, 1.5 and 5 mg/kg (i.e., 0, 10, 30 or 100 mg/m^2/day) SKI606 orally by gavage for four weeks. Doses were selected based on results from a 10-day dose-range finding study (0. 5, 37.5, 75 and 150 mg/kg, i.e., 0, 100, 750, 1500 or 3000 mg/m^2) in which animals administered ≥750 mg/m^2/day had emesis and/or fecal alterations that resulted in pre-scheduled euthanasia or cessation of dosing. Animals that were pre-scheduled euthanized in the dose range finding study had multifocal red linear discoloration of the colonic mucosa and/or the stomach duodenum, ileum and cecum. In this study ≥750 mg/m^2 SKI606 was not tolerated for >2 doses. At a dose of 100 mg/m^2/day, there were sporadic indications of GI toxicity (soft, mucoid, liquid stool) without concomitant decreases in body weight or food consumption. Based on the dose limiting emesis and fecal alterations a maximum dose of 100 mg/m^2/day (or, 5 mg/kg) was chosen for the 28 day study in dogs.

Evaluations in the 28 day study consisted of mortality, clinical observations, body weight, food consumption, ophthalmoscopy, ECG, serum chemistry, urinalysis, organ weights, and macro/microscopic examinations. Additionally, toxicokinetics was evaluated on day 1 and day 28.

Mortality in this study was limited to one male animal treated with 100 mg/m^2/day, which was euthanized on Day 21. The death was not bosutinib-related and was attributable to gavage errors and/or anaphylactoid response, based on thoracic radiologic report.

Dose dependent clinical observations in animals that survived to their scheduled were limited to fecal alterations (soft, mucoid, liquid, decreased and red) in males and females that were administered ≥30 mg/m2/day. Weight reductions were evident in both males (0.4-0.5 kg) and females (0.1-0.4 kg) throughout the treatment period in all dosing groups, including controls, however there did not appear to be treatment dependent changes in food consumption. There was no evidence of ocular or cardiac toxicity when evaluated with ophthalmoscopy and ECGs. Additionally there was not evidence for SKI606 induced toxicity in hematology, clinical chemistry or urinalysis measures. Macro/Microscopic findings were primarily limited to animals that experienced gavage error. Congestion of the cecum and colon were noted, however the incidence of these findings were similar in all dosing groups, including the control groups.

Toxicokinetic analysis indicated that exposure to SKI606, as measured by AUC and C_{max}, increased in a slightly greater than dose proportional manner with increasing dosage in both males and females when assessed after the first and 28th dose (see table below). There were no sex-related differences in exposure, and accumulation was minimal (≤2 fold).

Table 28 Toxicokinetic parameters: 4-week study in dogs

(PROTOCOL 05_1339)

Day	Dose (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (hrs)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	AUC/D[b]	$t_{1/2}$ (hr)	R[c]
1	0.5	M	13.0 ± 2.4	5.3 ± 2.3	149 ± 12	182 ± 8	364 ± 16	7.5 ± 0.9	NA
	0.5	F	16.0 ± 1.1	2.0 ± 0.0	154 ± 18	185 ± 23	370 ±47	7.7 ± 0.4	NA
	1.5	M	56.4 ± 5.2	4.0 ± 0.0	758 ± 102	1292 ± 313[d]	861 ± 209	19.0 ± 6.7[e]	NA
	1.5	F	52.3 ± 18.3	4.7 ± 3.1	599 ± 118	811 ± 98[d]	541 ± 66	13.1 ± 2.2[e]	NA
	5.0	M	257 ± 46	4.0 ± 0.0	2954 ± 322	3838 ± 483[d]	768 ± 97	11.6 ± 0.8[e]	NA
	5.0	F	177 ± 66	4.0 ± 0.0	2405 ± 892	3278 ± 1388[d]	656 ± 277	12.2 ± 2.6[e]	NA
28	0.5	M	16.7 ± 2.8	4.0 ± 0.0	229 ± 55	NA	458 ± 110	10.8 ± 4.4[e]	1.54 ± 0.40
	0.5	F	20.8 ± 5.6	2.0 ± 0.0	232 ± 81	NA	464 ± 161	8.2 ± 3.3[e]	1.52 ± 0.61
	1.5	M	77.6 ± 11.1	3.3 ± 1.2	985 ± 197	NA	656 ± 131	16.7 ± 5.7[e]	1.29 ± 0.09
	1.5	F	68.8 ± 5.4	3.3 ± 1.2	824 ± 48	NA	549 ± 32	13.7 ± 2.9[e]	1.40 ± 0.23
	5.0	M	364 (n=2)	3.0 (n=2)	3888 (n=2)	NA	778 (n=2)	11.4[e] (n=2)	1.29 (n=2)
	5.0	F	291 ± 77	2.7 ± 1.2	3450 ± 908	NA	690 ± 182	12.8 ± 2.1[e]	1.49 ± 0.21

NA: Not applicable
a: n=3; 5 mg/kg/day (Male), n=2
b: $AUC_{0-\infty}$/Dose, Day 1; AUC_{0-24}/Dose, Day 28
c: AUC_{0-24} Day 28/AUC_{0-24} Day 1
d: Estimated from the elimination rate constant (long $t_{1/2}$) obtained from a short sampling period that was too short to adequately characterize the terminal phase and hence should be interpreted with caution.
e: Values should be interpreted cautiously since the sampling period was too short (24 hrs) to adequately characterize the terminal half-life.

Study title: SKI-606: 9-month oral (gavage) toxicity study in FED dogs with a 28 day recovery

Study no.:	RPT-65542 (Protocol 05_1240)
Study report location:	Wyeth
Conducting laboratory and location:	Wyeth European Drug Safety & Metabolism Research Center, Catania, Italy
Date of study initiation:	August 29, 2005
GLP compliance:	The Applicant claimed GLP compliant: with signature pages attached, but without a QA page
QA statement:	Yes
Drug, lot #, and % purity:	Bosutinib (SKI-606), lot#RB5626, 95.6% (largest single impurity: 0.07%)

Key Study Findings

- Orally (gavage) administered SKI-606 at 1, 3 and 10 mg/kg/day in Beagle dogs for 9 months was tolerated. There were no treatment induced mortality or changes in

most of clinical and anatomic pathological parameters. The target organs were GI tract (mainly small intestine), as evidenced by findings in GI-related clinical signs, decreased mean group body weights as well as body weigh gain in compared to the control, changes in chemistry parameters (decreased total protein and albumin), and microscopic changes (crypt abscess of duodenum). Findings resolved.

- The systemic exposures to SKI-606 were increased with increased doses in a dose-proportional fashion. The AUC levels were higher on Day 273 than those on day 1, indicating potential accumulations of SKI-606 following repeated administration.
- There were no apparent gender differences in exposure.

Methods

Doses:	0 (control), 1, 3, 10 mg/kg/day as Groups 1, 2, 3, and 4, respectively.
Frequency of dosing:	Once daily
Route of administration:	Oral (gavage)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (4000 cps), 2% polysorbate 80 (NF), 0.06% glacial acetic acid and sterile water for injection
Species/Strain:	Beagle dogs
Number/Sex/Group:	Main study Groups: 4; Recovery: 3 (control and high dose); no extra groups; all animals were included in Toxicokinetic analysis
Age:	~ 8-9 months
Weight:	6.9-11.4 kg
Satellite groups:	Toxicokinetics groups
Unique study design:	Not remarkable
Deviation from study protocol:	Not remarkable

Observations and Results

Clinical signs:	At least twice daily for mortality, moribundity and gross abnormality during pretest (Day -15) and dosing period then once for animals scheduled for euthanasia. Detailed examinations were conducted twice pretest (on Days -15 and -2) in all animals; once weekly to Week 13, then once every 4 weeks thereafter and at Week 39; once per week during the recovery period.
Body weight:	Twice pretest (on Days -15, -8 and -1) in all Groups; once weekly to Week 13, then once every 2 weeks thereafter and at Week 39; once per week during the recovery period..
Food consumption:	Calculated daily starting Day 15 prior to treatment initiation.
Ophthalmology:	Once pretest (Day -9) and during Weeks 13 (Day 87) and 39 (Day 272) of dosing period, and during Week 4 (Day 28) of recovery period.
EKG	Once pretest (Day -9) and during Week 39 (Day 268) of dosing period, and during Week 4 (Day 28) of recovery period.

Hematology: Twice pretest (Days -13 and -2) and during Weeks 13 (Days 90), 39 (Day 269), and Week 4 of recovery (Day 28).
Clinical chemistry: See "hematology".
Urinalysis: Once pretest (Day -10), Week 39 (Day 268), and Week 4 of recovery (Day 24).
Bone marrow smears:
From all animals; samples prepared but not evaluated.
Gross pathology: At scheduled sacrifice in all animals.
Organ weights: At scheduled sacrifice in all animals. The following organs were weighted: adrenal, brain, heart, kidneys, liver, ovaries, pituitary, prostate, testes, and thyroid with parathyroid.
Histopathology: At scheduled sacrifice. All tissues collected from all animal. All fixed organs and tissues from all animals were processed and examined microscopically. See inventory list for organs examined.
Toxicokinetics: Blood samples, 0.5 and 1 mL, were collected on Days 1 and 273, prior to dosing (0 hour, Day 273 only), and at 1, 2, 4, 6, 9, 12 and 24 hours after dosing (n=2/sex/ group/time point). The control group was only sampled at 5 hours after doing on Day 180. Concentrations of SKI-606 were analyzing via LC/MS/MS. Lower limit of quantification (LLOQ): 5 ng/mL using 0.2 mL of rat plasma. (Study #RPT-66535)
Reviewer's note:
Two liver samples from all animals at necropsy were collected and stored for possible determination of drug metabolism enzyme activities. According to the Applicant, these samples were discarded because liver samples were not analyzed after all.

Mortality

No treatment related deaths.

Clinical Signs

SKI-606 induced dose-related (in terms of number of animals affected and frequency of incidence) clinical signs that were mainly GI-related findings. These findings included emesis and fecal changes. The finding resolved. As noted, GI signs did not result in apparent changes in body weight or food intake (see below)

Table 29 Clinical signs: 9-month study in dogs

The table below is the summary of clinical signs (table from the Applicant; the reviewer verifies and concurs with the content in the table):
Main study animals:

	Dosage (mg/kg/day)			
	0	1	3	10
		Male		
Emesis[a]	5 (1-3)	1 (1)	2 (2)	7 (1-23)
Liquid Feces[b]	1 (1)	2 (1-46)	3 (2-30)	7 (45-241)
Soft Feces[b]	1 (2)	4 (1-47)	4 (1-48)	7 (27-160)
Feces with Red Pigment (presumed blood)	0	0	0	6 (1-12)
Mucoid Feces	0	0	2 (1)	5 (1-15)
Mucoid Feces with Red Pigment (presumed blood)	0	0	0	7 (1-12)
		Female		
Emesis[a]	5 (1-6)	1 (2)	2 (2)	6 (1-171)
Liquid Feces[b]	1 (2)	1 (1)	3 (1-6)	7 (3-103)
Soft Feces[b]	1 (3)	2 (1-14)	4 (2-18)	7 (22-163)
Feces with Red Pigment (presumed blood)	0	0	1 (1)	3 (1-49)
Mucoid Feces	0	0	0	4 (1-3)
Mucoid Feces with Red Pigment (presumed blood)	1 (2)	0	0	6 (1-31)

( ). Range of number of days observed for affected animals.
a. Inclusive of emesis with food and emesis with dosing formulation.
b. Starting on weeks 1-3 of study and lasting throughout the entire dosing period.

Recovery animals:

	Dosage (mg/kg/day)	
	0	10
	Male	
Liquid Feces	0	2 (1-2)
Soft Feces	0	2 (1-2)
	Female	
Liquid Feces	0	2 (1-2)
Soft Feces	1 (1)	2 (2)
Mucoid Feces	0	1 (1)

( ). Range of number of days observed for affected animals.

Body Weights

SKI-606 induced a slight reduction in group mean body weight:

- During the dosing period: males (↓ 1-7% from the control), females (↓ 1-11% from the control). The effect increased with longer treatment duration.
- Changes were lack of apparent dose-dependence.
- Body weight gain from pre-test (Day -15) to the end of dosing phase was significantly compared to the control in females dosed at 10 mg/kg. Reduction also seen in males at 10 mg/kg, but the reduction was not statistically significant.
- Finding resolved.
- Overall weight gain:

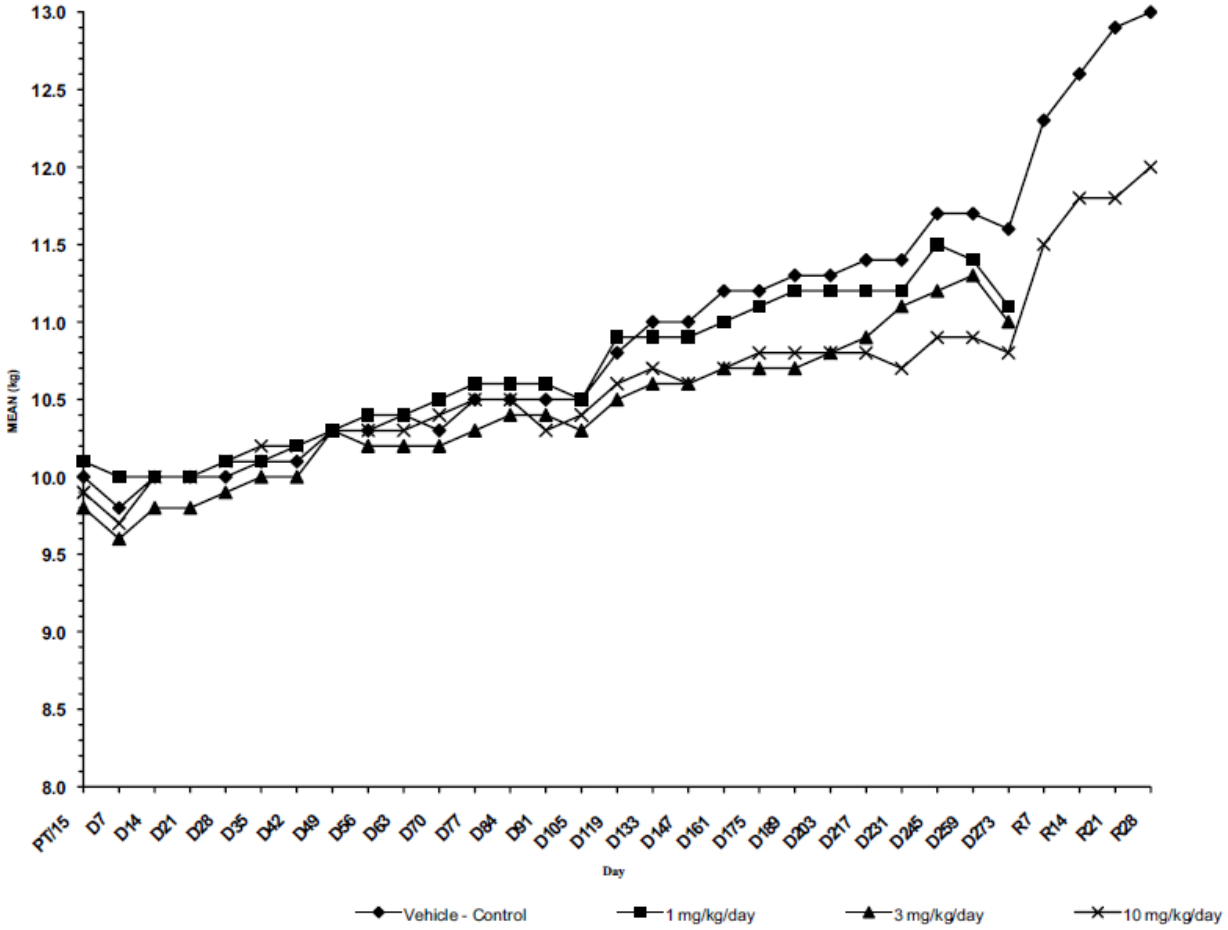
	Males				Females			
Group	1	2	3	4	1	2	3	4
Dose (mg/kg)	control	1	3	10	control	1	3	10
Day -15 to Day 273	1.6	1.0	1.2	0.9	1.2	1.3	1.1	0.2*

Day-15 to Day 28 (R)	2.1	NA	NA	1.4	0.4	NA	NA	0.7

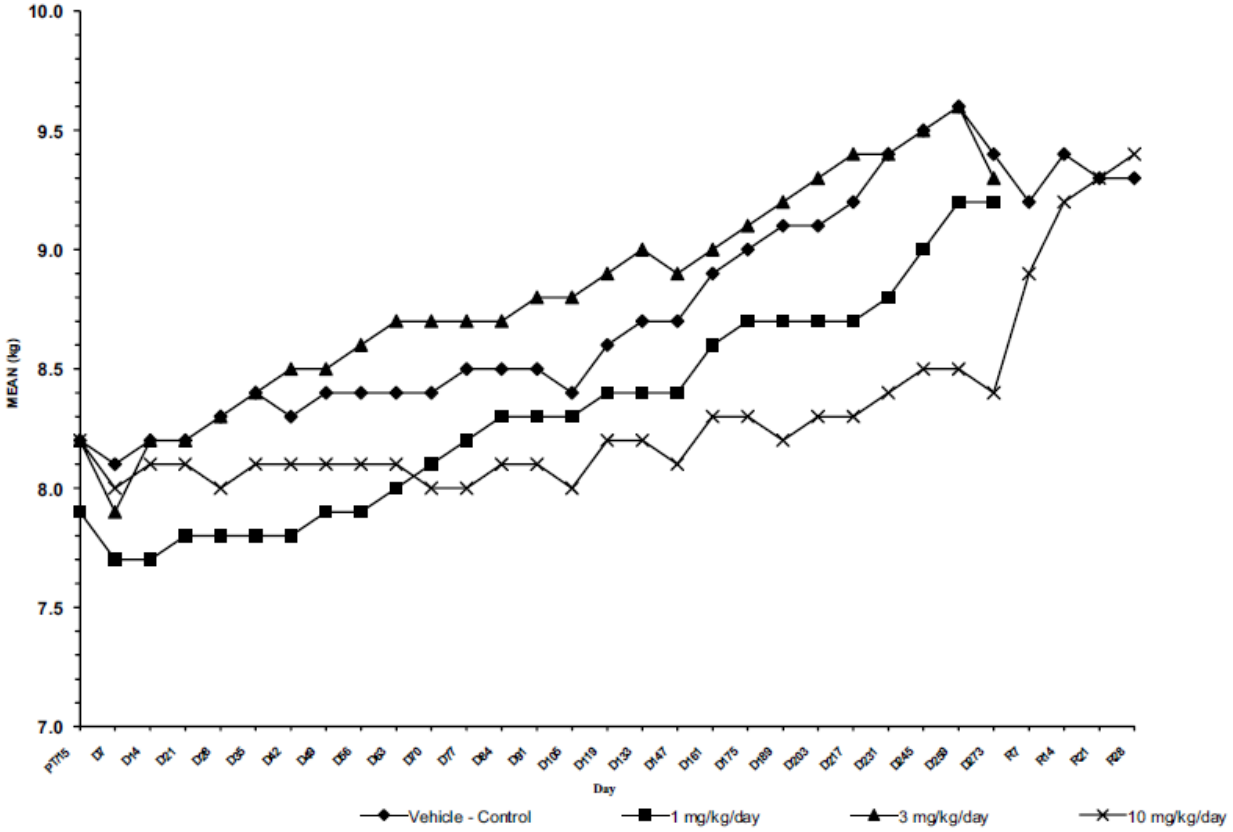
NA: not applicable
*** Statistically significant**

Figure 12 Group mean body weights: 9-month study in dogs

(from the Applicant)
Males:



Females:



Feed Consumption

Not remarkable.

Ophthalmoscopy

Not remarkable

ECG

Not remarkable.

Hematology

There were no treatment-related effects on hematology parameters. Statistically significant increases in WBC, neutrophil, large unstained cell (LUC) and platelet counts in males were sometimes due to higher pre-test values. These changes are unlikely adverse, because of small magnitude of changes, overlapping with the range of changes in the control, and lacking other supporting evidences in clinical signs or pathological findings. The toxicological meaning of increased values of fibrinogen in both treated male and female dogs was not clear.

Clinical Chemistry

Table 30 Clinical chemistry parameters: 9-month study in dogs

Clinical chemistry parameters on Days 90 and 269 (% changes from the control), and gains (from pretest Day -14 to Day 269, + greater gain, - less gain than gains found with the control):

	Males			Females		
Group	Group 4			Group 4		
Dose (mg/kg)	10			10		
Days	90	269	Gain	90	269	Gain
N	7	7	7	7	7	7
Total Protein ↓	7	10	-	5	11	-
Albumin ↓	12	10	-	10	18	-
A/G ↓	12§			14	18	-
Amylase ↑	29	47	+	36	33	+
Cholesterol ↓	17	24	-	26	37	-
Calcium ↓	6	4	-	6	7	-
Phosphorus ↓				21	11§	

§NS: Not statistically significant changes from the control.

The main findings, primarily at 10 mg/kg, include: decreased total protein, albumin, A/G ratio (due to reduction in albumin), cholesterol, calcium, and phosphorus, Decreased albumin and total protein may be the result of decreased nutrient absorption in the smaller intestine, as supported by the finding of crypt abscess in duodenum. Decreased calcium in plasma was possibly due to decreased albumin, a calcium carrier. In both male and female Group 4 dogs, amylase levels were higher than the control during pretest phase. This may contribute to higher amylase levels in the treated animals. Although the magnitude of elevation was not small, increases of amylase was not dose dependent. With the absence of pancreatic findings upon histopathological examination, the elevation of amylase is not likely toxicologically significant.

Urinalysis

Not remarkable.

Gross Pathology

Not remarkable.

Organ Weights

Not remarkable.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings: target organs: small intestine

Table 31 Histopathological findings: 9-month study in dogs

GI tract: small intestine

	Male				Female			
	Dosage (mg/kg/day)				Dosage (mg/kg/day)			
Finding	**0**	**1**	**3**	**10**	**0**	**1**	**3**	**10**
Duodenum[a]	4	4	4	4	4	4	4	4
- Crypt abscess	0	0	0	1	1	1	1	4
Slight	0	0	0	1	1	1	1	4
	(0.0)	(0.0)	(0.0)	(0.3)	(0.3)	(0.3)	(0.3)	(1.0)

a. Number examined.

( ). Average severity (number affected/total number examined; 0= no microscopic finding, 1 = slight).

Special Evaluation

None.

Toxicokinetics

Table 32 Toxicokinetic parameters: 9-month study in dogs

TK parameters are summarized in the following table (from the Applicant, verified by the reviewer):

Day	Dosage (mg/kg/day)	Sex	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	AUC[a] /Dose	$t_{1/2}$ (hr)	R
1	1	M	34.2	2.5	396	NR	NR	14.4 [b]	NA
			± 11.6	± 1.0	± 51			± 3.9	NA
		F	38.9	3.0	506	NR	NR	18.3 [b]	NA
			± 6.6	± 1.2	± 26			± 1.6	NA
	3	M	142	2.8	1670	2152	717	15.2 [b]	NA
			± 27	± 1.5	± 200	(n=1)	(n=1)	± 2.8	NA
		F	154	2.5	1686	NR	NR	13.2 [b]	NA
			± 20	± 1.0	± 176			± 2.6	NA
	10	M	652	2.6	6805	7393	739	13.7 [b]	NA
			± 166	± 1.4	± 1180	(n=1)	(n=1)	± 2.9	NA
		F	419	3.7	4891	5763	576	9.3	NA
			± 77	± 0.8	± 621	± 801	± 80	± 1.3	NA
273	1	M	34.9	3.5	575	NA	575	18.3 [b]	1.47
			± 9.4	± 1.0	± 141	NA	± 141	± 2.7	± 0.42
		F	66.4	2.3	823	NA	823	20.2 [b]	1.61
			± 44.6	± 1.3	± 313	NA	± 313	± 6.0	± 0.54
	3	M	133	3.5	2008	NA	669	17.6 [b]	1.23
			± 12	± 1.0	± 198	NA	± 66	± 5.4	± 0.26
		F	160	2.8	2372	NA	791	16.0 [b]	1.41
			± 20	± 1.5	± 280	NA	± 93	± 4.4	± 0.16
	10	M	583	3.3	8265	NA	826	14.7 [b]	1.25
			± 166	± 1.3	± 1981	NA	± 198	± 4.1	± 0.40
		F	513	2.1	6657	NA	666	14.3 [b]	1.36
			± 112	± 0.9	± 1340	NA	± 134	± 2.3	± 0.22

NR. Not reported; extrapolated portion > 25%
R. $AUC_{0-24\ (Day\ 273)}/AUC_{0-24\ (Day\ 1)}$
NA. Not applicable
a. $AUC_{0-\infty}$ on Day 1 and AUC_{0-24} on Day 273
b. These $t_{1/2}$ values are estimates and provided for informational purpose only and should be interpreted with caution since the $t_{1/2}$ values were relatively long compared to the sampling period (24 hrs)

C_{max} and AUC values increased with dose, in an approximately dose-proportional fashion. There were slight increases in AUC_{0-24} values on Day 273 compared to those on Day 1, indicating possible accumulation following repeat administration of SKI-606. There was no apparent gender difference in systemic exposure, although the AUC values on Day 273 were slightly higher in males.

Stability and Homogeneity

The stability and homogeneity was within the range of SOP. Based on the concentration, stability and uniformity analyses, all formulations were acceptable for use.

Special study:
Safety assessment of human metabolite M2:

Study title: WAY-198760 (SKI-606 M2 metabolite): 14-days oral toxicity study in rats

Study no.:	RPT-74244 (Protocol 08_0825)
Study report location:	Wyeth
Conducting laboratory and location:	Wyeth European Drug Safety & Metabolism Research Center, Catania, Italy
Date of study initiation:	May 16, 2008
GLP compliance:	The Applicant claimed GLP compliant: with signature pages attached, but without a QA page
QA statement:	Yes
Drug, lot #, and % purity:	WAY-198760 (SKI-606 metabolite M2), lot#MP040733, 95.6% (largest single impurity: 0.07%)

Key Study Findings

- Orally (gavage) administered SKI-606 metabolite M2 (WAY-198760) at 70 and 210 mg/kg/day in rats for 14 days was tolerated. There were no treatment induced mortality or changes in most of clinical and anatomic pathological parameters.
- The systemic exposures to M2 metabolite were increased with increased doses in an approximately dose-proportional fashion. There was no apparent gender difference in systemic exposure.

Methods

Doses:	0 (control), 70 and 210 mg/kg/day as Groups 1, 2, and 3, respectively.
Frequency of dosing:	Once daily
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (4000 cps), 2% polysorbate 80 (NF), and sterile water for injection
Species/Strain:	Rats (Crl:CD(SD))
Number/Sex/Group:	Main study Groups: 8; Toxicokinetic Groups: 9

	(except for n=3 in the Control group); note: no recovery groups
Age:	~ 6-7 weeks
Weight:	165-251 g
Satellite groups:	Toxicokinetics groups
Unique study design:	Not remarkable
Deviation from study protocol:	Not remarkable

Observations and Results

Clinical signs:	At least twice daily for mortality, moribundity and gross abnormality during pretest (Day -8) and dosing period then once for animals scheduled for euthanasia. Detailed examinations were conducted twice pretest (on Days -8 and -1) in all animals, then once weekly in main study animals (not TK animals).
Body weight:	Twice pretest (on Days -8 and -1), then once in all animals.
Food consumption:	Calculated once weekly starting Day 7 prior to treatment initiation. Food consumption data for TK animals were not included in the final report.
Ophthalmology:	Once pretest (Day -7) and during Week 2 (main study animals only).
EKG	Not conducted.
Hematology:	On Day 14 (Week 2); blood samples were collected via retro-orbital sinus.
Clinical chemistry:	See “hematology”.
Urinalysis:	Not conducted.
Bone marrow smears:	From all animals; samples prepared but not evaluated.
Gross pathology:	At scheduled sacrifice in all animals.
Organ weights:	At scheduled sacrifice in all animals. The following organs were weighted: adrenal, brain, heart, kidneys, liver, ovaries, pituitary, prostate, testes, and thyroid with parathyroid.
Histopathology:	At scheduled sacrifice. All tissues collected from all animal. All fixed organs and tissues from Groups 1 and 3 animals were processed and examined microscopically. See inventory list for organs examined.
Toxicokinetics:	Blood samples, 0.5 mL, were collected on Day 8 at 1, 2, 4, 7, 10 and 24 hours after dosing (n=3/sex/ group/time point. Concentrations of SKI-606 were analyzing via LC/MS/MS. Lower limit of quantification (LLOQ): 5 ng/mL using 0.2 mL of rat plasma.

Reviewer’s note:
Liver samples from all main study animals at necropsy were collected and stored for possible determination of drug metabolism enzyme activities. According to the Applicant these samples were discarded because liver samples were not analyzed after all.

Mortality

No treatment related deaths.

Clinical Signs

Not remarkable:

Body Weights

Not remarkable:

Feed Consumption

Not remarkable.

Ophthalmoscopy

Not remarkable

ECG

Not conducted.

Hematology

There were no treatment-related effects on hematology parameters.

Clinical Chemistry

There were no treatment-related effects on clinical chemistry parameters.

Urinalysis

Not conducted.

Gross Pathology

Not remarkable.

Organ Weights

Not remarkable.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

No remarkable histological findings.

Special Evaluation

None.

Toxicokinetics

Table 33 Toxicokinetic parameters: 2-week study in rats for metabolite M2

TK parameters are summarized in the following table (from the Applicant, verified by the reviewer):

Dosage (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	AUC/Dosage
70	Male	36.8 ± 9.8	2.0	354 ± 60	5.06 ± 0.86
	Female	110 ± 65	1.0	631 ± 346	9.01 ± 4.94
210	Male	93.8 ± 19.7	1.0	929 ± 98	4.42 ± 0.47
	Female	120 ± 19	1.0	1488 ± 657	7.09 ± 3.13

C_{max} and AUC values were increased with dose, with an approximately dose-proportional fashion. There was no apparent gender difference in systemic exposure.

Stability and Homogeneity

The stability and homogeneity was within the range of SOP. Based on the concentration, stability and uniformity analyses, all formulations were acceptable for use.

Safety assessment of impurity (b) (4)
In order to qualify the level of impurity (b) (4) greater than (b) (4) (the reporting threshold), two lots of SKI-606 which contain different levels of (b) (4) i.e., RB5626 (containing < (b) (4) of RRT (b) (4) and RB6609 ((b) (4) (b) (4) were used in the same study.

Study title: SKI-606: 14-days oral (gavage) impurity qualification study in rats

Study no.:	RPT-64729 (Protocol 06_1410)
Study report location:	Wyeth
Conducting laboratory and location:	Wyeth European Drug Safety & Metabolism Research Center, Catania, Italy
Date of study initiation:	May 12, 2006
GLP compliance:	The Applicant claimed GLP compliant: with signature pages attached, but without a QA page
QA statement:	No
Drug, lot #, and % purity:	SKI-606, lot#RB5626 (containing < (b) (4) impurity (b) (4) and RB6609 ((b) (4) impurity (b) (4) purity: 96.7% and 96.4%, respectively.

Key Study Findings

- Two lots of SKI-606, containing impurity (b) (4) at < (b) (4) (RB5626) and (b) (4) (RB6609), were orally (gavage) administered at 70 mg/kg/day in rats for 14 days. The two lots of SKI-606 elicited comparable findings, including reductions in body weight, food consumption, inflammation-related changes in hematology and clinical chemistry parameters. The target organs were GI tract (mainly small intestine), liver, and lymph nodes.
- The findings in this impurity qualification study were comparable with other repeat dose toxicology studies. Since two lots demonstrated similar effects, the impurity (b) (4) is considered qualified at levels up to (b) (4)

Methods

Doses:	0 (control) and 70 mg/kg/day (Lot #RB5626 and #RB6609) as Groups 1, 2 and 3, respectively.
Frequency of dosing:	Once daily x 14 days
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (4000 cps), 2% polysorbate 80 (NF), 0.06% acetic acid (w/v), and purified (Type 1) water
Species/Strain:	Rats (Crl:CD(SD))
Number/Sex/Group:	Main study Groups: 10; note: no recovery groups
Age:	~ 9 weeks
Weight:	191-353 g
Satellite groups:	None
Unique study design:	Not remarkable
Deviation from study protocol:	Not remarkable

Observations and Results

Clinical signs:	At least twice daily for mortality, moribundity and gross abnormality during pretest (starting at receipt) and dosing period then once for animals scheduled for euthanasia. Detailed examinations were conducted twice pretest (on Day -3 and -1) in all animals, then once weekly in main study animals.
Body weight:	Once per week including twice pretest (on Days -8 and -1.
Food consumption:	Calculated once weekly starting Day 3 prior to treatment initiation.
Ophthalmology:	Not conducted.
EKG	Not conducted.
Hematology:	On Day 14 (Week 2); blood samples were collected via retro-orbital sinus.
Clinical chemistry:	See “hematology”.
Urinalysis:	Not conducted.
Bone marrow smears:	From all animals; samples prepared but not evaluated.
Gross pathology:	At scheduled sacrifice in all animals.
Organ weights:	At scheduled sacrifice in all animals. The following organs were weighted: adrenal, brain, heart, kidneys, liver, ovaries, pituitary, prostate, spleen, testes (epidydymides), thyroid with parathyroid and uterus.
Histopathology:	At scheduled sacrifice. All tissues collected from all animal. All fixed organs and tissues from Groups 1 and 3 animals were processed and examined microscopically. See inventory list for organs examined.
Toxicokinetics:	Not conducted.

Mortality

No treatment related deaths. One Group 3 male rat (#42) was euthanized on Day 14 due to eye trauma from blood sampling.
The clinical signs and hematology findings were similar to those found in the same group. Macroscopically, the mesenteric lymph node was red and the Harderian gland was dark. Compound-related microscopic findings included slight hypertrophy of the liver and moderate erythrocytosis of the mesenteric lymph node.

Clinical Signs

Table 34 Clinical signs: study for impurity (b) (4)

The main findings were GI slinical signs and associated signs. The numbers in the table indicate number of animal affected and days of the incidence in the parentheses.

	M			F		
Group	1	2	3	1	2	3
Dose (mg/kg)	Control	70 (RB5626)	70 (RB6609)	Control	70 (RB5626)	70 (RB6609)
Number	10	10	10	10	10	10
Scheduled deaths	10	10	9	10	10	10
Euthanasia			1			
Appearance						
Exophthalmia: eye		1 (2)	1 (1)			
Focal lesion			2 (1-9)			
Mass					1 (Day 7)*	
Pale					1 (10)**	
Rales					1 (1)**	1 (8)
Red pigment around nose/mouth						1 (2-3)
Salivation			3 (1)		2 (1)	3 (1-3)

* A palpable firm mass (~2x2 cm) was found on the thorax of a female Group 2 rat (#37) on Day 7. According to the Investigator, the mass was a chronic interstitial hemorrhage in the mammary gland from a trauma. This animal was also found with pale appearance (Days 7-16) and rales on Day 9.

Except for salivation, most the clinical signs were not SKI-606 related. Salivation was not considered adverse, due to short duration of the incidence. The findings of clinical signs were comparable in two treated groups.

Body Weights

Table 35 Group mean body weights: study for impurity (b) (4)

Mean group body weights were slightly reduced in male rats of the two treated groups. The weight gains showed statistically significant reductions, based on the overall and pair-wise P values (P< 0.05). No remarkable changes in females. Weight changes in the two treated groups were comparable.

	Males			Females		
	Control	70 mg/kg (RB 5626)	70 mg/kg (RB 6609)	Control	70 mg/kg (RB 5626)	70 mg/kg (RB 6609)
N	10	10	10	10	10	10
Day -4	324.1	324.4	322.2	204.8	205.8	203.3
Day 7	374.4	363.9 (↓ 3)	362.9 (↓ 3)	216.9	219.0	216.8
Day 14	417.0	399.8 (↓ 4)	397.8 (↓ 5)	225.3	227.3	222.6
D -4 to D7	50.3	39.5 (↓ 21)	40.7 (↓ 19)	12.1	13.2	13.5
D-4 to D14	92.9	75.4 (↓ 19)	75.6 (↓ 19)	20.5	21.5	19.3

Feed Consumption

Table 36 Food consumption: study for impurity (b) (4)

	Males			Females		
	Control	70 mg/kg (RB 5626)	70 mg/kg (RB 6609)	Control	70 mg/kg (RB 5626)	70 mg/kg (RB 6609)
N	10	10	10	10	10	10
Day 4	25.42	25.20	25.03	17.14	16.19 (↓ 6)	16.73 (↓ 2)
Day 8	28.63	26.24 (↓ 8)	25.61 (↓ 11)	16.99	15.43 (↓ 9)	15.19 (↓ 11)
Day 14	29.93	27.43 (↓ 8)	26.83 (↓ 10)	17.03	15.33 (↓ 10)	15.83 (↓ 7)

Slight reductions in food consumption were observed in rats treated with lot#RB5626 or RB#6609. No apparent differences were found in the two treated groups.

Ophthalmoscopy

Not conducted.

ECG

Not conducted.

Hematology

Table 37 Hematology parameters: study for impurity (b) (4)

Hematology parameters on Days 14: expressed as the count (% changes from the control):

		M			F	
Group	1	2	3	1	2	3
Dose (mg/kg)	Control	70 (RB5626)	70 (RB6609)	Control	70 (RB5626)	70 (RB6609)
N	10	10	10	10	10	10
RBC ↓	7.3	7.28	7.24	7.47	**6.89 (8)**	**7.04 (6)**
HGB ↓	14.8	14.4	14.3	14.7	**13.4 (9)**	**13.6 (7)**
Hct ↓	45.0	44.0	43.9	44.6	**40.9 (8)**	**41.5 (7)**
Platelets ↑	1005.1	1051.6	1001.0	955.1	**1060.8 (11)**	**1050.5 (10)**
WBC ↑	13.7	14.0	13.1	9.2	11.0 (19)	10.4 (13)
Lymph (Ab) ↓	10.78	9.67 (10)	8.78 (19)	7.70	6.86 (11)	**5.46 (29)**
Neut (Ab) ↑	1.70	**2.78 (63)**	**2.96 (74)**	0.92	**3.06 (232)**	**3.77 (309)**
Mono (Ab) ↑	1.02	1.30 (27)	1.16 (14)	0.46	0.86 (87)	0.84 (84)
Eos (Ab) ↑	0.15	0.22 (48)	0.23 (56)	0.15	**0.22 (49)**	**0.36 (149)**
aPTT ↓	22.3	**19.9 (11)**	**18.8 (16)**	22.6	**18.8 (17)**	**20.1 (11)**
Fibrinogen ↑	191.1	**204.9 (7)**	**217 (14)**	181.3	**203.3 (12)**	**195.8 (8)**

Bolded printed number: Statistically significant changes from the control (based on the overall and pair-wise P values <0.05)

The hematological findings were primarily correlated with SKI 606-induce mild inflammation. These findings included increased white counts (except for lymphocyte), increased platelet count (females only), slight reduction in red cell mass (females only), and increased fibrinogen, with greater magnitude in changes in female rats than in males. Reduced red cell mass may be due to suppression of hematopoiesis secondary to inflammation; however, the magnitude of changes was small.

Clinical Chemistry

Table 38 Clinical chemistry parameters: study for impurity (b) (4)

Clinical chemistry parameters on Days 14: expressed as the count (% changes from the control):

	Males			Females		
Group	1	2	3	1	2	3
Dose (mg/kg)	Control	70 (RB5626)	70 (RB6609)	Control	70 (RB5626)	70 (RB6609)
N	10	10	10	10	10	10
ALP ↓	257.3	**195.8 (24)**	**194.6 (24)**	178.3	**94.9 (47)**	**98.2 (45)**
Total protein ↓	6.0	6.0	5.9	6.9	**6.2 (10)**	**6.2 (9)**
Albumin ↓	3.2	3.2	**3.1 (5)**	3.8	**3.3 (14)**	**3.4 (13)**
A/G ratio ↓	1.2	1.1	1.1	1.3	**1.1 (12)**	**1.2 (9)**
Glucose ↓	143.5	140.3	146.8	147.9	**134.9 (9)**	**139.1 (9)**
Cholesterol ↓	65.3	61.5	64.8	74.1	**45.3 (39)**	**49.3 (33)**
Potassium ↑	5.4	5.6	5.6	4.6	**4.9 (8)**	**4.9 (8)**
T4 ↓	3.5	3.4	3.1 (13)	1.4	1.0 (28)	1.3 (8)

Bolded printed number: Statistically significant changes from the control (based on the overall and pair-wise P values <0.05)

The main findings include: decreased total protein (due to reduction in albumin), A/G ratio, and cholesterol. Decreased albumin and total protein were in line with inflammation, as indicated in hematological findings above. The GI toxicities and decreased body weight may suggest malnutrition in these animals, which may also be attributable to decreased total protein levels. However, decreased body weight was observed mainly in male rats, while clinical chemistry parameters were, on the contrary, more prominent in the females.

In the 4-week study in rats (Study #RPT-52772), increased T3/T4 and decreased TSH were observed. No correlated histopathological findings were available in the thyroid. In another study (6-month toxicology study in rats, #RPT-63644), increased colloid in the thyroid was reported. The Applicant did not provide thyroid hormone data in this chronic study. In all these studies in rats, the thyroid-related findings have been inconsistent, and thus would not warrant the need to monitor of thyroid functions in the humans.

Urinalysis

Not conducted.

Gross Pathology

Table 39 Gross pathology: study for impurity (b) (4)

SKI-606 related macroscopic findings included discoloration of mesenteric lymph node and discoloration and thickened wall of squamous mucosal epithelium the stomach. These findings were supported by the histopathological findings (see below).

Finding	Male				Female			
	Ctrl	SKI-606	SKI-606 +	(b) (4)	Ctrl	SKI-606	SKI-606 +	(b) (4)
Number Examined	10	10	9		10	10	10	
Mesenteric Node Discoloration	0	10	9		0	9	9	
Squamous Stomach Mucosal Discoloration	0	0	0		0	1	1	
Thickened wall	0	0	0		0	1	0	

Organ Weights

In final necropsy, changed organ weights (mean absolute and/or relative weights) included: increased adrenal, heart, liver, spleen (females only), and ovary, and decreases in thymus. In general, changes in females were more than in the males. The two treated groups showed comparable results, except for decreased thymic weight: greater reduction was seen in treatment with lot#RB6609, especially in males. Summary in the following table was excerpted from the Applicant. The reviewer occurs with the content.

Table 40 Organ weights: study for impurity (b) (4)

	Dosage Group	Male					Female				
		N[a]	G[b]	% REF[c]	% REF (TBW)[d,e]	% REF (BNW)[f]	N[a]	G[b]	% REF[c]	% REF (TBW)[d,e]	% REF (BNW)[f]
Adrenals	Control	10	0.091	100	100	100	10	0.081	100	100	100
	SKI-606	10	0.087	95	99	95	10	0.097	120[g]	121[g]	120
	SKI-606 + (b) (4)	9	0.093	102	106	105	10	0.096	119[g]	123[g]	119
Heart	Control	10	1.493	100	100	100	10	0.909	100	100	100
	SKI-606	10	1.524	102	107	102	10	1.047	115[g]	117[g]	115[g]
	SKI-606 + (b) (4)	9	1.547	104	109	106	10	1.012	111[g]	115[g]	112[g]
Liver	Control	10	11.604	100	100	100	10	6.765	100	100	100
	SKI-606	10	12.254	106	110[g]	106	10	7.621	113[g]	114[g]	113[g]
	SKI-606 + (b) (4)	9	11.537	99	104	102	10	7.602	112[g]	116[g]	113[g]
Spleen	Control	10	0.944	100	100	100	10	0.558	100	100	100
	SKI-606	10	0.959	102	106	102	10	0.722	129[g]	131[g]	130[g]
	SKI-606 + (b) (4)	9	0.901	95	100	98	10	0.637	114[g]	118[g]	114[g]
Ovaries	Control	-	-	-	-	-	10	0.119	100	100	100
	SKI-606	-	-	-	-	-	10	0.139	117	119[g]	117[g]
	SKI-606 + (b) (4)	-	-	-	-	-	10	0.141	118	122[g]	118[g]
Thymus	Control	10	0.514	100	100	100	10	0.459	100	100	100
	SKI-606	10	0.489	95	99	95	10	0.384	84	85	84[g]
	SKI-606 + (b) (4)	9	0.378	73[g,h]	77[g,h]	76[g,h]	10	0.350	76[g]	78[g]	76[g]
Brain[i]	Control	10	2.134	100	100	-	10	1.950	100	100	-
	SKI-606	10	2 .131	100	104	-	10	1.946	100	101	-
	SKI-606 + (b) (4)	9	2.081	98	102	-	10	1.946	100	103	-
TBW[i]	Control	10	392.5	100	-	-	10	216.9	100	-	-
	SKI-606	10	376.8	96	-	-	10	214.5	99	-	-
	SKI-606 + (b) (4)	9	375.1	96	-	-	10	210.6	97	-	-

a. Number of animals.
b. Mean absolute weight in grams.
c. % of reference group (controls).
d. Terminal body weight.
e. % of body weight values for adrenals and ovaries are multiplied by 100.
f. Brain weight.
g. Mean absolute or relative weights statistically significant (Pairwise vs. Control, $p \leq 0.05$).
h. Mean absolute or relative weights statistically significant (Pairwise SKI-606 + (b) (4) vs. SKI-606, (b) (4).
i. Data for brain and terminal body weights included in the table for reference purposes to facilitate interpretation of organ to body and organ to brain weight ratios.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

The following tables are excerpted from the Applicant. The findings were organized by target organs/tissues:

Table 41 Histopathological findings: study for impurity (b) (4)

GI tract: especially small intestines

Finding	Male Ctrl	Male SKI-606	Male SKI-606 + (b) (4)	Female Ctrl	Female SKI-606	Female SKI-606 + (b) (4)
Ileum[a]	10	10	9	10	10	10
Goblet Cell Hypertrophy/Hyperplasia	0	8	8	0	10	10
Slight	0	8	7	0	10	10
Mild	0	0	1	0	0	0
	(0.0)	(0.8)	(1.0)	(0.0)	(1.0)	(1.0)
Jejunum[a]	10	10	9	10	10	10
Goblet Cell Hypertrophy/Hyperplasia	0	1	3	0	5	4
Slight	0	1	3	0	5	4
	(0.0)	(0.1)	(0.3)	(0.0)	(0.5)	(0.4)

a. Number examined
( ). Average severity (number affected/total number examined; 0 = no microscopic finding, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

Stomach:

Finding	Male Ctrl	Male SKI-606	Male SKI-606 + (b) (4)	Female Ctrl	Female SKI-606	Female SKI-606 + (b) (4)
Squamous Stomach[a]	10	10	9	10	10	10
Edema	2	1	0	1	5	3
Slight	2	1	0	1	3	2
Mild	0	0	0	0	1	0
Moderate	0	0	0	0	1	1
	(0.2)	(0.1)	(0.0)	(0.1)	(0.8)	(0.5)
Erosion	0	0	0	0	1	0
Slight	0	0	0	0	1	0
	(0.0)	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)
Hyperkeratosis	0	0	0	0	1	1
Mild	0	0	0	0	1	1
	(0.0)	(0.0)	(0.0)	(0.0)	(0.2)	(0.2)

a. Number examined.
( ). Average severity (number affected/total number examined; 0 = no microscopic finding, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe).

Liver:

Finding	Male				Female			
	Ctrl	SKI-606	SKI-606 -	(b) (4)	Ctrl	SKI-606	SKI-606 -	(b) (4)
Liver[a]	10	10	9		10	10	10	
Centrilobular Hepatocellular Hypertrophy	0	8	8		0	6	5	
Slight	0	8	8		0	6	5	
	(0.0)	(0.8)	(0.9)		(0.0)	(0.6)	(0.5)	

a. Number examined
( ). Average severity (number affected/total number examined; 0 = no microscopic finding, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe)

Centrilobular hyperplasia was correlated with increased liver weights. There was no supportive evidence in clinical chemistry parameters.

Lymph nodes:

Finding	Male				Female			
	Ctrl	SKI-606	SKI-606	(b) (4)	Ctrl	SKI-606	SKI-606 +	(b) (4)
Mesenteric Node[a]	10	10	9		10	10	10	
Sinus Erythrocytosis	0	10	9		0	10	10	
Mild	0	0	2		0	2	4	
Moderate	0	10	7		0	8	6	
	(0.0)	(3.0)	(2.8)		(0.0)	(2.8)	(2.6)	
Hemosiderin Pigment	0	6	5		0	7	5	
Slight	0	5	5		0	7	5	
Mild	0	1	0		0	0	0	
	(0.0)	(0.7)	(0.6)		(0.0)	(0.7)	(0.5)	
Lymphoid Atrophy	0	1	2		0	5	5	
Slight	0	1	1		0	2	3	
Mild	0	0	0		0	3	2	
Moderate	0	0	1		0	0	0	
	(0.0)	(0.1)	(0.4)		(0.0)	(0.8)	(0.7)	

a. Number examined.
( ). Average severity (number affected/total number examined; 0 = no microscopic finding, 1 = slight, 2 = mild, 3 = moderate, 4 = marked, 5 = severe).

Macroscopic findings of discoloration of mesenteric lymph node was corresponding with mild to moderate sinus erythrocytosis.

In general, the incidence and severity of histopathological findings were comparable in the to treated groups,

Special Evaluation

None.

Toxicokinetics

Not conducted.

Stability and Homogeneity

The stability and homogeneity was within the range of SOP. Based on the concentration, stability and uniformity analyses, all formulations were acceptable for use.

7 Genetic Toxicology

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

Study title: WAY-173606: Bacterial Reverse Mutation Test with *Salmonella typhimurium* and *Escherichia coli*

Study no.:	RPT-49003 (Protocol 02_1063)
Study report location:	Wyeth
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 16, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	WAY-173606 (bisutinib); Lot# L23445-136, 99.49%

Key Study Findings

WAY-173606 was not mutagenic, with and without S9 activation, under the condition of the study.

Methods

Strains:	*Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvrA*
Concentrations in definitive study:	1.0, 3.3, 10, 33, 100, 333 and 1000 μg* per plate.
Basis of concentration selection:	See below
Negative control:	Vehicle control (dimethyl sulfoxide,DMSO)
Positive control:	See below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	See below

***The dosing solutions were adjusted to compensate for the use-at value (95.20%) of the test article.**

<u>Concentration selection criteria</u>
Basis of concentration selection: based on previous experiments; data were not available in this submission. The sponsor followed ICH (1996 and 1997) and OEDC (1998) guideline and employed concentrations up to 5 mg/plate as the highest

concentration in the study. However, toxicity was observed beginning at 333 or 667 μg per plate. Precipitate was observed at 5000 μg per plate. Based on the findings of the toxicity assay, the maximum concentration plated in the mutagenicity assay was 1000 μg per plate.

Test agent stability: Stable in dimethyl sulfoxide (DMSO) for 48 hours at room temperature
Metabolic activation system: Aroclor 1254-induced rat liver microsome S-9 mix
Controls:
Vehicle: DMSO (100 μL/plate for plate incorporation test)
Negative controls: vehicle control
Positive controls: all dissolved in DMSO except for sodium azide, which was diluted with water.
With S-9: TA98, TA100, TA1535 and TA1537 and WP2 *uvrA*: 2-aminoanthracene (10 μg/plate).
Without S-9: TA98: 2-nitrofluorene (1.0 μg/plate), TA 1537: 9-aminoacridine (75 μg/plate), TA100 and TA1535: sodium azide (1.0 μg/plate), WP2 *uvrA*: methyl methanesulfate (1000 μg/plate).
Study design:
The assay was performed in two phases, using the plate incorporation and pre-incubation methods. The first phase, the preliminary toxicity assay, was used to establish the dose-range for the mutagenicity assay. The second phase, the mutagenicity assay (initial and confirmatory assays), was used to evaluate the mutagenic potential of the test article. The pre-incubation method was used in the confirmatory mutagenicity assay (i.e., Experiment B2) only. Due to contamination, WAY-173606 was retested in the repeat confirmatory assay (conducted using the pre-incubation method; Experiment B3) with tester strains TA1535, TA1537 and WP2 *uvrA* in the presence of S9 metabolic activation and with tester strain TA1535 in the in the absence of S9 metabolic activation at the same dose levels as Experiment B2.

Exposure conditions:
Incubation and sampling times:
- Plate incorporation: 48-72 hours
- Pre-incubation: 48-72 hours

Concentrations used:
- Preliminary assay (Experiment A): 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 μg per plate
- Mutagenicity assay (Experiment B1: initial and Experiment B2 and B3: confirmatory): 1, 3.3, 10, 33, 100, 333 and 1000 μg/plate
- WAY-173606: stock solution (in DMSO): 100 mg/mL; plating aliquot: 50 μL

Positive mutagenicity: the following is excerpted from the Applicant's report
For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article. Data sets for tester strains TA1535 and TA1537 were judged positive if the increase in mean revertants at the peak of the dose

response is equal to or greater than 3.0-times the mean vehicle control value. Data sets for tester strains TA98, TA100 and WP2 *uvrA* were judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2.0-times the mean vehicle control value.

Analysis:
Number of replicates:
Preliminary assay (Experiment A): one plate per dose
Mutagenicity assay (Experiment B): 3 plates for each test compound concentration
Counting method: automated colony counter (image analyzer).

Study Validity

The study is considered valid, because:

- Tester strain integrity was documented in the report.
- All tester strain culture titers were greater or equal to 0.3 x10^9 cells/mL to ensure that appropriate numbers of bacteria were plated.

Reviewer's note: In fact, lower titers such as 1.4 x 10^8 cells/mL were plated.

- Both negative (vehicle) and positive control data were within the laboratory historical range.
- The mean positive control value (± S9-mix) exhibited at least three fold increase over the respective mean vehicle control value for each tester strain.
- There was a minimum of three nontoxic concentrations. A concentration level was considered toxic if one or both of the following criteria were met: 1) ≥ 50% reduction in mean number of revertants/plate relative to the mean vehicle control value; or 2) at least a moderate reduction in the background lawn, i.e., background lawn code 3-5) in each tester strain, both in the absence and presence of S9-mix.

Results

- Experiment A (preliminary; plate incorporation)

Table 42 Number of revertant colonies: preliminary experiment

The numbers of revertant colonies/plate at each test compound concentration, and vehicle and positive controls are summarized in the table below:

Treatment	Concentration (mg/plate)	Revertant colonies/plate (n=1)				
Without S-9						
		TA98	TA100	TA1535	TA1537	WP2uvrA
DMSO	50 μL/plate	10	210	7	8	12
WAY-173606	6.7	13	163	10	9	13
	10	15	185	10	11	12
	33	13	189	6	10	10
	67	14	196	10	8	10

	100	11	227	7	12	11
	333	11	0*	0*	0*	12
	667	10*	0*	0*	0*	0*
	1000	0*	0*	0*	0*	0*
	3333	0*	0*	0*	0*	0*
	5000	0*IP	0*NP	0*NP	0*NP	0*NP
With S-9						
DMSO	50 μL/plate	17	238	9	12	11
WAY-173606	6.7	12	183	10	13	10
	10	17	208	10	12	12
	33	15	212	8	12	10
	67	15	222	10	14	10
	100	11	220	13	12	10
	333	13	131	7	11	10
	667	5*	0*	0*	0*	0*
	1000	0*	0*	0*	0*	0*
	3333	0*	0*	0*	0*	0*
	5000	0*NP	0*NP	0*NP	0*NP	0*NP

***: Bacteriotoxic: reduced background bacterial lawn or no bacteria background (Grade 3, 4 or 5)**
Precipitation (NP: non-interfering precipitation; IP: interfering precipitation)

- Experiment B1 (initial mutagenicity; plate incorporation)

Table 43 Number of revertant colonies: initial mutagenicity experiment

Treatment	Concentration (μg/plate)	Revertant colonies/plate (mean ± SD, n=3)				
Without S-9						
		TA98	TA100	TA1535	TA1537	WP2uvrA
DMSO	50 μL/plate	15 ± 2	168 ± 11	12 ± 4	11 ± 2	11 ± 1
WAY-173606	1.0	11 ± 1	153 ± 4	12 ± 2	13 ± 1	11 ± 2
	3.3	14 ± 2	160 ± 6	13 ± 1	13 ± 2	10 ± 2
	10	10 ± 2	152 ± 18	14 ± 3	12 ± 1	10 ± 2
	33	14 ± 3	157 ± 10	11 ± 1	12 ± 3	10 ± 2
	100	11 ± 1	161 ± 6	16 ± 3	13 ± 1	12 ± 1
	333	12 ± 3	124 ± 6	8 ± 2	9 ± 2	9 ± 1
	1000	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
Positive control	(see above for + controls)	87 ± 5	688 ± 44	392 ± 24	115 ± 2	105 ± 9
With S-9						
DMSO	50 μL/plate	22 ± 4	158 ± 8	12 ± 2	11 ± 3	11 ± 1
WAY-173606	1.0	21 ± 2	167 ± 9	13 ± 3	11 ± 1	10 ± 2
	3.3	18 ± 1	170 ± 5	14 ± 2	13 ± 1	10 ± 2
	10	19 ± 4	170 ± 17	11 ± 3	11 ± 4	10 ± 1
	33	19 ± 1	181 ± 14	11 ± 2	14 ± 2	9 ± 1
	100	19 ± 7	193 ± 8	10 ± 3	8 ± 1	9 ± 1
	333	19 ± 3	171± 13	8 ± 1	12 ± 3	9 ± 1
	1000	10 ± 2	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
Positive control	(see above for + controls)	1079 ± 49	816 ± 57	226 ± 38	224 ± 40	866 ± 98

***: Bacteriotoxic: reduced background bacterial lawn or no bacteria background (Grade 3, 4 or 5)**

Experiment B2 (confirmatory, pre-incubation)

Table 44 Number of revertant colonies: confirmatory experiments

Treatment	Concentration (μg/plate)	Revertant colonies/plate (mean ± SD, n=3)			
Without S-9					
		TA98	TA100	TA1537	MP2 uvrA
DMSO	50 μL/plate	12 ± 3	139 ± 11	10 ± 4	12 ± 2
WAY-173606	1.0	11 ± 2	164 ± 15	13 ± 3	9 ± 3
	3.3	10 ± 3	150 ± 4	14 ± 3	11 ± 2
	10	11 ± 2	154 ± 11	17 ± 3	13 ± 2
	33	12 ± 4	157 ± 18	15 ± 4	10 ± 7
	100	13 ± 3	118 ± 15	7 ± 4	12 ± 0
	333	6 ± 1	0 ± 0*	0 ± 0*	0 ± 0*
	1000	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
Positive control	(see above for + controls)	305 ± 34	733 ± 19	84 ± 3	472 ± 83
With S-9					
DMSO	50 μL/plate	12 ± 1	161 ± 12		
WAY-173606	1.0	18 ± 4	176 ± 32		
	3.3	20 ± 6	177 ± 9		
	10	19 ± 2	176 ± 6		
	33	19 ± 2	177 ± 17		
	100	20 ± 3	195 ± 23		
	333	19 ± 3	61± 5		
	1000	4 ± 2	0 ± 0*		
Positive control	(see above for + controls)	862 ± 13	1666 ± 165		

Experiment B3 (repeat confirmatory, pre-incubation):

Treatment	Concentration (mg/plate)	Revertant colonies/plate (mean ± SD, n=3)			
		Without S-9	With S-9		
		TA1535	TA1535	TA1537	MP2 uvrA
DMSO	50 μL/plate	10 ± 2	9 ± 2	10 ± 2	11 ± 1
WAY-173606	1.0	10 ± 2	9 ± 1	10 ± 2	11 ± 2
	3.3	11 ± 1	10 ± 2	9 ± 1	11 ± 1
	10	9 ± 1	9 ± 1	10 ± 3	12 ± 2
	33	11 ± 1	9 ± 2	12 ± 2	12 ± 1
	100	10 ± 2	11 ± 1	7 ± 1	13 ± 1
	333	0 ± 0*	10 ± 1	10 ± 2	13 ± 1
	1000	0 ± 0*	0 ± 0*	0 ± 0*	11 ± 3
Positive control	(see above for + controls)	391 ± 28	122 ± 7	146 ± 12	550 ± 50

The following study is reviewed and summarized:
Study title: WAY-173606 (Batch 11): *Salmonella typhimurium* reverse mutation screening assay (Ames Test) (Study #RPT-46058; Protocol 02_0225) (Non-GLP)
Conducting laboratory: Wyeth Research, Chazy, NY (Study initiation date: April 2, 2002)

Summary:
The mutagenic activity of WAY-173606 (Batch 11) was assessed via Ames test, using the plate incorporation method (incubation duration: 48-72 hours). The bacterial strains used were *Salmonella typhimurium* TA98 and TA100, and the revertant colonies were counted via an automatic counter. DMSO (50 μL/plate) was used as the vehicle control, and the positive control is described below:

With S-9: 2-aminoanthracene (2.5 μg/plate).

Without S-9: TA98: 2-nitrofluorene (1.0 μg/plate), TA100: sodium azide (5.0 μg/plate).

Concentrations of WAY-173606 were 10, 33, 100, 333, 1000, 3330 and 5000 μg/plate, respectively. Precipitation of test article occurred in both strains with and without metabolic activation at 1000 μg/plate. Toxicity occurred in TA98 with metabolic activation at 1000 μg/plate. Toxicity occurred in TA100 with and without metabolic activation and in TA98 without metabolic activation at 333 μg/plate.

The study is considered valid, because:

- Tester strain integrity was documented in the report.
- The mean positive control value (± S9-mix) exhibited at least three fold increase over the respective mean vehicle control value for each tester strain.
- There was a minimum of three nontoxic concentrations (≤ 50% reduction in mean number of revertants/plate relative to the mean vehicle control value; or at least a moderate reduction in the background lawn, i.e., background lawn code 3-5) in each tester strain, both in the absence and presence of S9-mix.

However, the following information was not found in the report:

- The tester strain culture titers.
- The laboratory historical range of negative (vehicle) and positive control data.

It was found that no positive increases in the number of revertants in the plates treated with WAY-173606, in the presence or absence of S-9 metabolic activation. Thus, WAY-173606 (Batch 11) was not mutagenic under the condition of the study.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: WAY-173606: In vitro mammalian chromosome aberration test in human peripheral blood lymphocytes

Study no.:	RPT-50322 (Protocol 03_0252)
Study report location:	Wyeth
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 16, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	WAY-173606 (bisutinib); Lot# L23445-136, 99.49%

Key Study Findings

Under the condition of the study, WAY-173606 was not clastogenic, in the absence and presence of S9 activation

Methods

Cell line:	Human peripheral blood lymphocytes (HPBL) (from a healthy adult male)
Concentrations in definitive study:	0.25, 0.5, 1, 2.5, 5, 7.5 and 10 μg/mL
Basis of concentration selection:	See below
Negative control:	Vehicle control (dimethyl sulfoxide,DMSO)
Positive control:	See below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	See below

Concentration selection criteria

Based on reduced cell growth (cytotoxicity studies) and on the depression of mitotic index during the chromosomal aberration test. A total of two experiments were performed.

Range finding studies: none.

Test agent stability: Stable in DMSO up to 500 mg/mL. Precipitation of test article was observed at concentrations > 50 μg/mL.

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

Controls:

Vehicle: DMSO

Negative controls: vehicle (solvent) control.

Positive controls:

With S-9: Cyclophosphamide (CP: 20 μg/mL)

Without S-9: Mitomycin (MMC, 0.3 μg/mL)

Table 45 Historical control data: chromosome aberration in HPBL

Historical control data (2005-2007):

Without S9 activation:

Historical Values	Percent Aberrant Cells (%)	
	Solvent Control[1]	Positive Control[2]
Mean	0.0	17.6
Standard Deviation	±0.1	±4.4
Range	0.0-0.5	5.5-40.0

With S9 activation:

Historical Values	Percent Aberrant Cells (%)	
	Solvent Control[1]	Positive Control[3]
Mean	0.0	17.0
Standard Deviation	±0.1	±3.1
Range	0.0-0.5	9.0-24.0

1. **Solvents include water, saline, DMSO, ethanol, acetone, and other non-standard and Sponsor-supplied vehicles.**
2. **Positive control for non-activated studies is mitomycin C (MMC).**
3. **Positive control for S9 activated studies is cyclophosphamide (CP).**

Exposure conditions:

Incubation and sampling times:

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

Concentrations used in the Experiments:

- Preliminary cytotoxicity assay: 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 μg/mL.
- Chromosome aberration assay: based on the preliminary toxicity assay (i.e., toxicity response, see below) the condition and concentrations in this assay were shown in the following table:

Treatment condition	Treatment time	Recovery time	Concentrations (μg/mL)
Without S9	4 hr	16 hr	0.25, 0.5, 1, 2.5, 5, 7.5, 10
	20 hr	0 hr	0.25, 0.5, 1, 2.5, 5, 7.5, 10
With S9	4 hr	16 hr	0.25, 0.5, 1, 2.5, 5, 7.5, 10

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

Evaluation of metaphase cells:

To ensure that an acceptable response is obtained from the positive controls, 25-50 cells from each positive control culture in both the non-activated and the S9 activated groups were assessed, prior to the analysis of the slides by a scorer. At least 6% aberrant cells from each culture is required in order to proceed with analysis of the study. A minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations.

- Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements.
- Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings.
- Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange.
- Pulverized chromosome(s), pulverized cells and severely damaged cells (≥10 aberrations) also were recorded.
- The mitotic index was recorded as the percentage of cells in mitosis per 500 cells counted.
- The percent polyploid and endoreduplicated cells was evaluated per 100 cells.

Evaluation of test result:

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

Study Validity

The study is considered valid, because:

- The percentage of cells with chromosome aberrations in the positive control had to be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control in each assay, with or without S9.
- The vehicle control data were within the laboratory historical range.

Results

- Preliminary toxicity assay: in three experiments.

Table 46 Preliminary toxicity assay

The table below is the summary of the preliminary assay:

Experiment		Experiment 1		Experiment 2		Experiment 3	
Condition*		4 hr treatment without S9		4 hr treatment with S9		20 hr treatment without S9	
		Mitotic index (%)	Percent change (%)	Mitotic index (%)	Percent change (%)	Mitotic index (%)	Percent change (%)
DMSO	100 μL	9.6		9.0		7.2	
WAY-173606	0.5 μg/mL	8.0	-17	8.4	-7	6.6	-8
	1.5	6.8	-29	7.8	-13	5.4	-25
	5	4.6	-52	4.2	-53	3.2	-56
	15	2.0	-79	3.6	-60	2.0	-72
	50	0.6	-94	0.6	-93	0.0	-100
	150	0.0	-100	0.0	-100	0.0	-100
	500	0.0	-100	0.0	-100	0.0	-100
	1500	0.0	-100	0.0	-100	0.0	-100
	5000	0.0	-100	0.0	-100	0.0	-100

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

Toxicity (mitotic inhibition) in excess of 50% (i.e., over 50% depression from the control) was observed at WAY-173606 concentrations of ≥ 5 μg/mL in all three treatment conditions.

- Chromosome aberration assay: in three experiments.

The group means of the mitotic index determination and the cytogenetic analysis of three treatment conditions are summarized in the table below (from the Applicant; data of individual experiment are not shown). The results from cells treated at 0.25, 5, 7.5 and 10 μg/mL of WAY-173606 were not scored. At the highest concentration evaluated microscopically for chromosome aberrations, 2.5 μg/mL, mitotic inhibition was 53%. The percentage of cells with structural or numerical aberrations in the WAY-173606 treated groups was not significantly increased above that of the solvent levels at any of the concentrations evaluated. The percentage of structurally damaged cells in the positive control samples was all statistically significant, in comparison to the solvent control.

Table 47 Group means of mitotic index and cytogenetic analysis

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-	4	8.7	200	0.000	±0.000	0.0	0.0
WAY-173606								
0.5	-	4	7.1	200	0.000	±0.000	0.0	0.0
1	-	4	6.2	200	0.005	±0.071	0.0	0.0
2.5	-	4	4.1	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-	4	5.4	200	0.145	±0.380	0.0	13.5**
DMSO	+	4	7.2	200	0.000	±0.000	0.0	0.0
WAY-173606								
0.5	+	4	6.0	200	0.005	±0.071	0.0	0.5
1	+	4	5.3	200	0.000	±0.000	0.0	0.0
2.5	+	4	3.4	200	0.000	±0.000	0.5	0.0
CP, 20	+	4	3.3	200	0.195	±0.478	0.0	16.5**
DMSO	-	20	10.0	200	0.000	±0.000	0.0	0.0
WAY-173606								
0.5	-	20	6.8	200	0.000	±0.000	0.0	0.0
1	-	20	7.3	200	0.000	±0.000	0.0	0.0
2.5	-	20	4.7	200	0.000	±0.000	0.0	0.0
MMC, 0.3	-	20	4.9	200	0.090	±0.287	0.0	9.0**

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

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

Study title: SKI-606: Single dose oral (garage) bone marrow micronucleus study in male mice

Study no.:	RPT-52501 (Protocol 03_1445)
Study report location:	Wyeth
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 23, 2003 (TK study: October 14, 2003)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SKI-606 (WAY-173606); Lot# MP030103, 99.49% (for TK study: Lot# MP030203, 94.2%)

Key Study Findings

SKI-606 was negative in the mouse bone marrow micronucleus assay, under the condition of the study. The AUC_{0-24} value at the highest dose used, 2000 mg/kg, was 172495 ± 26050 ng·hr/mL.

Methods

Doses in definitive study:	0, 500, 1000, and 2000 mg/kg (as Groups 1, 2, 3 and 4)
Frequency of dosing:	Single dose
Route of administration:	Oral (gavage)
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose (4000 cps) (w/v), 2% polysorbate 80, NF (w/v), 0.06% glacial acetic acid (w/v) and purified (Type 1) water
Species/Strain:	Male CD mouse (Crl:CD-1® (ICR) BR mice)
Number/Sex/Group:	N=6, except for Group 4 (n=7) (see table below)
Satellite groups:	None. A toxicokinetics study (Study #RPT-52338) (n=24) was conducted separately.
Basis of dose selection:	Not reported
Negative control:	Vehicle
Positive control:	Cyclophosphamide (CP, 50 mg/kg)

Test agent stability: the stability and uniformity (homogeneity) of test article dosing formulation were determined and demonstrated to be stable in the concentration range of 0.1 to 200 mg/mL for at least 14 days when stored refrigerated and protected from light.

Study design: Table from the Applicant

- Male mice: ~6-8 weeks, weight: 25.8-31.2 g

Males only were used in the current study because there was no difference in the observed toxicity between male and female CD-1 mice in the previously conducted oral acute toxicity study (Study # RPT-52017; data not shown).

Reviewer's note: both male and female mice were used in this single dose toxicology study (control and WAY-173606 at 2000 μg/kg) and the NOAEL was determined to be 2000 μg/kg. According to the PK studies, exposures in female mice were higher than those in male mice. Since no bone marrow lesions or hematological findings (especially erythrocyte parameters and reticulocytes) were reported for both genders at 2000 μg/kg, a maximum dose level indicated in ICH S2 (R1) for *in vivo* micronucleus assay, the use of male mice only in the current study is acceptable.

- Dose schedule: single oral administration at 0, 500, 1000 and 2000 mg/kg (as Groups 1, 2, 3 and 4).

Treatment (10 mL/kg)	[a]Number of Male Mice Dosed and Bone Marrow Collected at	
	24 hr postdose	48 hr postdose
Group 1, Vehicle Control: Specified by Sponsor	6	6
Test Article (SKI-606):		
Group 2, Low dose (500 mg/kg)	6	6
Group 3, Mid Dose (1000 mg/kg)	6	6
Group 4, High Dose (2000 mg/kg)	7	7
Group 5, Positive Control: CP (50 mg/kg)	6	0

a: Bone marrow analysis was conducted using 5 animals/treatment.

- Micronucleus assay:
  - Bone marrow harvest time points: Twice samplings in Groups 1 to 4 at 24 and 48 hours, respectively, after dosing; and for positive controls, only one sampling at 24 hours post-dosing.
  - Slide analysis: The bone marrow cells collected from both femurs of each mouse were used to prepare smears on slides. Two smears were prepared from each animal. Cells (polychromatic erythrocytes [PCEs: immature erythrocytes] and normochromatic erythrocytes [NCEs: mature erythrocytes]) on slides were stained, and scored (5 randomly selected animals/treatment) for micronuclei and the MPCEs (micronucleated PCEs) and MNCEs (micrnucleated NCEs).
  - The extent of treatment-induced chromosome damage, indicated by the number of MNCEs, was determined by analyzing 2000 immature erythrocytes (NCEs) per animals for the presence of micronuclei.
  - The number of MPCEs, was the mean value expressed per 2000 mature erythrocytes (PCEs) examined. The data were not used to evaluate the response of the test article; however, the data are included in the study file but are not presented in the current report.
  - In addition, the proportion of immature erythrocytes (NCEs), expressed as percent NCE/(NCE + PCE), was assessed by examination of a total of 1000 erythrocytes per animal.

- ✧ Historical background frequency of micronuclei was: individual animal mean ratio of PCE/EC: 0.39 (0.13-0.76); MPCE/1000PCE scored/animal: 23.95 (5.5-79); MPCE/50000 PCE scored/group: 119.73 (46-250).

- ➢ In-life observation: All animals were observed following dosing for mortality, clinical signs and reaction to treatment. Individual body weights were measured prior to randomization, prior to treatment and at termination.
- ➢ Toxicokinetics: (Study #RPT-52825, GLP; study started on) (Protocol 03_1826) Plasma samples were obtained via cardiac puncture at 0.5, 1, 2, 4, 8, 12, 24 and 36 hours (n=3/time point) after dosing (at 2000 μg/kg, male CD-1 mice with comparable ages and body weights as the main study animals, n=24). Plasma samples were analyzed using a validated LC/MS/MS method for the quantification of SKI-606 and the pharmacokinetic parameters were calculated.

Assay acceptance criteria:

- ➢ Statistical significance was determined using the Kastenbaum-Bowman tables which are based on the binomial distribution (Kastenbaum and Bowman, 1970). All analyses were performed separately for each sampling time.
- ➢ The test article was considered to induce a positive response if the incidence of micronucleated polychromatic erythrocytes (PCEs) for the test article treatment was statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman Tables) at any sampling time. However, values which were statistically significant but did not exceed the range of historical negative or vehicle controls (see above) were judged as not biologically relevant.
- ➢ The test article was judged negative if no statistically significant increase in micronucleated polychromatic erythrocytes above the concurrent vehicle control was observed at any sampling time.
- ➢ A positive response was the detection of a statistically significant dose-related increase in the incidence of micronucleated PCEs (MPCEs) above the control level ($p \leq 0.01$). Individual and/or group mean values should also exceed the laboratory historical control range.
- ➢ In order to quantify the test article effect on erythropoiesis, as an indicator of bone marrow toxicity, the proportion of polychromatic erythrocytes to total erythrocytes (PCEs/ECs ratio) was determined for each animal and treatment group.

Result:

Study validity:

The study is considered valid, because of:

- Acceptable controls: the incidence of MPCE for the vehicle control was close to or within the laboratory historical vehicle/negative control range, while the positive control had a statistically significantly higher number of MPCE's than the vehicle control ($p \leq 0.05$).
- The mean incidence of micronucleated polychromatic erythrocytes (MPCE) did not exceed 5/1000 polychromatic erythrocytes (0.5%) in the negative (vehicle) control.

- Acceptable high dose: the high dose used 25 mg/kg, was the MTD of dose range-finding study.

Study Validity

Study validity:
The study is considered valid, because of:

- Acceptable controls: the incidence of MPCE for the vehicle control was close to or within the laboratory historical vehicle/negative control range, while the positive control had a statistically significantly higher number of MPCE's than the vehicle control ($p \leq 0.05$).
- The mean incidence of micronucleated polychromatic erythrocytes (MPCE) did not exceed 5/1000 polychromatic erythrocytes (0.5%) in the negative (vehicle) control.
- Acceptable high dose: the high dose used 25 mg/kg, was the MTD of dose range-finding study.

Results

Study outcome:

- ✧ There were no remarkable findings in mortality or clinical signs. Piloerection was observed at ≥ 1000 μg/kg, and lethargy at 2000 μg/kg.
- ✧ Micronucleus analysis (mean ± SD) at 24 and 48 hr sampling time: table from the Applicant.

Table 48 Micronucleus analysis

Treatment (10 mL/kg)	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes: Number per 1000 PCEs (Mean +/- SD)	Micronucleated Polychromatic Erythrocytes: Number per PCEs Scored[1]
Vehicle: Specified by Sponsor	M	24	5	0.511 ± 0.09	---	0.6 ± 0.22	6 / 10000
SKI-606							
500 mg/kg	M	24	5	0.439 ± 0.08	-14	0.7 ± 0.45	7 / 10000
1000 mg/kg	M	24	5	0.457 ± 0.04	-11	0.7 ± 0.45	7 / 10000
2000 mg/kg	M	24	5	0.464 ± 0.08	-9	0.7 ± 0.27	7 / 10000
CP							
50 mg/kg	M	24	5	0.342 ± 0.02	-33	18.8 ± 3.55	*188 / 10000
Vehicle: Specified by Sponsor	M	48	5	0.485 ± 0.06	---	0.3 ± 0.27	3 / 10000
SKI-606 (WAY-173606)							
500 mg/kg	M	48	5	0.482 ± 0.08	-1	0.7 ± 0.27	7 / 10000
1000 mg/kg	M	48	5	0.451 ± 0.03	-7	0.6 ± 0.42	6 / 10000
2000 mg/kg	M	48	5	0.433 ± 0.07	-11	0.6 ± 0.42	6 / 10000

[1]*Statistically significant, $p \leq 0.05$ (Kastenbaum-Bowman Tables)

- ✧ SKI-606 did not increase the incidence of micronucleated immature erythrocytes (MICE) at either sampling time. Individual and group mean values for treated animals were within the range of historical control values (data not shown).
- ✧ The incidence of MPCE for all groups was uniformly low, confirming the absence of micronucleus-like artifacts.
- ✧ SKI-606 treatment did not reduce the proportion of immature erythrocytes, indicating a lack of bone marrow toxicity.

Toxicokinetics:

Table 49 Toxicokinetic parameters: in vivo micronuleus assay

The PK parameters were summarized as the following (Table from the Applicant):

Dose (mg/kg)	Sex	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)
2000	M	9811 ± 3998	2.0	172495 ± 26050

7.4 Other Genetic Toxicity Studies

Mutagenicity assessment of human metabolite M2:

Study title: SKI-606: Bacteria reverse mutation test of M2 metabolite with *Salmonella typhimurium* and *Escherichia coli*

Study no.:	RPT-73086 (Protocol 08_0036)
Study report location:	Wyeth
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 15, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	WAY-198760 (SKI-606 metabolite M2), lot#MP040733, 95.6%

Key Study Findings

- Elevated numbers of revertants with tester strain TA100, at WAY-198760 ≥ 3333 μg/plate in the presence of S9 activation, were observed in the preliminary and initial mutagenicity assays (> 3 fold and ~2.1 fold of the vehicle control, respectively). Such results were not reproduced in a confirmatory mutageniciity assay.
- Because the number of revertants was considerably smaller at concentrations ≥ 1500 μg/plate in the confirmatory mutagenicity assay, with comparable revertant numbers with TA 100 at other concentrations of WAY-198760 and controls in all experiments, the result of the confirmatory mytagenicity assay was questionable.
- Thus, WAY-198760 (M2 metabolite) may be positive in the Ames test with tester strain TA100 in the presence of S9 activation.

Methods

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

***The dosing solutions were adjusted to compensate for the use-at value (88.7%) of the test article.**

<u>Concentration selection criteria</u>
Basis of concentration selection: based on previous experiments; data were not available in this submission. The sponsor followed ICH (1996 and 1997) and OEDC (1998) guideline and employed concentrations up to 5 mg/plate as the highest

concentration in the study. No toxicities were observed in the preliminary assay. Precipitate was observed at 3333 or 5000 μg per plate. Based on non-toxic findings in the preliminary assay, the maximum concentration plated in the mutagenicity assay was 5000 μg per plate. However, toxicity was observed beginning at 1500, 3000 or 5000 μg per plate in the confirmatory mutagenicity assay. Precipitate was observed at 1500 or 5000 μg per plate (and 3000 μg/plate for TA100) in the confirmatory mutagenicity assay. Since precipitate was observed in the top 2 concentrations, this indicates that the test system was saturated with the test article. Based on these results, the regulatory-required top dose level was achieved in each case and the results support the validity of the study conclusion.

Test agent stability: Stable in dimethyl sulfoxide (DMSO) under frozen conditions for 6 days
Metabolic activation system: Aroclor 1254 induced rat liver microsome S-9 mix
Controls:
Vehicle: DMSO (50 μL/plate)
Negative controls: vehicle control
Positive controls: all dissolved in DMSO except for sodium azide, which was diluted with water.
With S-9: TA98, TA100, TA1535 and TA1537 and WP2 *uvrA*: 2-aminoanthracene (1 and 10 μg/plate for all the *Salmonella* strains and WP2 *uvr*A, respectively).
Without S-9: TA98: 2-nitrofluorene (1.0 μg/plate), TA 1537: 9-aminoacridine (75 μg/plate), TA100 and TA1535: sodium azide (1.0 μg/plate), WP2 *uvr*A: methyl methanesulfate (1000 μg/plate).
Study design:
The assay was performed in two phases, using the plate incorporation and pre-incubation methods. The first phase, the preliminary toxicity assay, was used to establish the dose-range for the mutagenicity assay. The second phase, the mutagenicity assay (initial and confirmatory assays), was used to evaluate the mutagenic potential of the test article. The pre-incubation method was used in the confirmatory mutagenicity assay (i.e., Experiment B2) only.

Replicate plate procedure:
This procedure was used when increases in revertant counts were observed on plates that exhibited background lawn toxicity or when there were intermediately sized colonies on the plates that made determination of the true revertants difficult. The procedure was used to ensure that only true revertant colonies were included in the revertant counts reported on the data tables. The colony in question was marked and transferred to a fresh sterile minimal glucose bottom agar plate containing 50 μM biotin. The plate was incubated for 24 to 48 hours at 37 ± 2°C and stored at 2-8°C until counted. Each plate was evaluated for revertant colony distribution and number.

Exposure conditions:
Incubation and sampling times:
- Plate incorporation: 48-72 hours
- Pre-incubation: 48-72 hours

Concentrations used:

- Preliminary assay (Experiment A): 6.7, 10, 33, 67, 100, 333, 667, 1000, 3333 and 5000 μg per plate.
- Mutagenicity assay (Experiment B1: initial and Experiment B2: confirmatory): 50, 150, 500, 1500 and 5000 μg per plate. One additional concentration, 3000 μg per plate was used for TA100 in the presence of S9 activation
- WAY-173606: stock solution (in DMSO): 100 mg/mL; plating aliquot: 50 μL

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

An equivocal response was a biologically relevant increase in a revertant count that partially met the criteria for evaluation as positive. This could have been a dose-responsive increase that achieved the respective threshold cited above or a non-dose responsive increase that was equal to or greater than the respective threshold cited.

Analysis:
Number of replicates:
Preliminary assay (Experiment A): one plate per dose
Mutagenicity assay (Experiment B): 3 plates for each test compound concentration
Counting method: automated colony counter (image analyzer).

Study Validity

The study is considered valid, because:

- Tester strain integrity was documented in the report.
- All tester strain culture titers were greater or equal to 0.3 x10^9 cells/mL, to ensure that appropriate numbers of bacteria were plated.

Reviewer's note: In fact, lower titers were plated in Experiment B2 (0.6 - 2 x 10^8 cells/mL were used).

- Both negative (vehicle) and positive control data were within the laboratory historical range.
- The mean positive control value (± S9-mix) exhibited at least three fold increase over the respective mean vehicle control value for each tester strain.
- There was a minimum of three nontoxic concentrations. A concentration level was considered toxic if one or both of the following criteria were met: 1) ≥ 50% reduction in mean number of revertants/plate relative to the mean vehicle control value; or 2) at least a moderate reduction in the background lawn, i.e.,

background lawn code 3-5) in each tester strain, both in the absence and presence of S9-mix.

Results

- Experiment A (preliminary; plate incorporation)

Table 50 Number of revertant colonies: preliminary, metabolite M2

The numbers of revertant colonies/plate at each test compound concentration, and vehicle and positive controls are summarized in the table below:

Treatment	Concentration (mg/plate)	Revertant colonies/plate (n=1)				
Without S-9						
		TA98	TA100	TA1535	TA1537	MP2 uvrA
DMSO	50 μL/plate	33	248	24	8	27
WAY-173606	6.7	28	246	26	7	29
	10	19	253	22	9	21
	33	26	232	15	11	32
	67	23	193	23	13	21
	100	28	229	25	12	18
	333	26	227	24	7	32
	667	25	223	30	8	27
	1000	18	230	23	12	25
	3333	21	251	24	7	20
	5000	27	334	20	9	29
With S-9						
DMSO	50 μL/plate	37	148	12	14	17
WAY-173606	6.7	32	264	21	12	28
	10	28	244	26	7	15
	33	36	231	24	10	18
	67	34	245	37	12	22
	100	45	191	29	12	22
	333	26	324	22	13	25
	667	38	305	31	15	28
	1000	36	291	22	12	23
	3333	37	**499**	19	8	18
	5000	38	**936**	31	12	22

Reviewer's note: There were > 3 fold increases in the number of revertants (in bold prints; compared to the vehicle control) with TA100 at concentrations ≥3333 μg/plate in the presence of S9 activation. A 2.1 fold increase was observed again at 5000 μg/plate in the initial mutagenicity assay (Experiment B1, see below). According to the Applicant (no data included in the current report), the replicate plates for the vehicle and 5000 μg per plate concentration for tester strain TA100 in the presence of S9 activation confirmed that the correct colonies were evaluated as revertants.

- Experiment B1 (initial mutagenicity; plate incorporation)

Table 51 Number of revertant colonies: initial mutagenicity, metabolite M2

The result is summarized in the table below (from the Applicant); the respective positive controls for the bacterial strains are described above.

Average Revertants Per Plate ± Standard Deviation

Activation Condition	: None														
Dose (µg/plate)	TA98			TA100			TA1535			TA1537			WP2 *uvr*A		
Vehicle	12	±	5	198	±	24	23	±	6	8	±	2	15	±	5
50	12	±	5	221	±	17	20	±	4	7	±	3	17	±	3
150	15	±	5	183	±	19	21	±	1	4	±	2	17	±	3
500	14	±	3	210	±	25	19	±	12	3	±	2	10	±	6
1500	12	±	6	240	±	26	17	±	10	3	±	3	7	±	2
5000	11	±	4	317	±	24	13	±	5	9	±	2	11	±	1
Positive	135	±	8	612	±	50	293	±	49	578	±	119	140	±	23

Activation Condition	: Rat Liver S9														
Dose (µg/plate)	TA98			TA100			TA1535			TA1537			WP2 *uvr*A		
Vehicle	23	±	9	199	±	16	16	±	5	4	±	2	16	±	4
50	20	±	0	200	±	24	15	±	3	8	±	2	18	±	3
150	19	±	9	142	±	7	10	±	4	6	±	2	14	±	4
500	25	±	2	175	±	13	16	±	4	8	±	5	12	±	5
1500	19	±	6	186	±	15	11	±	4	7	±	3	13	±	4
3000				176	±	7									
5000	15	±	5	424	±	109	20	±	3	5	±	3	18	±	6
Positive	600	±	191	643	±	120	82	±	30	183	±	108	349	±	12

Vehicle = Vehicle Control

Positive = Positive Control (50 µL plating aliquot)

Plating aliquot = 50 µL

Experiment B2 (confirmatory, pre-incubation)

Table 52 Number of revertant colonies: confirmatory, metabolite M2

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None																		
Dose (µg/plate)	TA98			TA100			TA1535			TA1537			WP2 *uvr*A		
Vehicle	16	±	2	93	±	2	18	±	6	9	±	1	12	±	1
50	13	±	3	108	±	12	19	±	6	6	±	4	10	±	2
150	16	±	4	105	±	7	18	±	4	10	±	3	13	±	3
500	13	±	3	102	±	2	14	±	4	8	±	1	13	±	2
1500	10	±	1	47	±	6	7	±	2	5	±	2	11	±	3
5000	9	±	2	58	±	16	6	±	5	1	±	1	11	±	2
Positive	314	±	33	468	±	18	382	±	51	677	±	143	397	±	89

Activation Condition : Rat Liver S9															
Dose (µg/plate)	TA98			TA100			TA1535			TA1537			WP2 *uvr*A		
Vehicle	18	±	6	122	±	23	23	±	4	10	±	3	16	±	4
50	23	±	11	132	±	16	14	±	4	9	±	2	13	±	4
150	25	±	6	115	±	12	16	±	5	12	±	3	15	±	8
500	26	±	11	109	±	4	18	±	7	11	±	5	17	±	5
1500	22	±	3	97	±	14	17	±	2	11	±	3	20	±	4
3000				84	±	21									
5000	14	±	2	78	±	5	14	±	1	8	±	1	13	±	2
Positive	1341	±	243	1096	±	172	183	±	32	187	±	42	235	±	98

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 50 µL

According to the Applicant, the 2.1-fold increase in the number of revertants per plate observed with tester strain TA100 in the presence of S9 in Experiment B1 was not reproduced in Experiment B2. Therefore, the overall response for WAY-198760 (metabolite M2) was negative for mutagenicity in the study. However, the number of revertants at concentrations ≥ 1500 µg/plate in Experiment B2 were considerably smaller than those in Experiment A and Experiment B1, in the absence or presence S9 activation. As noted, the number of revertants at other concentration and for the controls (negative and positive) remained comparable in all three experiments. Furthermore, the revertant colonies with TA100 in the presence of S2 in Experiment A were confirmed during replicate plate procedure. The Applicant's conclusion regarding the mutagenicity of WAY-198760 (metabolite M2) was questionable. The review team submitted a request to the CDER Computational Toxicology Consulting Service, and a negative result was recommended.

In vitro chromosomal aberration assay in mammalian cells for M2 metabolite:

Study title: SKI-606: Bacteria reverse mutation test of M2 metabolite with *Salmonella typhimurium* and *Escherichia coli*

Study no.:	RPT-73087 (Protocol 08_0037)
Study report location:	Wyeth
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 19, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	WAY-198760 (SKI-606 metabolite M2), lot#MP040733, 95.6%

Key Study Findings

Under the condition of the study, WAY-198760 was not clastogenic, in the absence and presence of S9 activation

Methods

Strains:	Human peripheral blood lymphocytes (HPBL) (from a healthy adult female)
Concentrations in definitive study:	10, 15, and 25 μg/mL* (selected for microscopic evaluation)
Basis of concentration selection:	See below
Negative control:	Vehicle control (dimethyl sulfoxide,DMSO)
Positive control:	See below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	See below

***The dosing solutions were adjusted to compensate for the use-at value (88.7%) of the test article.**

Concentration selection criteria

- Based on reduced cell growth (cytotoxicity studies) and on the depression of mitotic index during the chromosomal aberration test. A total of three experiments were performed (a preliminary toxicity assay and initial and repeat chromosome aberration assays).
- Range finding studies: none.

Test agent stability and solubility: Stable in DMSO up to 500 mg/mL. At the beginning of treatment period, visible precipitation of test article was observed at concentrations > 500 μg/mL., and concentrations ≤ 150 μg/mL of the test article were soluble in treatment medium. At the conclusion of the treatment period, in the non-activated 4 and 20-hour exposure groups, visible precipitate was observed at WAY-198760 concentration of ≥ 1500 μg/mL and WAY-198760 concentrations of ≤ 500 μg/mL were soluble in treatment medium.

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

Controls:

Vehicle: DMSO

Negative controls: vehicle (solvent) control.

Positive controls:
With S-9: Cyclophosphamide (CP: 20 μg/mL)
Without S-9: Mitomycin (MMC, 0.3 μg/mL)

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

Historical Values	Percent Aberrant Cells (%)	
	Solvent Control[1]	Positive Control[2]
Mean	0.0	17.6
Standard Deviation	±0.1	±4.4
Range	0.0-0.5	5.5-40.0

With S9 activation:

Historical Values	Percent Aberrant Cells (%)	
	Solvent Control[1]	Positive Control[3]
Mean	0.0	17.0
Standard Deviation	±0.1	±3.1
Range	0.0-0.5	9.0-24.0

1. **Solvents include water, saline, DMSO, ethanol, acetone, and other non-standard and Sponsor-supplied vehicles.**
2. **Positive control for non-activated studies is mitomycin C (MMC).**
3. **Positive control for S9 activated studies is cyclophosphamide (CP).**

Exposure conditions:
Incubation and sampling times:
- Preliminary toxicity assay: in the absence of S9: Pulse treatment 4 hr and continued for 20 hr (~1.5 cell cycle); in the presence of S9: incubation for 4 hours
- Chromosome aberration assay: see table below

Concentrations used in the Experiments:
- Preliminary cytotoxicity assay: 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 μg/mL.
- Chromosome aberration assay: based on the preliminary toxicity assay (i.e., toxicity response, see below) the condition and concentrations in this assay were shown in the following table:

Treatment condition	Treatment time	Recovery time	Concentrations (μg/mL)
Without S9	4 hr	16 hr	10, 15, 25, 50, 75, 100, 125 and 150
	20 hr	0 hr	5, 10, 15, 25, 35 and 50
With S9	4 hr	16 hr	5, 10, 15, 25, 35, 50, 60 and 75

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

Evaluation of metaphase cells:
To ensure that an acceptable response is obtained from the positive controls, 25-50 cells from each positive control culture in both the non-activated and the S9 activated groups were assessed, prior to the analysis of the slides by a scorer. At least 6% aberrant cells from each culture is required in order to proceed with analysis of the study. A minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations.

- Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements.
- Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings.
- Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange.
- Pulverized chromosome(s), pulverized cells and severely damaged cells (≥10 aberrations) also were recorded.
- The mitotic index was recorded as the percentage of cells in mitosis per 500 cells counted.
- The percent polyploid and endoreduplicated cells was evaluated per 100 cells.

Evaluation of test result:

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

Study Validity

The study is considered valid, because:

- The percentage of cells with chromosome aberrations in the positive control had to be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control in each assay, with or without S9.
- The vehicle control data were within the laboratory historical range.

Results

- Preliminary toxicity assay: in three experiments.

Table 53 Preliminary toxicity assay: metabolite M2

The table below is the summary of the preliminary assay:

Experiment		Experiment 1		Experiment 2		Experiment 3	
Condition*		4 hr treatment without S9		4 hr treatment with S9		20 hr treatment without S9	
		Mitotic index (%)	Percent change (%)	Mitotic index (%)	Percent change (%)	Mitotic index (%)	Percent change (%)
DMSO	100 μL	12.2		8.0		12.2	
WAY-198760	0.5 μg/mL	10.8	-11	7.0	-13	11.6	-5
	1.5	10.8	-11	7.2	-10	10.4	-15
	5	8.8	-28	6.2	-23	9.2	-25
	15	6.6	-46	4.6	-43	5.8	-52
	50	4.4	-64	4.0	-50	5.0	-59
	150	2.6	-79	2.2	-73	0.4	-97
	500	0.4	-97	0.4	-95	0.0	-100
	1500	0.0	-100	0.0	-100	0.0	-100
	5000	0.0	-100	0.0	-100	0.0	-100

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

Toxicity (mitotic inhibition) in excess of 50% (i.e., over 50% depression from the control) was observed at WAY-198760 dose levels of 50, 150, 500, 1500, and 5000 μg/mL in the non-activated and S9-activated 4-hour treatment groups, and at WAY-198760 dose levels of 15, 50, 150, 500, 1500 and 5000 μg/mL in the non-activated 20-hour treatment group.

- Chromosome aberration assay: in three experiments.

The group means of the mitotic index determination and the cytogenetic analysis of three treatment conditions are summarized in the table below (from the Applicant; data of individual experiment are not shown). The highest concentrations scored in the three experiments varied slightly: 25 μg/mL for 4 hour incubation without S9, and 15 μg/mL for 4 hour incubation with S9 and 20 hour without S9, respectively. At the highest concentration evaluated microscopically for chromosome aberrations was 53-54%. The results from cells treated at less than 10 μg/mL or greater than 25 or 15 of μg/mL WAY-198760 were not scored. The percentage of cells with structural or numerical aberrations in the WAY-198760 treated groups was not significantly increased above that of the solvent levels at any of the concentrations evaluated. The percentage of structurally damaged cells in the positive control samples was all statistically significant, in comparison to the solvent control.

Table 54 Chromosome aberration assay: metabolite M2

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored Numerical	Cells Scored Structural	Aberrations per Cell (Mean +/- SD)		Cells with Aberrations Numerical (%)	Cells with Aberrations Structural (%)
DMSO	-S9	4	6.1	200	200	0.000	±0.000	0.0	0.0
WAY-198760									
10	-S9	4	5.6	200	200	0.000	±0.000	0.0	0.0
15	-S9	4	3.7	200	200	0.005	±0.071	0.0	0.5
25	-S9	4	2.8	200	200	0.000	±0.000	0.0	0.0
MMC, 0.6	-S9	4	1.9	200	100	0.310	±0.662	0.0	21.0**
DMSO	+S9	4	5.1	200	200	0.000	±0.000	0.0	0.0
WAY-198760									
5	+S9	4	5.2	200	200	0.000	±0.000	0.0	0.0
10	+S9	4	4.7	200	200	0.000	±0.000	0.0	0.0
15	+S9	4	2.4	200	200	0.000	±0.000	0.0	0.0
CP, 20	+S9	4	1.2	200	100	0.350	±0.730	0.0	22.0**
DMSO	-S9	20	5.0	200	200	0.000	±0.000	0.0	0.0
WAY-198760									
5	-S9	20	3.7	200	200	0.005	±0.071	0.0	0.5
10	-S9	20	3.2	200	200	0.000	±0.000	0.0	0.0
15	-S9	20	2.3	200	200	0.005	±0.071	0.0	0.5
MMC, 0.3	-S9	20	2.4	200	100	0.270	±0.664	0.0	18.0**

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

8 Carcinogenicity

A report of 2-year carcinogenicity study in rats is reviewed by Dr. Shawna Weis. The report will filed separately by Dr. Weis..

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: SKI-606: Oral (gavage) fertility study in rats

Study no.:	PRT-63257 (Protocol 06_0073)
Study report location:	Wyeth
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 16, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	WAY-173606 (bisutinib); Lot# RB-5626, 96.7% (total impurities: 0.08%)

Key Study Findings

- SKI-606 treatment induced mortality (1/25, at 70 mg/kg), reduction in body weight, weight gain and food consumption, and GI-related clinical signs in males. No effects were found in male reproductive organs or mating index, but reduced fertility index was reported at 70 mg/kg.
- SKI-606 did not elicit adverse effects on estrous cycles, mating index or fertility index in treated females. Slight clinical signs (salivation, at ≥ 10 mg/kg) were noted during treatment period.
- At 30 mg/kg, SkI-606 induced reduction in adjusted weights, gravid uterine weights, and number of viable fetuses in the dams. Increases in proportion of resorption (at ≥ 3 mg/kg), number of dead fetuses and number of dams without viable fetuses (latter two: 30 mg/kg) were attributable to SKI-606 treatment.
- The no observed adverse effect level (NOAEL) for paternal and maternal reproductive performance was 30 mg/kg and 3 mg/kg, respectively.

Methods

Doses:	Males: 0, 10, 30 and 70 mg/kg/day (Groups 1, 2, 3 and 4) Females 0, 3, 10 and 30 mg/kg/day (Groups 1, 2, 3 and 4) (see table below)
Frequency of dosing:	Once daily for 7 weeks in males and once daily for 4 (3-6) weeks in females
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% methylcellulose (4000 cps) (w/v), 2% polysorbate 80, NF (w/v), 0.06% glacial acetic acid (w/v) and purified (Type 1) water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	N=25 (see table below)
Satellite groups:	None
Study design:	See below
Deviation from study protocol:	Not remarkable

The allocation of rats and dosing levels (table from the Applicant):

Dosage Group	Dosage[a] (mg/kg/day)	Dosage[b] (mg/m^2)	Concen-tration[a] (mg/mL)	Dosed Male Numbers	Cohort Female Numbers	Dosed Female Numbers	Number of Breeder Males
Male Fertility Assessment							
1 Control	0	0	0	1-25	101-125		
2 Low	10	60	1	26-50	126-150		
3 Middle	30	180	3	51-75	151-175		
4 High	70	420	7	76-100	176-200		
Female Fertility Assessment							
5 Control	0	0	0			201-225	25
6 Low	3	18	0.3			226-250	25
7 Middle	10	60	1			251-275	25
8 High	30	180	3			276-300	25

a. Dosages were administered based on the active moiety of the test article at a constant dose volume of 10 mL/kg.
b. The calculation for dosage (mg/m^2)=(Km) x (dosage in mg/kg), where Km is a constant value of 6.

Study design:

- Age of animals: Dosed males and females were approximately 7 to 9 and 10 to 12 weeks old, respectively, at the initiation of dosing. Cohort females were 13 to 17 weeks old on GD 0. Breeder males were approximately 10 to 12 weeks old at initiation of cohabitation.
- Body weights: 266 to 329 g for dosed males, and 206 to 279 g for dosed females at initiation of dosing. Cohort females weighed 224 to 333 g on GD 0.
- Treatment and mating:
  Males: dosed once daily for 7 weeks (i.e., 4 weeks prior to mating with untreated cohort females, during the 2 week mating period, and until terminal necropsy that was following the completion of the mating period and fertility examination in pregnant cohort females, i.e., GD 7).

Females: dosed once daily for 3-6 weeks, depending on day of mating (2 weeks of premating period, during mating with untreated breeder males and through gestation day (GD) 7). Sperm-negative females were sacrificed.

Mating: Animals in each dose group were co-housed one female: one male and mating continued for up to two weeks.

Note: Gestation Day (GD) 0: the day when evidence of mating was identified; while Study Day 1 was designated to the day on which SKI-606/vehicle treatment started (i.e., two weeks before mating).

Dose justification:
The highest dose used in male groups was 70 mg/kg/day. At this dose 10% (2/20 animals during Day 43-Day 176) mortality has been observed in the six month toxicology study (#PRT-63644, see above). On the other hand, the high dose used in the females, 30 mg/kg/day, also induced 1/20 mortality during Day 43-Day 176 in the 6-month study. In current study, the rats were treated for 7 weeks and 3-6 weeks in male and females, respectively; the selection of the high doses is reasonable.

According to the Applicant, a decrease (66%) in numbers of pregnant rats given 30 mg/kg/day was observed in the previous fertility and developmental toxicity dose-ranging study (Dose-range finding study, PRT-61450).

Observations and Results

Clinical signs:	Mortality and moribundity (twice daily) and clinical signs (once daily): beginning on the first day of cohabitation (cohort females) or dosing (once on day of euthanasia).
Body weights:	Dosed Males: Body weights were recorded prior to initiation of dosing, then twice weekly thereafter until euthanasia (including terminal to allow calculation of organ to body weight ratios). Dosed Females: Body weights were recorded prior to initiation of dosing, then twice weekly until mating (or euthanasia for females with no record of copulation), and on GDs 0, 4, 8, and 12. Cohort Females: Body weights were recorded on GDs 0, 6, and 12.
Food consumption:	Dosed Males: Individual food consumption was determined weekly during dosing prior to cohabitation with females. Dosed Females: Individual food consumption was determined weekly during dosing prior to cohabitation with males and for GDs 0-3, 4-7, and 8-11.
Estrous cycles	Evaluation of vagina smears in dosed and cohort females was conducted daily, beginning 15 days prior to mating, continuing throughout the 14-day pre-cohabitation dosing period (all stages), and until mating or until conclusion of the 14-day cohabitation period (estrus stage only).
Gross and histopathology:	

Males: following determination of female fertility or at scheduled necropsy; testes and prostate were weighed; prostate, epididymides, and seminal vesicles from all surviving animals were fixed but only control and high dosage animals were evaluated microscopically.
Females: GD 12 or 14 days after the end of the cohabitation period if no prior evidence of copulation; gravid uterus (with ovaries) was weighed; corpora lutea in each ovary were counted; number, type, and position of implantation sites were determined. Another female that had previously cohabited with a male was examined for evidence of implantation.

Reproductive parameters: Males: weights of testes and epididymis, sperm counts, and % motile sperm.
Females: fecundity parameters (mating index, fertility index [corpora lutea, pregnancy rate], time to mating), estrous cycles, gravid uterine weight, and hysterotomy parameters (viable fetuses, live fetal sex and weights, early/late resorption, pre/post-implantation loss).

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

- Body weights, food consumption and hysterotomy parameters (in terms of number per litter and proportion per Litter): Analysis for a trend among dosage groups using Jonckheere's test, and for a difference among groups using a nonparametric one-way analysis of variance, with appropriate follow up tests.
- Percent of reference values for proportion dead embryos: were calculated based on the group mean values for this parameter; the SAS system was used for this analysis.
- Organ weights: A two-sided nonparametric trend test and a nonparametric one-way ANOVA using all groups were conducted, with follow up pairwise comparisons of all other groups to the reference group.

Study outcome:

Mortality

One Group 4 male (70 mg/kg/day, #97) was euthanized on Day 38, due to SKI-606 related clinical signs and reduction in body weight (loss of 56 grams between Days 32 and 35). The clinical signs included: appearance and activity (pale and thin, salivation, red pigment around nose/mouth, yellow discoloration of the perineal pelage), fecal changes, feces adhered to fur, and positive skin tent.

There was no treatment-related mortality in treated females.

Clinical Signs

Table 55 Clinical signs: fertility and early embyonic development

Summary of clinical signs in the treated males and females: Not remarkable in cohort females.

	Males					Females			
Group	1	2	3	4	#97	1	2	3	4
Dose (mg/kg/day)	Control	10	30	70	70	Control	3	10	30
N	25	25	25	24	1	25	25	25	25
Appearance/activity									
Dyspnea				1					
Thin					1				
Salivation	1		3	22	1			1	11
Salivation prior to dosing			1	4					
Fecal changes									
Decreased feces				2	1				
Feces adhered to fur					1				
Loose feces			1	6	1				
Staining/fur/skin									
Positive skin tent					1				
Red pigment around:									
Eyes				3					
Nose/mouth			1	10	1				
Genitalia				1					
Yellow discoloration of perineal pelage	1	1			1		2	2	3

Body Weight:

- Dosed males
- ✧ Body weights: There was a treatment-related decrease in group mean body weights in Group 4 (↓19% in comparison with the control, Days 1-28).
- ✧ Changes in weight gain were summarized in the table below (n=25/group): group mean body weight (g) and weight gains (g) are shown in the table.

Table 56 Body weight: gestation body weight, weight gains, adn gravid uterine weights

Study Day	1-7	8-14	15-21	22-28	1-28	29-49	1-49
Control	50.1	38.4	33.7	32.2	154.4	60.7	215.2
10 mg/kg	50.2	35.5	34.1	31.8	151.6	58.4	210.0
30 mg/kg	47.8	38.8	33.3	31.9	151.8	**52.5 (↓ 14%)**	204.2
70 mg/kg	44.3	**28.0**	28.6	**24.4**	**125.3 (↓ 19%)**	**29.8 (↓ 51%)**	**155.5 (↓ 28%)**

Statistically significant changes, compared to the control, were indicated by bold numbers.

- Dosed females
- ➢ Premating and during gestation days (all females in Study Days 1-14; only pregnant females were included in data for gestation period):

- ✧ Body weights: By the end of premating period (on Day 15) slight increases in group mean body weights were seen in all SKI-606 treated females compared to controls (up to 2.6%). Increased body weight and weight gains (see below) were likely due to increased food intake during premating period.
- ✧ Changes in weight gain are summarized in the table below (n=25/group or as indicated for pregnant females during gestation days):

Study Day	1-7	8-14	1-14	GD 0-3	GD 4-7	GD 0-7	GD 8-11	GD0-11
Control	4.8	5.6	10.3	21.6 (n=24)	15.8	37.5	23.7	61.2
3 mg/kg	5.2	7.2	12.4 (↑ 20%)	19.6 (n=25)	17.5	37.1	23.3	60.4
10 mg/kg	**10.1**	6.5	**16.6 (↑ 61%)**	22.5 (n=24)	14.8	37.3	21.8	59.1
30 mg/kg	**13.0**	4.4	**17.4 (↑ 69%)**	17.3 (n=25)	13.0	30.4 (↓19%)	20.8	**51.2 (↓16%)**

- Dams:

Gravid uterine weight and adjusted pregnancy weight gain (GD 0-11 weight gain minus gravid uterine weight):

	N	Gravid uterine weight	GD0-11 (gestation weight gain)	Adjusted weight gain
Control	24	7.4	61.2	53.8
3 mg/kg	25	7.5	60.4	52.9
10 mg/kg	24	7.6	59.1	51.5
30 mg/kg	25	**5.8 (↓ 22%)**	**51.2 (↓16%)**	**45.4 (↓ 16%)**

Comment:

- ➢ At 30 mg/kg, there was SKI-606 induced reduction in gravid uterine weight.
- ➢ After adjusting gravid uterine weights, it is shown a decrease in the adjusted weight indicating a direct SKI-606 related maternal toxicity.

- Cohort females:

No remarkable changes were found in group mean body weights, weight gains or gravid uterine weights.

Feed Consumption

SKI-606 induced decreases of food consumption which were observed in high dose males (70 mg/kg, D1-D28: 7-10% reduction from the control). On the contrary, food consumption was increased slightly in dosed females during premating period. During gestation, no remarkable changes in food consumption were seen in all treated females. Data of group mean food consumption (g/animal/day), and % deviation from controls wherever the deviation reached statistical significance are presented in the table below.

Table 57 Food consumption: fertility and early embyonic development

Dosed males: Not remarkable at 10 and 30 mg/kg.

Study Day	1-7	8-14	15-21	22-28	1-28
Control	28.1	28.7	29.3	29.5	28.9
70 mg/kg	26.1 (↓7%)	5.9 (↓10%)	27.1 (↓8%)	27.5 (↓7%)	26.6 (↓8%)

Dosed females:

Premating day	1-7	8-14	1-14
Control	18.8	18.4	18.6
3 mg/kg	19.3 (↑ 2%, NS)	19.0 (↑ 3%, NS)	19.1 (↑ 3%, NS)
10 mg/kg	20.0 (↑ 6%, NS)	19.6 (↑ 6%, NS)	19.8 (↑ 6%)
30 mg/kg	20.6 (↑ 9%)	19.7 (↑ 7%)	20.2 (↑ 8%)

NS: not statistically significant

Cohort females: Not remarkable

Toxicokinetics

Not conducted.

Stability and Homogeneity

The test article and control article were acceptable for use. The stability and homogeneity of dosing formulation were acceptable, based on concentration (strength) and pH values.

Necropsy

Dosed males:
There were no treatment related changes in organ weight or microscopic findings (male reproductive system)

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

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

- Males: no changes in time to mate or mating index.
  Fertility index: statistically significant reduction in fertility index was observed in males treated at 70 mg/kg (↓ 16% from the control).
- Female reproductive parameters:
  - ✧ Vaginal cytology and mating indices: There were no remarkable findings in estrous cycle, time to mate, mating index or fertility index (see table below).
  - ✧ Hysterotomy findings:
    - ➢ Mating parameters: no remarkable effects
    - ➢ Uterine/implantation data: The SKI-606 treatment did not induce remarkable changes in corpora lutea, but induced dose-dependent increases in resorptions. Viable fetal numbers were affected accordingly. The data are summarized in the table below:

Table 58 hysterotomy findings: fertility and early embryonic development

	Control	3 mg/kg	10 mg/kg	30 mg/kg
Number of females mated	25	25	25	25
Number positive for mating (mating index %)	24 (96)	25 (100)	25 (100)	25 (100)
Number of females pregnant (fertility index %)	24 (100)	25 (100)	24 (96)	25 (100)
Corpora lutea Total Average/pregnant animal (mean)	 400 16.67	 427 17.08	 407 16.96	 419 16.76
Implant sites Total Average/pregnant animal (mean)	 366 15.25	 404 16.16	 393 16.38	 354 (↓3%) 14.16
Embryonic resorptions Total % (resorptions/implantation sites x 100%) Average/pregnant animal (mean)	 15 4 0.625	 26 (↑ 0.7 fold) 15.54 (↑ 2.9 fold) 1.04	 56 (↑ 2.7 fold) 14.25 (↑ 2.56 fold) 2.33	 275 (↑ 17 fold) 77.68 (↑ 18 fold) 11
Embryos Live Dead % (viable/total implants x 100%) Average/pregnant animal (mean) Number of dams without viable fetuses	 353 1 96.4 14.7 0	 364 2 90.1 15.04 0	 335 (↓5%) 1 85.2 (↓11%) 13.96 (↓5%) 1 (4% of pregnant dams)	 75 (↓78.8%) 4 20.1 (↓79%) 3 (↓79%) 8 (32% of pregnant dams)

<u>Summary of individual study findings:</u>

- SKI-606 treatment induced mortality (1/25 treated male at 70 mg/kg), GI related clinical signs, body weight loss (both absolute BW and weight gain) and decreased food consumption in male rats, mainly at ≥ 30 mg/kg, during treatment period. Minor clinical sighs (salivation) were noted in females at ≥ 10 mg/kg. Deficits in absolute body weights and weight gains were observed in the dams during gestation.
- In dams treated at 30 mg/kg, both adjusted (for gravid uterine weight) body weight gains and gravid uterine weights were decreased: ↓ 16% and 22% from the control, respectively. Reduced gravid uterine weight was likely attributable to increased resorption and reduced numbers of viable fetuses.
- SKI-606 did not exhibit remarkable effects on estrous cycle in females (dosed or cohort), or mating indices in male and females. While no treatment –related effect on female's fertility index, statistically significant reduction in fertility index was observed in males treated at 70 mg/kg (↓ 16% from the control).
- Although no changes in corpora lutea, SKI-606 induced dose-dependent increases in resorptions. Such effects were observed at ≥ 3 mg/kg (the proportion of embryonic resorptions). Fetal viability decreased dose-dependently (litter size: 21% of the control), mainly at 30 mg/kg.
- Increased number of dams without viable fetuses increased at ≥ 10 mg/kg, i.e., 1/25 (4%) and 8/25 (32%), respectively.

9.2 Embryonic Fetal Development

Rats:

Study title: SKI-606: Oral (gavage) developmental toxicity study in rats

Study no:	PRT-63107 (Protocol 06_0072)
Study report location:	Wyeth
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 19, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	WAY-173606 (bisutinib); Lot# RB5626, 96.7% (total impurities: 0.08%)

Key Study Findings

- No remarkable embryonic toxicity (numbers of resorptions, viable fetuses and litter size) and fetal toxicity (external, visceral and skeletal malformations and/or variations) were observed up to 10 mg/kg of SKI-606, the highest dose used.
- No apparent maternal toxicities were observed under the condition of the study.
- The derived NOAEL for maternal and embryo-fetal developmental toxicities is 10 mg/kg, the highest dose used, with a corresponding AUC_{0-24} level on GD 15 at 4463 ng·hr/mL (dose normalized: 446 ng·hr/mL/kg/mg).

Methods

Doses:	0 (control), 1, 3 and 10 mg/kg (as Groups 1, 2, 3 and 4)
Frequency of dosing:	Daily for 12 days (GD 6-17); in TK study: daily for 10 days (GD 6-15).
Dose volume:	10 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% methylcellulose (4000 cps) (w/v), 2% polysorbate 80, NF (w/v), 0.06% glacial acetic acid (w/v) and purified (Type 1) water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	N=25
Satellite groups:	None. A toxicokinetics study (Study #RPT-64286) (n=9/group) was conducted separately.
Study design:	See below
Deviation from study protocol:	Not remarkable

Study design:

- Age of animals: 9 to 11 weeks old.
- Body weights: 212 to 273 g.
- Time-mated females were treated during gestation days (GDs) 6-17. The animals in the main study were sacrificed on GD 21, while the TK animals on GD 15 or GD 16.

Dose justification: dose selection was based on the result of a dose range finding study in rats (RPT-61450; not reviewed). According to the Applicant, reduced number of dams with viable fetuses was observed at 30 mg/kg, the dose where maternal toxicities were noted (e.g., weight loss up to 90% and reduced gravid uterine weight). Adverse fertility effects (increased incidence of resorption and decreased numbers of implantation) were observed at doses ≥10 mg/kg. No dose justification is necessary, since maternal toxicity and embryo-fetal toxicity was reached in this study.

Observations and Results

Clinical signs:	Mortality and moribundity (twice daily, starting GD 6), clinical signs (a minimum of once daily, starting GD 6).
Body weights:	Main study animals on GDs 6-18 and 21/ TK animals on GDs 6-15.
Food consumption:	Main study animals on GDs 6-8, 9-12, 13-15, 16-17, and 18-20; not for TK animals.
Gross pathology:	At scheduled necropsy: major viscera of all main study animals including gross evaluation of placenta
Histopathology:	<u>All organs/tissues were considered normal unless otherwise indicated</u>
Toxicokinetics:	On Day 15 at 1, 3, 5, 7, 10, and 24 hr postdose; n=3/sex/group (blood samples were collected from jugular vein or caudal vena cava). Blood samples were collected from 3 control animals (numbers 1R*, 2, and 3) at 5 hours after GD 15 dosing (jugular vein). (*1R: an animal replaced for one conrol animal #1 that were euthanized due to clinical signs)
Cesarean section:	GD 21
Reproductive parameters:	Dams: gravid uterine weight, uterine site description (live fetus, early or late resorption), number, type, and position of implantation sites, corpora lutea (main study animals) Fetal examination (live fetuses): weights, sexes, external findings, visceral examination on approximately 50% of the fetuses from each litter, skeletal examination on the rest of 50% fetuses.

<u>Reviewer's note:</u>
The following statement is excerpted from the Applicant's protocol:
The number per litter and proportion per litter were analyzed for early resorptions, late resorptions, dead fetuses, total of resorptions and dead fetuses, and preimplantation loss (corpora lutea minus total implantations). The number per litter was analyzed for live fetuses, total implantations, and corpora lutea. The number of corpora lutea was used as the denominator when calculating the proportion preimplantation loss and the number of total implantations was used as the denominator when calculating all other proportions. If a litter had more implantations than corpora lutea, then for the purpose of the analysis, the preimplantation loss was set equal to 0.0.

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

- Maternal body weights and weight gains, food consumption and hysterotomy parameters (litter size, embryo/fetal mortality) (in terms of number per litter and proportion per Litter) and postmortem observations (fetal sex, weight, external and palatal anomalies): Analysis for a trend among dosage groups using Jonckheere's test, and for a difference among groups using a nonparametric one-way analysis of variance, with appropriate follow up tests.
- Skeletal or visceral anomalies were not analyzed.

Hysterotomy findings on GD 21 (corpora lutea, litter size, embryo/fetal mortality), and postmortem observations; fetal sex, weight, external and palatal anomalies, and skeletal or visceral anomalies; and placental appearance.

Mortality

None

Clinical Signs

Not remarkable.

Body Weight

Not remarkable in maternal body weight, weight gains (GD 6-20), gravid uterine weight, and adjusted pregnancy weight gain.

Feed Consumption

Not remarkable.

Toxicokinetics

Table 59 Toxicokinetic parameters: embryonic fetal development

The table below is the summary of PK parmeters on GD 15 (from the Applicant, concurred by the reviewer):

Dosage (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	C_{max}/Dose	AUC_{0-24}/Dose
1	17.3 ± 5.1	7.0	204 ± 42	17.3 ± 5.1	204 ± 42
3	70.4 ± 12.8	7.0	857 ± 87	23.5 ± 4.3	286 ± 29
10	377 ± 33	7.0	4463 ± 227	37.7 ± 3.3	446 ± 23 [a]

a: Significantly different than corresponding values at other doses

Comment:

- AUC values increased with increased doses in a greater than dose-proportional fashion. This finding was not reported in the chronic study (6-month study, see below).
- The mean AUC level at 10 mg/kg is comparable with that in the 6-month repeated dose toxicology study in rats (Study# RPT-63644, 4387 ng·hr/mL at 10 mg/kg on Day 180; see above).

Stability and Homogeneity

The test article and control article were acceptable for use. The stability and homogeneity of dosing formulation were acceptable, based on concentration (strength) and pH values. According to the investigator, stability and uniformity of dosing formulations were previously established by the Sponsor (i.e., the Applicant).

Necropsy

Not remarkable.

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

Table 60 Hysterotomy findings: embryonic fetal development

	Control	1 mg/kg	3 mg/kg	10 mg/kg
Females mated	25	25	25	25
Number of females pregnant (%)	25 (100%)	24 (96%)	25 (100%)	24 (96%)
Pregnant at C-section	25	24	25	25
Dams with viable fetuses	25	24	25	25
Dams with all resorption	0	0	0	0
Corpora lutea				
Total	354	343	363	354
Average/animal (mean)	14.16	14.29	14.52	14.75
Implantation sites				
Total	325	315	342	325
Average/animal (mean)	13	13.13	13.68	13.54
Preimplantation loss				
Total	29	28	21	29
(%) (total/corpora lutea x 100%)	8.19	8.16	5.79	8.19
Average/animal (mean)	1.16	1.17	0.84	1.20
Postimplantation loss (%)	0	0	0	0
Dead fetuses	0	0	0	0
Total resorptions (early + late resorptions)				
Total	17	23	18	14
% (resorptions/implantation sites x 100%)	5.23	7.30	5.26	4.31
Average/animal (mean)	0.68	0.96	0.72	0.58
Early resorptions				
Total	17	23	18	14
% (resorptions/implantation sites x 100%)	5.23	7.30	5.26	4.31

	Control	1 mg/kg	3 mg/kg	10 mg/kg
Average/animal (mean)	0.68	0.96	0.72	0.58
Late resorptions				
Total	0	0	0	0
% (resorptions/implantation sites x 100%)	0	0	0	0
Average/animal (mean)	0	0	0	0
Viable fetuses				
Total	308	292	324	311
% (viable/implantation sites x 100%)	94.77	92.70	94.74	95.69
Average/animal (mean)	12.32	12.17	12.96	12.96
Viable male fetuses (%)	157 (51.0)	158 (54.1)	167 (51.5)	161 (51.8)
Live fetal body weight (g) (mean): absolute	5.34	5.33	5.39	5.28
Mean male fetal weight (g)	5.46	5.49	5.54	5.44
Mean female fetal weight (g)	5.21	5.16	5.23	5.11
Live fetal body weight (g) (mean): Adjusted	5.32	5.32	5.40	5.29
Mean male fetal weight (g)	5.44	5.48	5.56	5.45
Mean female fetal weight (g)	5.20	5.15	5.24	5.12

Absolute fetal weight: average fetal weights based on average numbers of viable fetuses
Adjusted fetal weight: average fetal weights based on average numbers of implantation sites

Offspring (Malformations, Variations, etc.)

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

Table 61 Offspring: external, visceral and skeletal variations

- External malformation and variations:

	Fetus				Litter			
Group (mg/kg)	0	1	3	10	0	1	3	10
Number evaluated	308	292	324	311	25	24	25	24
Variations								
Eye-open (%)	0 (0)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4.2)
Protruding tongue (%)	0 (0)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4.2)
Exencephaly (%)	0 (0)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (4.2)
Total incidence (%)	0 (0)	0 (0)	0 (0)	3 (1)	0 (0)	0 (0)	0 (0)	3 (12.5)

Due to the occurrence of such findings in a single fetus, a relationship to SKI-606 could not be established.

- Visceral malformation and variations:

	Fetus				Litter			
Group (mg/kg)	0	1	3	10	0	1	3	10
Number evaluated	154	145	160	156	25	24	25	24
Variations								
Innominate artery absent (%)	2 (1.3)	0 (0)	1 (0.6	1 (0.6)	2 (8)	0 (0)	1 (4)	1 (4.2)
Renal pelvis, dilated (%)	0 (0)	0 (0)	1 (0.6)	5 (3.2)	0 (0)	0 (0)	1 (4)	5 (20.8)
Spleen, small (%)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (4.2)	0 (0)	0 (0)
Total incidence (%)	2 (1.3)	1 (0.7)	2 (1.3)	6 (3.8)	2 (8)	1 (4.2)	2 (8)	6 (25)

Note: incidences of dilated renal pelvis were found in one each litter of five different individual dams at 10 mg/kg (dam # 77, 78, 81, 85 and 91), and in another fetus in one dam at 3 mg/kg (dam # 75). Other findings were also found in an individual fetus of different litter in different dams. The increased occurrence of dilated renal pelves at 3

and 10 mg/kg/day was not considered adverse because the ureter undergoes a recanalization process at the end of the embryonic period.

- Skeletal malformations and variations:

	Fetus				Litter			
Group (mg/kg)	0	1	3	10	0	1	3	10
Number evaluated	154	147	164	155	25	24	25	24
Variations								
Frontal, reduced ossification (%)	0 (0)	0 (0)	0 (0)	**2 (1.3)**	0 (0)	0 (0)	0 (0)	**2 (8.3)**
Interparietal, reduced ossification (%)	0 (0)	0 (0)	0 (0)	**2 (1.3)**	0 (0)	0 (0)	0 (0)	**2 (8.3)**
Parietal, reduced ossification (%)	0 (0)	1 (0.7)	1 (0.6)	**3 (1.9)**	0 (0)	1 (4.2)	1 (4)	**3 (12.5)**
Total incidence (%)	0 (o)	1 (0.7)	1 (0.6)	7 (4.5)	0 (0)	1 (4.2)	1 (4)	7 (29.2)

Bolded prints: statistically significant changes.

Reviewer's note:
The table below is the Applicant's summary of findings in visceral and skeletal examinations; historical data were included for reference. (M0dule 2, Section 2.6.6: Toxicology written summary, Table 4)

	0 Litters / Fetuses	1 mg/kg/day Litters / Fetuses	3 mg/kg/day Litters / Fetuses	10 mg/kg/day Litters / Fetuses	Historical Maximum[a] Litters / Fetuses
Visceral Examination					
Number Examined	**25 / 154**	**24 / 145**	**25 / 160**	**24 / 156**	
Renal Pelvis, Dilation	0	0	1 / 1	5 / 5	7 / 11
Skeletal Examination					
Number Examined	**25 / 154**	**24 / 147**	**25 / 164**	**24 / 155**	
Frontal, Reduced ossification	0	0	0	2 / 2	1 / 2
Intraparietal, Reduced ossification	0	0	0	2 / 2	2 / 2
Parietal Reduced ossification	0	1 / 1	1 / 1	3 / 3	2 / 3

a The highest per study control group incidence, based on historical control data from studies, conducted by Wyeth over the last 5 years, that included developmental toxicity endpoints.

Comments:

- Statistically significant increases in skeletal variations were noted at 10 mg/kg; however, the changes were within the range of historical controls.
- Reduced ossification in the skeletons was observed in two fetuses (the first one [Dam #81, fetus #01] with reduced frontal and parietal bone ossification; and the second fetus [Dam #84, fetus# 14] with reduced ossification of frontal, parietal, and interparietal bones). Weight reductions were also noted in both fetuses (4.4 grams and 3.4 grams, respectively), corresponding approximately 14% and 38% decreased compared to the group average within the same. Decreased ossification of skeletal bones was considered a temporary and reversible indication of delayed maturation in the affected fetuses. It is not unexpected that smaller fetuses display findings of delayed maturation.

- One fetus (#08) from a Group 4 dam (#98) displayed reduced numbers of ossified phalanges - front, bipartite vertebral centrum (atlas), small vertebral arch, misshapen cervical and thoracic vertebrae, hemicentra (thoracic). The skull of this fetus displayed nearly complete absence of frontal and parietal bones, absent interparietal bones, bipartite supraoccipital bones, and small squamosals. These skull findings correlated externally to exencephaly (see above). Due to a single incidence, the correlation between SKI-606 treatment and the finding was not certain.
- The adverse fertility effects at SKI-606 dose levels ≥ 10 mg/kg observed in Study #RPT-63257 (increased resorptions), and in Study#RPT-.61450 (decreases in implantation, early resorption and numbers of viable fetuses) were not reproduced in the current study. In these two studies, females were treated starting two weeks before mating until GD 7 (RPT-63257)-GD 20 (RPT-61450). However, the time mated females in the current study were treated from GD 6-GD 17. It is not certain whether the different outcomes in these studies may be attributable to different timing of SKI-606 treatment. No treatment-related effects on placenta or mating indices were reported in these studies, regardless whether SKI-606 were administered before or after mating. It is possible that a longer treatment period may be required in females before mating in order to demonstrate adverse hysterotomy findings.

Rabbits:

The following DRF study is reviewed. Study result is summarized as the following:
Study title: SKI-606: Oral (gavage) developmental toxicity dose ranging study in mated rabbits (Study #RPT-62710; Protocol 05_2094; TK study: RPT-64285) (non-GLP; according to GLP, without details of QA information) (Module 4)

Key Study Findings:

- Oral administration of SKI-606 (GD 6-19) in time mated rabbits elicited maternal toxicities, including: mortality at 60 mg/kg, at ≥ 30 mg/kg, clinical signs, reduction in body weight and food consumption, were observed.
- Decreased gestation weight gains, gravid uterine weights, and embryonic toxicity (increases in numbers of resorptions, decreases in implantation, viable fetuses and litter size, fetal body weights) were observed at ≥ 10 mg/kg.
- Postmortem findings in the uterus (abnormal content) associated with pregnant interruption, were observed at 60 mg/kg/day.
- No apparent embryo-fetal toxicities, i.e., fetal external morphology in this DRF study, were observed at the dose where maternal toxicities and adverse embryonic effects occurred.

- Agents: SKI-606 (WAY-173606 monohydrate; Lot #RB5626, estimated purity: 96.7%); vehicle: 0.5% methylcellulose (4000 cps), 2.0% polysorbate 80, 0.06% acetic acid, and purified (Type I) water.
- Study design: time mated female New Zealand white rabbits (n=8/group) were administered orally with SKI-606 at 0 (control), 10, 30 and 60 mg/kg/day (as Groups 1-4, respectively) for 14 days (starting gestation day (GD) 6 through GD 19). Surviving animals were euthanized (cesarean section) on GD 29. The following

evaluations were conducted: Maternal mortality, clinical observations, body weight, food consumption, gravid uterine weight, hysterotomy findings on GD 29 (corpora lutea, litter size, embryo/fetal mortality), and postmortem observations; fetal weight, and external and palatal anomalies, and placental appearance. Plasma toxicokinetics of SKI-606 were also evaluated: blood samples collected (via ear artery or saphenous vein) at 0 (predose), 1, 2, 4, 6, 9, 12 and 24 hours after dosing on GD 15.

Table 62 Summary of study result: embryonic fetal development study in rabbits (dose range finding study)

<table>
<tr><th>Evaluation</th><th>Outcomes</th></tr>
<tr><td colspan="2">Maternal findings (Dams)</td></tr>
<tr><td>TK (C_{max} and AUC)</td><td>
<table>
<tr><th>Dosage (mg/kg/day)</th><th>C_{max} (ng/mL)</th><th>t_{max} (hr)</th><th>AUC_{0-24} (ng•hr/mL)</th><th>AUC/Dose</th></tr>
<tr><td>10</td><td>706 ± 41</td><td>2.0</td><td>6235 ± 493</td><td>624 ± 49</td></tr>
<tr><td>30</td><td>2325 ± 161</td><td>2.0</td><td>20750 ± 485</td><td>692 ± 16</td></tr>
<tr><td>60</td><td>3389 ± 892</td><td>2.0</td><td>40293 ± 287</td><td>672 ± 5</td></tr>
</table>
a. The 14 days were gestation days (GDs) 6 to19 and GD 15 corresponds to day 10 of dosing.
b. SE = standard error
</td></tr>
<tr><td>Mortality</td><td>2/8 dams at 60 mg/kg (#27 and #30, on GD 15 and 16, respectively) due to:
• Clinical signs: decreased feces, thin
• Weight loss; reduced absolute body weight, up to 492 and 286 grams, respectively.
• Reduction in food consumption: ↓ 32 grams to no food intake.
• GI related gross pathology findings: distended cecum, large intestine.</td></tr>
<tr><td>Clinical sign</td><td>Mainly at ≥30 mg/kg: decreased feces, focal lesion, red pigment in urine (correlated with abnormal content in the uterus, indicating pregnancy interruption)</td></tr>
<tr><td>Body weights and weight gains</td><td>
<table>
<tr><td>Group (F0)</td><td>Group 1</td><td>Group 2</td><td>Group 3</td><td>Group 4</td></tr>
<tr><td>Dose (mg/kg)</td><td>Control</td><td>10</td><td>30</td><td>60</td></tr>
<tr><td>N</td><td>7</td><td>8</td><td>8</td><td>2</td></tr>
<tr><td>Gestation weight gain* Mean, g (↓ %)
GD 6-8
GD 6-19
GD 6-28</td><td>
55.6
285.9
474.1</td><td>
39.5 (29)
252.5 (12)
369.1 (22)</td><td>
5.1 (91)
217 (24)
380.5 (20)</td><td>-61.5
(211)
35.5 (88)
236.5 (50)</td></tr>
<tr><td>Gravid uterine weight (↓ %)</td><td>505.3</td><td>452.5 (11)</td><td>433.8 (14)</td><td>287 (43)</td></tr>
<tr><td>Adjusted weight (GD 6-28)</td><td>-31.1</td><td>-83.4</td><td>-53.3</td><td>-50.5</td></tr>
</table>
***Greatest loss in the term of weight gain occurred GD 6-8 (first three days of administration): weight loss was noted at 60 mg/kg/day.**
</td></tr>
<tr><td>Food consumption (g/day)</td><td>
<table>
<tr><td>Group (F0)</td><td>Group 1</td><td>Group 2</td><td>Group 3</td><td>Group 4</td></tr>
<tr><td>Dose (mg/kg)</td><td>Control</td><td>10</td><td>30</td><td>60</td></tr>
<tr><td>N</td><td>7</td><td>8</td><td>8</td><td>2</td></tr>
<tr><td>Food consumption: Mean (↓ %)
GD 6-8
GD 6-19
GD 20-28</td><td>
164.1
154.7
129.5</td><td>
153.1 (7)
146.8 (5)
104.9 (19)</td><td>
138.1 (16)
138.0 (11)
118.1 (9)</td><td>
82.5 (50)
89.5 (42)
128.2 (1)</td></tr>
</table>
Reduction in food consumption recovered partially after cessation of dosing.
</td></tr>
</table>

Hysterotomy findings (group means) (↓ %)	Group (F0)	Group 1	Group 2	Group 3	Group 4
	Dose (mg/kg)	Control	10	30	60
	N (number of does)	7	8	8	2
	Number of corpora lutea per doe	8.25	8.00 (3)	7.75 (6)	9.00 (↑ 9)
	Total implantation per doe	8.00	7.38 (7.8)	7.38 (7.8)	8.50 (↑ 6.3)
	Preimplantation loss (a)	3.4	7.8 (↑ 129)	4.8 (↑ 41)	5.6 (↑ 68)
	Post implantation loss (b)	3.6	1.7 (53)	3.4 (6)	29.4 (↑ 717)
	Early resorption per doe	0.14	0.13	0.13	2.50 (↑1686)
	Late resorption per doe	0.14	0.00	0.13	0.00
	Dead fetuses per doe	0.00	0.00	0.00	0.00
	Viable fetuses per doe	7.71	7.25 (6.0)	7.13 (7.5)	6.00 (28.5)
	Body weight of all live fetuses (g)	44.84	43.65	41.61 (7)	30.08 (32.9)
	(a): (Total Corpora Lutea - Total Implantations) * 100 / Total Corpora Lutea **(b): (Total Implantations - Total Viable Fetuses) * 100 / Total Implantations** Decreased gravid uterine weight correlated with increased implantation loss in all treated groups; increased early resorption at 60 mg/kg enhanced such deficits in Group 4 does.				
Placenta	SKI-606 induced increased numbers (2 from litter 28 and 1 from litter 26) of granular placentas at 60 mg/kg/day.				
Feto-embryonic findings (postmortem observations)					
External morphology	Not remarkable				

Pivotal study:

Study title: SKI-606: Oral (gavage) developmental toxicity study in mated rabbits

Study no:	PRT-65533 (Protocol 06_0074)
Study report location:	Wyeth
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 17, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	WAY-173606 (bisutinib); Lot# MP030203, 96.7% (total impurities: 1.53% and 1.12%; largest single impurity:0.48% and 0.32%)

Key Study Findings

- SKI-606 induced maternal toxicities, manifested as GI related clinical signs, reduced gestation body weight gains and gravid uterine weights, as well as reduced food consumption, were observed at 30 mg/kg.
- No remarkable embryonic toxicity (numbers of resorptions, viable fetuses and litter size) were observed up to 30 mg/kg of SKI-606, the highest dose used.
- Fetal toxicities (visceral changes and skeletal variations, such as fused sternebra and vertebral variations) were observed mainly at 30 mg/kg.
- The NOAEL for both maternal and fetal developmental toxicities is 10 mg/kg.

Methods

Doses:	0 (control), 3, 10 and 30 mg/kg (as Groups 1, 2, 3 and 4)
Frequency of dosing:	Daily for 14 days (GD 6-19)
Dose volume:	2 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	0.5% methylcellulose (4000 cps) (w/v), 2% polysorbate 80, NF (w/v), 0.06% glacial acetic acid (w/v) and purified (Type 1) water
Species/Strain:	New Zealand white (SPF) rabbits
Number/Sex/Group:	N=20
Satellite groups:	None. All animals were used in the toxicokinetics study (Study #RPT-66820).
Study design:	See below
Deviation from study protocol:	Not remarkable

Study design:

- Age of animals: approximately 5.5 months old.
- Body weights: 2818 to 4216 g.
- Time-mated* females were treated during gestation days (GDs) 6-19. The animals were sacrificed on GD 29. *Mating was performed by cohabitation of does with breeder males; the day of mating was designated GD 0.

Dose justification: No dose justification is necessary, since maternal toxicity and embryo-fetal toxicity was reached in this study.

Observations and Results

Clinical signs:	Mortality and moribundity (twice daily, starting GD 6), clinical signs (a minimum of once daily, starting GD 6).
Body weights:	Main study animals on GDs 6-20, 24 and 29.
Food consumption:	Main study animals on GDs 6-8, 9-11, 12-15, 16-19, 20-23, 24-28, 6-19, and 20-28; not for TK animals.
Gross pathology:	At scheduled necropsy: major viscera of all main study animals and all offspring (external, visceral, and skeletal examination), including gross evaluation of placenta
Histopathology:	<u>All organs/tissues were considered normal unless otherwise indicated</u>
Toxicokinetics:	On Day 15 at 1, 2, 4, 6, 9, 12 and 24 hr postdose; n=4/group (blood samples were collected (via ear artery or saphenous vein).
Cesarean section:	GD 29
Reproductive parameters:	Dams: gravid uterine weight, uterine site description (live fetus, early or late resorption), number, type, and position of implantation sites, corpora lutea (main study animals)

Fetal examination (live fetuses): weights, sexes, external findings, visceral and skeletal examination on all fetuses from each litter.

Reviewer's note:
The following statement is excerpted from the Applicant's protocol:
The number per litter and proportion per litter were analyzed for early resorptions, late resorptions, dead fetuses, total of resorptions and dead fetuses, and preimplantation loss (corpora lutea minus total implantations). The number per litter was analyzed for live fetuses, total implantations, and corpora lutea. The number of corpora lutea was used as the denominator when calculating the proportion preimplantation loss and the number of total implantations was used as the denominator when calculating all other proportions. If a litter had more implantations than corpora lutea, then for the purpose of the analysis, the preimplantation loss was set equal to 0.0.

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

- Maternal body weights and weight gains, food consumption and hysterotomy parameters (litter size, embryo/fetal mortality), postmortem observations (fetal sex, weight, external and palatal anomalies) and fetal examination findings: Analysis for a trend among dosage groups using Jonckheere's test, and for a difference among groups using a nonparametric one-way analysis of variance, with appropriate follow up tests.
- Analyses of hysterotomy findings were done with respect to number per litter and proportion per litter. Analyses of fetal examination findings were done with respect to proportion of affected fetuses per litter and proportion of litters with at least one affected fetus.

Mortality

There was no SKI-606 related mortality. One doe (#21, 10 mg/kg) was euthanized due to gavage trauma on Day 11.

Clinical Signs

Table 63 Clinical signs: EFD study in rabbits (pivotal)

The clinical signs were noted mainly at 30 mg/kg. However, the dose-relationship was not well established; the incidences were small also.

Group	1	2	3	4
Dose (mg/kg/day)	Control	3	10	30
N	20	20	20	20
Appearance/activity Tachypnea Fecal changes			1	1

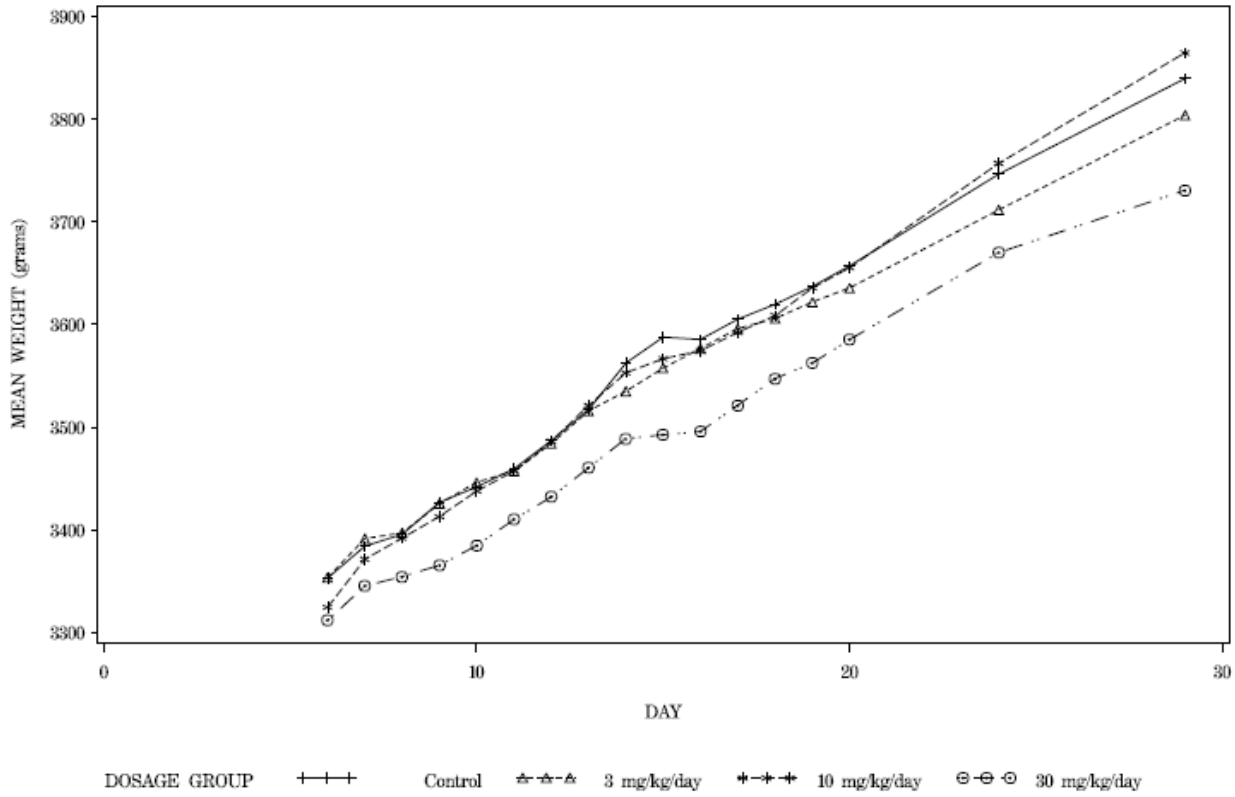
Decreased feces	1		1	1
Discolored feces		1		
Feces adhered to fur				1
Loose feces	4	6	3	7
No feces				1
Focal lesion		1		2
Red pigment in:				
Drop pan/in cage			1	1
Feces			1	
Urine		1		

Body Weight

Reduction in maternal body weight, weight gains (GD 6-20), gravid uterine weight, and adjusted pregnancy weight gain were observed at 30 mg/kg.

Figure 13 Group mean gestatiob body weights: EFD study in rabbits (pivotal)

Group mean maternal (gestation) body weights (figure from the Applicant):



Weight gains:

Group (F0)	Group 1	Group 2	Group 3	Group 4
Dose (mg/kg)	Control	3	10	30
N	19	18	18	18
Gestation weight gain* Mean, g (↓ %)				
GD 6-8	73.95	71.83 (3)	88.28 (↑ 19)	53.56 (28)
GD 6-19	304.26	281.89 (7)	330.72 (↑ 9)	273.56 (10)
GD 20-28	182.68	168.56 (8)	209.28 (↑ 15)	145.22 (21)

GD 6-28	486.95	450.44 (7)	540.00 (↑ 11)	418.78 (14)
Gravid uterine weight (↓ %)	500.47	461.50 (8)	487.44 (3)	458.06 (8)
Adjusted weight (GD 6-28)	-13.53	-11.06	52.56	-39.28

Feed Consumption

Group (F0)	Group 1	Group 2	Group 3	Group 4
Dose (mg/kg)	Control	3	10	30
N	19	13-17	17-18	18
Food consumption, Mean, g (↓ %)				
GD 6-8	170.5	166.1 (3)	170.5	152.9 (10)
GD 6-19	163.1	158.1 (3)	163.5	150.7 (8)
GD 20-28	182.7	168.6 (8)	209.3 (↑ 15)	145.2 (21)

All treated groups showed reduced food intake, except for Group 3 (10 mg/kg). The findings correlated with the weight gain changes, i.e., only Group 3 dams showed increased gestation weight gains. In this group, there was the least reduction in gravid uterine weights in comparison with other groups.

Toxicokinetics

Table 64 Toxicokinetic parameters: EFD study in rabbits (pivotal)

Dosage (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	AUC_{0-24} /Dosage
3	129 ± 22	4.0	1308 ± 282	436 ± 94
10[a]	505 ± 98	2.0	5451 ± 1074	545 ± 107
30	1857 ± 155	2.0	14002 ± 1344	467 ± 45

GD. Gestation Day
a. A 2 hour sample obtained from one animal (no. 43) was obtained at 1 hour 20 minutes; the nominal time used for pharmacokinetic analysis.

Stability and Homogeneity

The test article and control article were acceptable for use. The stability and homogeneity of dosing formulation were acceptable, based on concentration (strength) and pH values. According to the investigator, stability and uniformity of dosing formulations were previously established by the Sponsor (i.e., the Applicant).

Necropsy

Table 65 Postmortem observations in does

Group	1	2	3	4
Dose (mg/kg/day)	Control	3	10	30
N	20	20	20	20
Esophagus; abnormal content Urinary bladder		1		

Calculus				1
Distended				1
Uterus: abnormal content*		*	2	

***Red clotted material in the left horn and dark red fluid (10 mg/kg).**

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

Table 66 Hystero findings: EFD study in rabbits (pivotal)

Hysterotomy findings: There were no apparent SKI-606 related effects on fertility, implantation, resorption or fetal parameters.

	Control	1 mg/kg	3 mg/kg	10 mg/kg
Females mated	20	20	20	20
Number of females pregnant (%)	19 (95)	18 (90)	18 (90)	18 (90)
Pregnant at C-section	19	18	18	18
Dams with viable fetuses	19	18	18	18
Dams with all resorption	0	0	0	0
Abortion Index	0	0	0	0
Corpora lutea				
Total	171	156	172	168
Average/animal (mean)	9	8.67	9.56	9.33
Implantation sites				
Total	160	138	162	153
Average/animal (mean)	8.42	7.67	9	8.5
Preimplantation loss				
Total	11	18	10	15
(%) (total/corpora lutea x 100%)	6.43	11.5	5.81	8.93
Average/animal (mean)	0.58	1	0.56	0.83
Postimplantation loss (%)	0	0	0	0
Dead fetuses	1	0	0	0
Total resorptions (early + late resorptions)				
Total	3	3	20	8
% (resorptions/implantation sites x 100%)	1.88	2.17	12.35	5.23
Average/animal (mean)	0.16	0.17	1.11	0.44
Early resorptions				
Total	1	3	15	4
% (resorptions/implantation sites x 100%)	0.63	2.17	9.26	2.61
Average/animal (mean)	0.05	0.17	0.83	0.22
Late resorptions				
Total	2	0	5	4
% (resorptions/implantation sites x 100%)	1.25	0	3.09	2.61
Average/animal (mean)	0.11	0	0.28	0.22
Viable fetuses				
Total	157	135	142	145
% (viable/implantation sites x 100%)	98.13	97.83	87.65	94.78
Average/animal (mean)	8.21	7.5	7.89	8.06
Viable male fetuses (%)	80 (51.0)	70 (51.9)	76 (53.5)	73 (50.3)
Live fetal body weight (g) (mean): absolute	42.09	42.67	43.08	39.40
Mean male fetal weight (g)	42.46	43.37	43.85	39.42
Mean female fetal weight (g)	41.71	41.96	42.31	39.37
Live fetal body weight (g) (mean): Adjusted	42.14	41.73	43.76	39.56
Mean male fetal weight (g)	42.58	42.36	44.46	39.66

	Control	1 mg/kg	3 mg/kg	10 mg/kg
Mean female fetal weight (g)	41.69	41.09	43.06	39.46

Absolute fetal weight: average fetal weights based on average numbers of viable fetuses
Adjusted fetal weight: average fetal weights based on average numbers of implantation sites

Offspring (Malformations, Variations, etc.)

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

Table 67 Offspring: external, visceral and skeletal variations

- External malformation and variations:

	Fetus				Litter			
Group (mg/kg)	0	3	10	30	0	3	10	30
Number evaluated	157	135	142	145	19	18	18	18
Variations								
Abdominal discoloration-dark (%)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (5.6)
Abdomen-distended	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (5.6)
Total incidence (%)	0 (0)	0 (0)	0 (0)	2 (1.4)	0 (0)	0 (0)	0 (0)	1 (5.6)

Although only a single incidence in one fetus (62:07) in a Group 4 dam, the findings of distended abdominal and dark abdominal discoloration were correlated with visceral findings at 30 mg/kg 9see below), thus it was considered SKI-606 related. Other external findings, such as front octrodactyly, distended abdomen, omphalocele, bifide spina and short tail, were considered incidental, due to absence of dose-relationship, small numbers of incidences, or incidence within the historical range of the laboratory.

- Visceral malformation and variations:

	Fetus				Litter			
Group (mg/kg)	0	3	10	30	0	3	10	30
Number evaluated	157	135	142	145	19	18	18	18
Variations								
Ascites (%)	0 (0)	0 (0)	0 (0	1 (0.7)	0 (0)	0 (0)	0 (0	1 (5.6)
Cardiomegaly (%)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (5.6)
Gall bladder-absent (%)	0 (0)	0 (0)	0 (0)	4 (2.8)	0 (0)	0 (0)	0 (0)	2 (11.1)
Gall bladder-small (%)	1 (0.6)	0 (0)	0 (0)	2 (1.4)	1 (5.3)	0 (0)	0 (0)	2 (11.1)
Gall bladder-supernumerary (%)	0 (0)	0 (0)	0 (0)	2 (1.4)	0 (0)	0 (0)	0 (0)	2 (11.1)
Hydrocephaly	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (5.6)
Liver-enlarged; dark (%)	0 (0)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	1 (5.6)
Total incidence (%)	1 (0.6)	0 (0)	0 (0)	12 (8.3)	1 (5.3)	0 (0)	0 (0)	10 (55.6)

Note: In the fetus (62:07), following variations were found: ascites, cardiomegaly, changes in gal bladder, and liver (dark and enlarged). Gall bladder supernumerary was found in another fetus (62:02) of the same litter. Hydrocephaly and absent gall bladder were observed in other two litters (#68 and 66, respectively).

- Skeletal malformations and variations:

	Fetus				Litter			
Group (mg/kg)	0	3	10	30	0	3	10	30
Number evaluated	157	135	142	145	19	18	18	18
Variations								
Pelvic girdle, reduced ossification (%)	0 (0)	2 (1.5)	1 (0.7)	4 (2.8)	0 (0)	1 (5.6)	1 (5.6)	2 (11.1)
Rib, fused (%)	0 (0)	0 (0)	3 (2.1)	1 (0.7)	0 (0)	0 (0)	3 (16.7)	1 (5.6)

	Fetus				Litter			
Group (mg/kg)	0	3	10	30	0	3	10	30
Number evaluated	157	135	142	145	19	18	18	18
Sternebra, misaligned (%)	0 (0)	2 (1.5)	**8 (5.6)**	1 (0)	0 (0)	2 (11.1)	5 (27.8)	1 (5.6)
Sternbra, misshapen (%)	0 (0)	0 (0)	1 (0.7)	1 (0)	0 (0)	0 (0)	1 (5.6)	1 (5.6)
Sternebra, extra ossification site (%)	0 (0)	0 (0)	1 (0.7)	4 (2.8)	0 (0)	0 (0)	1 (5.6)	1 (5.6)
Sternebra, fused (%)	0 (0)	1 (0.7)	2 (1.4)	**7 (4.8)**	0 (0)	1 (5.6)	2 (11.1)	**4 (22.2)**
Caudal vertebrae, decreased number (%)	1 (0.6)	2 (1.5)	2 (1.4)	3 (2.1)	1 (0)	1 (5.6)	2 (11.1)	3 (16.7)
Hemicentrum (%)	0 (0)	0 (0)	**3 (2.1)**	0 (0)	0 (0)	0 (0)	3 (16.7)	0 (0)
Vertebral arches, misaligned (%)	0 (0)	1 (0.7)	2 (1.4)	1 (0.7)	0 (0)	1 (5.6)	2 (11.1)	1 (5.6)
Sternebral morphological anomalies (%)	0 (0)	**3 (2.2)**	**11 (7.7)**	**11 (7.6)**	0 (0)	**3 (16.7)**	**8 (44.4)**	**5 (27.8)**
Vertebral morphological anomalies (%)	0 (0)	4 (3.0)	5 (3.5)	4 (2.8)	0 (0)	3 (16.7)	4 (22.2)	2 (11.1)

Bolded prints: statistically significant changes.

The following table is excerpted from the Applicant's report, indicating summary of sternebral and vertebral morphological anomalies with the comparison to the historical maximum of respective findings.

	----------Dosage (mg/kg/day)----------				Historical
	0	3	10	30	Maximum[a]
Sternebral Findings					
- Fused	0	1	2 (2)	7 (4)[b]	3 (3)
- Misshapen	0	0	1	1	2 (1)
- Misaligned	0	2 (2)	8 (5)[b]	1	4 (3)
- Extra Ossification Site	0	0	1	4 (1)	6 (5)
Vertebral Findings					
- Gap (caudal)	0	1	0	0	1 (1)
- Misshapen (caudal and one or other regions)	0	1	1	0	1 (1)
- Misaligned (caudal)	0	1	0	2 (1)	7 (5)
- Hemicentrum	0	0	3 (3)[b]	0	2 (1)
- Hemivertebra/Absent Vertebral Element	0	2 (2)	2 (2)	1	4 (4)
- Small Arch	0	1	0	0	1 (1)
- Fused Arches	0	0	1	1	1 (1)
- Misaligned Arches	0	1	2 (2)	1	1 (1)
- Fused Centra	0	1	1	1	1 (1)

a. Historical control data based on 10 studies conducted at the Wyeth Chazy site over the last 5 years.
b. $P \leq 0.05$ (Overall and/or Trend)
#( ). Number observed (litters affected)

<u>Reviewer's note:</u>
The table below is the Applicant's summary of findings in visceral and skeletal examinations; historical data were included for reference. (M0dule 2, Section 2.6.6: Toxicology written summary, Table 5)

	0 Litters / Fetuses	3 mg/kg/day Litters / Fetuses	10 mg/kg/day Litters / Fetuses	30 mg/kg/day Litters / Fetuses	Historical Maximum[a] Litters / Fetuses
Visceral Examination					
Number Examined	**19 / 157**	**18 / 135**	**18 / 142**	**18 / 145**	
Gall Bladder					
Absent	0	0	0	2 / 4	1 / 1
Heart,					
Cardiomegaly	0	0	0	1 / 1	1 / 1[b]
Liver					
Enlarged	0	0	0	1 / 1	0 / 0
Head					
Hydrocephaly	0	0	0	1 / 1	1 / 1
Skeletal Examination					
Number Examined	**19 / 157**	**18 / 135**	**18 / 142**	**18 / 145**	
Sternebrae					
Fused	0	1 / 1	2 / 2	4 / 7	3 / 3

a. The highest per study control group incidence, based on historical control data from studies, conducted by Wyeth over the last 5 years, that included developmental toxicity endpoints.
b. Identified as enlarged atrium and ventricle in historical data.

Comments:

- Statistically significant increases in skeletal variations were noted at 10 and 30 mg/kg; however, some of the changes were lacking dose-relationship or were within the range of historical controls. Fused sternebra were significantly increased at 30 mg/kg.
- Based on the statistical analysis, there were significant increases in sternebral morphological anomalies, in terms of numbers of fetuses and litters.

9.3 Prenatal and Postnatal Development

Not conducted.

10 Special Toxicology Studies

Phototoxicity:
Study title: Bosutinib: multiple (2-day) dose phototoxicity study to determine the effects of oral (gavage) administration on eyes and skin in pigmented rats (Study #RPT-74360; Wyeth Protocol 08_1326) (b) (4) GLP compliant) (Module 4)

Key study findings:
Bosutinib-treatment did not induce phototoxicity in eyes or the skin of Long Evans pigmented rats, under the condition of the study. The NOAEL under the study condition was 100 mg/kg/day, the highest dose used.

Background: (Statement excerpted from the Applicant)

Bosutinib absorbs light within a wavelength range of 290 to 700 nm (UVB, UVA, and visible light). Bosutinib shows high affinity for melanin-containing tissues as indicated by higher exposure in the skin and uveal tract of pigmented rats (Long Evans) than in non-pigmented rats (Sprague-Dawley). Thus, the potential existed for bosutinib to elicit compound-related phototoxicity.

Study design:

- Animals: rats (Crl:LE (Long Evans)) (n=5/sex/group in the bosutinib groups, n=3/sex/group, in the 8-MOP group)
- Agents: test article: bosutinib (SKI-606, Lot# RB5626; purity: 96.7%); vehicle control: 0.5% methylcellulose (4000 cps) (w/v), 2% polysorbate 80, NF (w/v), and 0.06% acetic acid (w/v) in reverse osmosis membrane processed deionized water; positive control (comparator): 8-methoxypsoralen (8-MOP; in corn oil) (Lot# 035K1267)
- Dose: bosutinib: 0 (vehicle control), 10, 100 mg/kg/day and 8-MOP (50 mg/kg) (as Groups 1, 2, 3 and 4, respectively)
- Administration: bosutinib, oral (gavage at 10 mL/kg) once daily for two consecutive days; 8-MOP: one dose
- Light exposure: The skin and eyes of each rat administered vehicle or bosutinib were exposed to UVR approximately 4 hours (± 15 minutes) after the second dose when the concentration of bosutinib is at its highest in these tissues. The 8-MOP group was exposed to ultraviolet radiation (UVR) 1 hour (± 10 minutes) after the 8-MOP treatment.

Reviewer's note: justification for intervals between agent administration and UVR exposure (statement excerpted from the Applicant):
The interval between test article administration and UVR exposure was based on the amount of time that it took concentrations of the test article to reach a peak in the skin and/or eyes of rats after a single administration. In the skin and eyes, the peaks are at 4 and 24 hours, respectively. In the eyes the tissue concentration remains unchanged between 24 and 672 hours after a single dose. In contrast, elimination occurs in the skin over the first 24 hours after dosing. Therefore, the timing of UVR exposure is determined by the 4 hour peak in the skin. Thus, the skin and eyes were exposed to UVR at approximately 4 hours after the second dose, when skin concentration is at peak. High concentrations are present in the eye at this time point as well. The one hour interval between comparator article administration and UVR exposure is based on previous experience at the Testing Facility.
An instrumental UVR exposure dose equivalent to approximately 0.5 minimal erythema dose (MED, a UVR dose adequate to elicit a barely perceptible response in skin) was delivered to each rat in 30 ± 5 minutes (i.e., the minimal erythema dose, MED=0.5 hr). This dose was used because it was below the response level and permitted observation of phototoxic effects of the test article.

- All animals were euthanized upon completion of the final ophthalmological examination. Evaluations consisted of adult mortality, clinical observations, skin evaluation, body weight, ophthalmological evaluation (once pretest and three days after UVR exposure), and histopathologic evaluation of eyes.

Results:

- There were no remarkable effects on mortality, clinical signs or body weights.

Table 68 Skin reactions adn ophthalmological observations

	Males				Females			
Group	1	2	3	4	1	2	3	4
N	5	5	5	3	5	5	5	3
Dose	Control	10	100	MCP 50	Control	10	100	MCP 50
Skin reactions (N/N)								
Light site:								
Edema, Grade 1	0/0	0/0	0/0	2/2	0/0	0/0	0/0	6/3
Erythema, Grade 1					0/0	0/0	0/0	6/3
Grade 2					0/0	0/0	0/0	2/2
Dark site:								
Light site:								
Edema, Grade 1					0/0	0/0	0/0	5/3
Erythema, Grade 1					0/0	0/0	0/0	3/3
Eye examined (N1/N2)	10	10	10	6	10	10	10	6
Diffused corneal edema, Superficial	0/0	0/0	0/0	3/6	0/0	0/0	0/0	3/6
Focal superficial corneal scarring	1/1	0/0	0/0	0/0	1/1	0/0	0/0	0/0
Focal retinopathy	1/2	2/2	5/8	0/0	3/4	3/5	5/6	0/0
Focal myelinated retinal nerve fibers					0/0	1/1	0/0	0/0

N/N= total number of observations/number of rats with observation; N1/N= rats affected/eye affected.

Table 69 Histopathological fidnigs in the eyes

	Males				Females			
Group	1	2	3	4	1	2	3	4
N	5	5	5	3	5	5	5	3
Dose (mg/kg)	0	10	100	MCP 50	0	10	100	MCP 50
Left eye								
Cornea:								
Edema, corneal stroma								
Moderate								1
Marked				3				2
Edema, intercellular corneal epithelium								
Minimal				1				2
Mild				1				1
Moderate				1				
Infiltration, neutrophils, corneal stroma								
Minimal	1	1			1			
Mild	1			3				3
Necrosis, corneal epithelium, focal								
Minimal				1				
Lens:								
Hyperplasia, lenticular epithelium, focal								
Minimal				1				1
Mild				2				

Necrosis, subcapsular, focal								
Minimal				2				
Mild				1				1
Bulbar conjunctiva:								
Hyperplasia, mucosa								
Minimal								1
Mild								1
Infiltration, mixed inflammatory cell								
Minimal								2
Right eye								
Cornea:								
Edema, corneal stroma								
Moderate				2				1
Marked				1				2
Edema, intercellular corneal epithelium								
Minimal				1				1
Mild				1				1
Moderate				1				1
Infiltration, neutrophils, corneal stroma								
Minimal			2		1	1	1	
Mild		1		3				3
Moderate		1						
Lens:								
Hyperplasia, lenticular epithelium, focal								
Minimal				2				3
Necrosis, subcapsular, focal								
Minimal				2				3
Retina								
Macrophages/hemosiderin, focal								
Minimal								1
Necrosis, focal								
Minimal								1
Bulbar conjunctiva:								
Hyperplasia, mucosa								
Minimal								1
Mild								1

Most of the histopathological findings were in 8-MOP treated rats. Incidental findings such as neutrophil infiltration in corneal stroma in the control and bosutinib treated rats were possibly the result of UVR exposure, since the findings occurred in both control and treated rats with no apparent difference.

11 Integrated Summary and Safety Evaluation

A full battery of toxicology studies that supported the safety evaluation for the NDA of bosutinib were conducted in *in vitro* systems as well as in mice, rats, rabbits, and dogs. The target organs of bosutinib are GI tract and lymphoid tissues. The general toxicology studies were conducted in appropriate animal species, following administration route and dosing regimens that adequately addressed safety concerns in human usage. In general, the toxicity profile was similar in rodent and nonrodent species. Prolonged treatment did not identify different target organs.

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

Bosutinib induced GI-related clinical signs such as salivation, oral discharge, fecal changes (including diarrhea) and/or emesis, in rats and dogs. Lesions in the GI tract, mainly in the small intestine upon histopathological examination were obvious in rats: mucosal hyperplasia, distention and hemorrhage; and in dogs: crypt abscess in duodenum. GI tract being the major target of bosutinib-induced toxicity is supported by the PK data, i.e., high distribution of bosutinib radioactivity to the GI tract in rats. Toxicities associated with lesions in the liver were not noticeable in the non-rodent species dogs, and were only minimal in the rats: slight centrilobular hepatocytic hyperplasia at 70 mg/kg in a 2-week study (Study #RPT-64729, an impurity qualification study) and in one high dose rat in the 4-weeek study. In 4-week study, one male rat at 70 mg/kg was found with bile duct hyperplasia. There was no correlating increased in liver enzymes. Changes in the clinical chemistry parameters indicative of hepatobiliary toxicities were reported in patients with CML who were treated with bosutinib.

In the 6-month toxicology study in rats, increased colloid in thyroid was reported. However, this finding was not considered adverse because it was not severe and it was not accompanied by any degenerative, hyperplastic or regenerative changes. In addition, the rat is particularly sensitive to the effects of altered thyroid hormone metabolism. Increased T4 and decreased TSH levels were found in 4-week study in rats; such changes were not reproduced in the 6-month study. Thus, the thyroid finding is likely not relevant to humans. The only findings of CNS safety pharmacology study in rats were increased incidence of impaired gait and decreased pupil size. However, in a distribution study, radioactivity was not detected when rats received and oral dose [^{14}C]bosutinib.

Metabolite M2 and impurity (b) (4) were evaluated in repeat dose toxicity studies. Up to 210 mg/kg/day orally administered metabolite M2 (WAY-198760) in rats for 14 days was well tolerated. No M2 related toxicities were observed. Similarly, a lot of SKI-606 containing (b) (4) (b) (4) elicited similar toxicities as another lot with much less of (b) (4) (< (b) (4) indicating no remarkable toxicities were attributed to impurity (b) (4)

See Section 1.2 for discussion of genetic toxicity and reproductive and developmental toxicities of SKI-606.

In general, ocular toxicities were not significant in animals treated with bosutinib. Bosutinib-treatment did not induce phototoxicity in eyes or the skin of Long Evans pigmented rats. Thus, although pigmented tissues (such as eyes) showed high bosutinib radioactivity distribution, the potential of phototoxicity of bosutinib in humans is low.

12 Appendix/Attachments

None

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/s/

SHWU LUAN LEE
07/17/2012

HALEH SABER
07/17/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 203341 **Applicant: Wyeth Pharmaceuticals, Inc.** **Stamp Date: November 17, 2011**

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

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		The carcinogenicity, pre- and post-natal development studies and toxicology studies in juvenile animals are not included in the current submission. This is in line with the ICH S9 guidance for the critical oncologic indication as proposed.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		Several excipients in the drug product, i.e., the formulation to be marketed, were not included in the vehicle used in pivotal toxicology studies. However, these ingredients are compendial compounds. The acceptance of the content of excipients will be determined after the review of the submission.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		Comparisons of human exposures to animal exposures are expressed as safety factors in terms of AUC.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		A two-week toxicology study in rats was designated to evaluate safety of impurity (b) (4). Whether additional non-clinical studies are required for impurity (b) (4) and/or for other impurities will be a review issue.
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.

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

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Shwu-Luan Lee — December 19, 2011

Reviewing Pharmacologist — Date

Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

SHWU LUAN LEE
12/23/2011

HALEH SABER
12/28/2011