CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

203469Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Iclusig (ponatinib)

Date: November 19, 2012
To: File for NDA 203469
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 Drs. Ricci and Del Valle and labeling and secondary memorandum provided by Dr. Saber. I concur with Dr Saber's conclusion that Iclusig may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
11/19/2012

MEMORANDUM

Date: November 19, 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: 203469
Drug: ICLUSIG (ponatinib) tablet
Indication: Treatment of adult patients with chronic phase, accelerated phase, or blast phase chronic myeloid leukemia (CML) or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL), resistant or intolerant to prior tyrosine kinase inhibitor therapy
Applicant: ARIAD Pharmaceuticals, Inc.

Ponatinib is a small molecule tyrosine kinase inhibitor developed for the treatment of CML. It has activity against BCR-ABL and multiple mutant forms of BCR-ABL, including the T315I mutation. Ponatinib also inhibits several other kinases, including VEGFRs, FGFRs, PDGFRs, RET, KIT, FLT3, and SRC family members. Ponatinib showed anti-tumor activity in mice bearing tumor xenografts expressing native BCR-ABL or the T315I mutant. The pharmacologic class assigned to ponatinib is "kinase inhibitor" consistent with other drugs of the same class, such as imatinib, dasatinib, nilotinib, and bosutinib.

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. Ponatinib-related toxicities in rats and monkeys included: lymphoid depletion, necrosis involving the exocrine pancreas with low incidence lipase elevation, and elevated liver enzymes (ALT and AST). Cardiovascular findings in animals included systolic heart murmurs and myocardial necrosis; however, these findings were of low incidence and/or non-dose-dependent.

In patients treated with ICLUSIG, serious safety concerns include the findings in the cardiovascular, hepatic and pancreatic systems.

Ponatinib was not mutagenic or clastogenic when tested in the battery of genotoxicity studies. At a maternally toxic dose of 3 mg/kg/day, ponatinib was teratogenic when administered to pregnant rats during the period of organogenesis. Systemic exposure in animals at this dose was equivalent to that reported for patients treated with the recommended ponatinib dose (45 mg/day). Ponatinib also caused embryofetal toxicities in rats at systemic exposures below those observed in patients treated with the recommended dose. A pregnancy category D has been assigned to this drug. Due to the

teratogenicity findings in rats, an embryofetal developmental study in a second species was deemed not necessary.

Fertility studies using ponatinib have not been conducted; however, based on findings in the reproductive organs in the general toxicology studies, ponatinib may impair male and female fertility. Findings in animals included: degeneration of epithelium of the testes and follicular atresia in ovary and associated endometrial atrophy.

The nonclinical studies needed to support product labeling were reviewed by Dr. Stacey Ricci and Dr. Pedro Del Valle. The nonclinical findings are summarized in the "Executive Summary" of the NDA review and reflected in the product label.

Recommendation: I concur with Drs. Ricci and Del Valle that from a nonclinical perspective, ICLUSIG may be approved for the proposed indication. No additional nonclinical studies are needed to support approval of ICLUSIG for the proposed indication.

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/s/

HALEH SABER
11/19/2012

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:	NDA 203,469
Supporting document/s:	1
Applicant's letter date:	July 30, 2012
CDER stamp date:	July 30, 2012
Product:	Iclusig® (Ponatinib)
Indication:	Chronic phase, accelerated phase, or blast phase chronic myeloid leukemia (CML) or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) resistant or intolerant to prior tyrosine kinase inhibitor therapy.
Applicant:	ARIAD Pharmaceuticals, Inc. 26 Landsdowne Street Cambridge, MA 02139
Review Division:	Hematology and Oncology Toxicology on behalf of the Division of Hematology Products
Reviewers:	M. Stacey Ricci, M.Eng., Sc.D. Pedro L. Del Valle, Ph.D.
Supervisor/Team Leader:	Haleh Saber, Ph.D.
Division Director:	John K. Leighton, Ph.D.
Project Manager:	Monsurat O. Akinsanya, M.S.

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 203469 are owned by ARIAD Pharmaceuticals, Inc. or are data for which ARIAD Pharmaceuticals, Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 203469 that ARIAD Pharmaceuticals, Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 203469.

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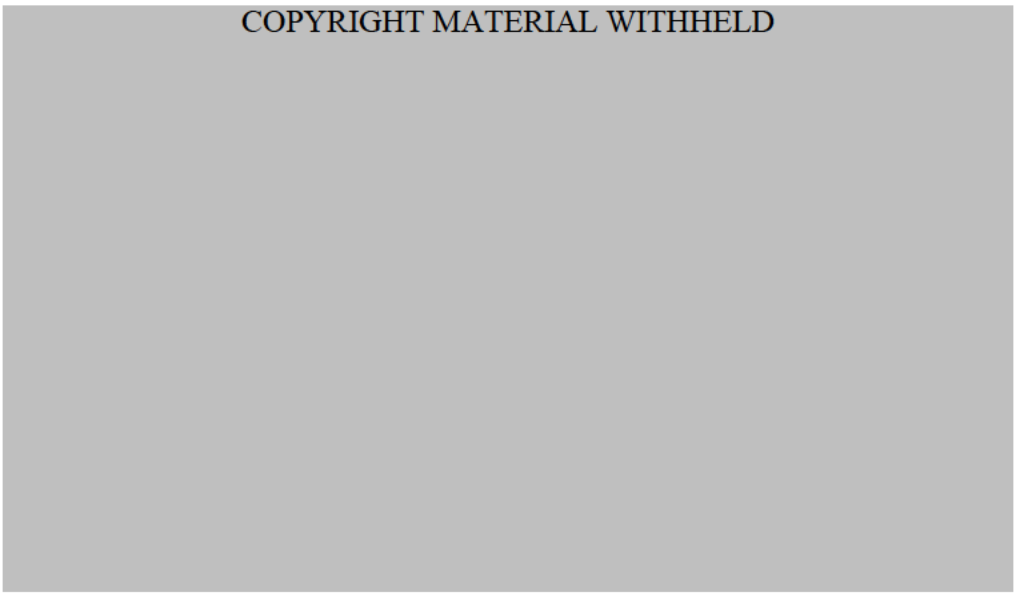
1 Executive Summary

1.1 Introduction

Ponatinib (Iclusig®; AP24534) is an orally administered kinase inhibitor with activity against BCR-ABL and multiple mutant forms of BCR-ABL, including the T315I mutation (threonine to isoleucine substitution at amino acid position 315). BCR-ABL is a constitutively active tyrosine kinase found in most cases of chronic myeloid leukemia (CML) or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL). The BCR-ABL oncogene forms when the ABL gene from chromosome 9 joins the BCR gene on chromosome 22. The resulting chromosome 22 is called the 'Philadelphia chromosome'. The BCR-ABL fusion protein accelerates cell division and inhibits DNA repair, thereby promoting genomic instability.



Three tyrosine kinase inhibitors are approved for use to treat newly diagnosed patients with chronic phase CML, the first generation inhibitor imatinib and the second generation inhibitors dasatinib and nilotinib. Bosutinib was recently approved for the treatment of adult patients with chronic, accelerated, or blast phase Ph+ CML with resistance, or intolerance to prior therapy. BCR-ABL inhibitor resistance is a major cause of treatment failure, and none of the currently approved tyrosine kinase inhibitors (TKIs) can inhibit the T315I mutant form of BCR-ABL at clinically achievable serum concentrations.

1.2 Brief Discussion of Nonclinical Findings

In support of the commercial development program for ponatinib, in vitro studies and animal studies (in the mouse, rat, dog, and monkey) were conducted to evaluate the pharmacology, general toxicology, reproductive effects and genotoxicity of ponatinib.

Ponatinib pharmacology was evaluated using a series of in vitro, cell based and in vivo studies, results of which include:

- Ponatinib inhibits the kinase activity of native BCR-ABL or different mutant BCR-ABL proteins, including the T315I mutation, as demonstrated in vitro using recombinant proteins or cell-based survival assays.
- Comparative studies were conducted using ponatinib, dasatinib, nilotinib or imatinib that demonstrated ponatinib alone has activity towards inhibiting T315I mutant activity at sub-micromolar concentrations.
- Ponatinib was tested against a panel of kinases comprising approximately half of the human kinome, and it inhibited 41 kinases (other than BCR-ABL and its variants) with IC_{50} values ≤ 20 nM. These kinases include RET, FLT3, KIT and members of the VEGFR, FGFR, PDGFR, EPH and SRC families of kinases (see the Appendix for a full list of kinases tested).

Safety pharmacology studies[1] conducted included studies in mice, rats and dogs and the hERG assay. There were no dose-dependent ponatinib-related effects noted on pulmonary function in conscious rats, neurologic effects in mice, or cardiac function in telemeterized dogs. A transient increase in QTc was observed in one dog that received the highest dose used (10 mg/kg; 200 mg/m^2). Ponatinib inhibited hERG current in a dose-dependent manner beginning at the 1000 nM concentration and had an estimated IC_{50} = 2330 nM. ARIAD estimates that the mean C_{max} plasma concentration of a human dose of 45 mg ponatinib is 145 nM (77 ng/ml), which is ~20-fold lower than the hERG IC_{50} value. The potential for ponatinib to block hERG channel activity is low.

Pharmacokinetic parameters were measured in single dose (intravenous or oral) PK studies using rats and monkeys and as part of the repeat dose toxicology studies using rats and monkeys (oral). Ponatinib was absorbed slowly with a T_{max} of 6 and 4 hours, respectively, in the rat and monkey following an oral dose. The oral bioavailability in rats and monkeys was 54 % and 21%, respectively. The terminal half-life of ponatinib in plasma after an intravenous dose was 9.7 hours in rats and 5.3 hours in monkeys. Blood clearance was moderate in rats but was slow in monkeys. In vitro plasma protein binding was high (>99.7%) in all species tested (mouse, rat, monkey and human). Qualitatively, all metabolites observed in human plasma were also detected in either rat or monkey. AP24600 was the major metabolite in plasma of humans and rats but not monkeys. AP24600 had no effect on cells expressing native or T315I mutant BCR-ABL. Ponatinib was eliminated predominantly by metabolism in rats, monkeys and humans. Tissue distribution studies using [^{14}C]-ponatinib in rats demonstrated that ponatinib is widely distributed throughout the body with maximum tissue concentration observed by 8h post-dose. Tissues with the highest relative tissue concentrations were small intestine, uveal tract of the eye, brain (meninges), lung, liver, pituitary and adrenal glands, white and red pulp of spleen, Harderian gland, kidney cortex and thyroid.

[1] Note that the Safety Pharmacology, single dose Pharmacokinetic and General Toxicology studies and the ADME studies were not reviewed in their entirety; descriptions of these studies results are summaries from information provided in the NDA's Common Technical Documents or from the individual study summary sections.

Single-dose and repeat-dose general toxicology studies using mice, rats and monkeys were conducted. The repeat dose 28-Day or 6-Month studies administered AP24534 to either Sprague-Dawley (SD) rats or cynomolgus monkeys daily. An embryo-fetal development (EFD) toxicology study was conducted using the SD rat, and a phototoxicity study was conducted using Long Evans rats.

Single dose studies in rats resulted in mortality or morbidity of 80% of males and 100% females that received the high dose of 100 mg/kg. Histopathology results in these animals indicated immunosuppression as the likely cause of death (due to lymphoid depletion) and associated bacterial sepsis. Necrosis involving the exocrine pancreas and intestinal crypt epithelial cells was also observed. No mortalities were observed following single dose studies in monkeys administered single doses up to 45 mg/kg. Ponatinib-related mortalities were also observed in the repeat-dose studies: rats receiving ≥ 0.75 mg/kg/day (4.5 mg/m^2) and in monkeys receiving 5 mg/kg/day (60 mg/mg^2). A common cause for the moribundity and early mortalities in repeat-dose studies was not established, but toxicities common to both rat and monkey repeat-dose studies included immunosuppression manifest as lymphoid depletion of the thymus, spleen and lymph nodes, increased neutrophils, eosinophils and monocytes and clinical signs of weight loss and skin effects. Hyper- and hypo-plastic changes were noted in femoral bone in both rat studies.

Serious clinical safety issues related to ponatinib use include the cardiovascular, hepatic and pancreatic systems. Nonclinical findings relevant to these events include the following:

- Physical examination findings in the 28-Day monkey study showed systolic heart murmurs in one low dose male (low Grade I/VI), one mid dose female (Grade III/VI) and two high dose animals (male: Grade II/VI; female: Grade I/VI). No murmurs were noted pre-study in the animals with these findings or in monkeys during the 6 month toxicology study. Physical exams performed on Day 13 post-dose on monkeys during the single dose toxicology study revealed systolic heart murmurs (Grade II/VI) in one male in the 45 mg/kg dose group and one female in the 5 mg/kg group. These findings combined and evidence of a possible dose-dependent effect on necrosis of myocardial cells in the 6-month monkey toxicology study may be relevant to the clinically observed cardiovascular toxicities observed.
- Pancreatic alterations were observed in high dose males (acinar cell necrosis, fibrinous inflammation, fibrosis and acinar atrophy) and lipase was elevated in two males from this group. These were the only pancreatic changes observed in the toxicology studies and their relevance to clinically observed cases of pancreatitis is unknown.
- Elevated ALT and AST values occurred in treated monkeys at the end of the 6-month dosing period at all doses that ranged from 2- to 4.5-fold in ALT and 2- to 9.2-fold in AST, relative to Day -6 values. There were no microscopic correlates

observed in the affected monkeys and elevations in transaminases were not present during the recovery period.

None of these findings demonstrated a clear dose-dependent relationship that corresponded with other histopathological or serum chemistry findings.

In the embryo-fetal development study, ponatinib was administered orally to pregnant rats at doses of 0, 0.3, 1, and 3 mg/kg. Systemic exposures (AUC) at 3 mg/kg were equivalent to the AUC in patients receiving the recommended human dose. Soft tissue and skeletal alterations and differences in the number of ossification site averages were observed in the mid and high dose groups and maternal toxicity, including mortality, was observed at the high dose. Additional fetal toxicities observed at the high dose included increased post-implantation loss (early, late and total resorptions); reduced body weight; gross external alterations; multiple soft tissue and skeletal alterations, as well as differences in the number of ossification site averages.

Ponatinib was not genotoxic when evaluated in three separate assays (Ames assay for mutagenicity, in vitro chromosomal aberration assay or in vivo mouse micronucleus assay). Carcinogenicity studies were not completed because of the short life-expectancy of CML and Ph+ ALL patients that have failed prior TKI therapy. Results from a phototoxicity study indicate that ponatinib does not cause dermal toxicity but ocular effects were observed at the mid and high doses used (5 and 10 mg/kg).

1.3 Recommendations

1.3.1 Approvability

RECOMMEND APPROVAL: The submitted pharmacology and toxicology studies using ponatinib (Iclusig®) support the safety of its use in chronic phase, accelerated phase, or blast phase chronic myeloid leukemia (CML) or Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) patients resistant or intolerant to prior tyrosine kinase inhibitor therapy.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies using ponatinib are necessary for the proposed indication.

1.3.3 Labeling

A separate labeling review will be completed if necessary.

2 Drug Information

2.1 Drug

CAS Registry Number
- 1114544-31-8 (HCl salt)
- 943319-70-8 (free base)

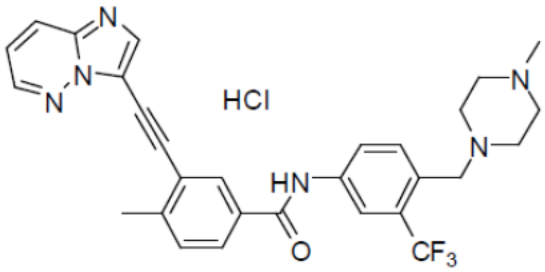
Generic Name
Ponatinib Hydrochloride (USAN); ponatinib (INN)

Code Name
- AP24534 Hydrochloride
- [redacted] (b) (4) (used by Drug Substance manufacturer)

Chemical Name
- IUPAC: 3-(imidazo[1,2-b]pyridazin-3-ylethynyl)-4-methyl-N-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide hydrochloride
- CAS Index Name: Benzamide, 3-(2-imidazo[1,2-b]pyridazin-3-ylethynyl)-4-methyl-N-[4-[(4-methyl-1-piperazinyl)methyl]-3-(trifluoromethyl)phenyl]-, hydrochloride (1:1)

Molecular Formula/Molecular Weight
- $C_{29}H_{28}ClF_3N_6O$ / 569.02 g/mol (HCl salt)
- $C_{29}H_{27}F_3N_6O$ / 532.56 g/mol (free base)

Structure or Biochemical Description



Pharmacologic Class
- Kinase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

- Clinical investigation of ponatinib was conducted under IND 78,375.
- The following DMFs were included in the NDA under Quality Section 1.4.1: [redacted] (b) (4)

2.3 Drug Formulation

The proposed commercial oral formulation of ponatinib is an immediate release, film-coated tablet supplied in two strengths, 15 mg and 45 mg. Ponatinib HCl is manufactured by contract manufacturers and released for use in the manufacture of ponatinib drug product by ARIAD Pharmaceuticals, Inc.

The composition of the proposed commercial formulation was copied from NDA Section 2.3:

Table 1 Target Composition of Ponatinib Film-Coated Tablets

Description	Component	Percent (w/w)	Quantity (mg)		Function	Quality Standard
			15 mg	45 mg		
Core Tablet	Ponatinib free base (added as ponatinib HCl)[a]	15.0	15.0	45.0	Active Ingredient	In-house (see 2.3.S Drug Substance, Section 2.3.S.4 Control of Drug Substance)
	Lactose monohydrate[b]	(b) (4)				NF, Ph. Eur., JP
	Microcrystalline cellulose					NF, Ph. Eur., JP
	Sodium starch glycolate, Type B					NF, Ph. Eur., JP
	Colloidal silicon dioxide					NF, Ph. Eur., JP
	Magnesium stearate (b) (4)					NF, Ph. Eur., JP
	Total	**100%**	**100 mg**	**300 mg**		
Film Coat	(b) (4) White film coating[c]	(b) (4)	(b) (4)			In-house (Section 2.3.P.4 Control of Excipients)
	Purified Water [d]		q.s.[e]	q.s.[e]	Solvent	USP, Ph. Eur., JP
Total Tablet Weight (mg)			102.5	307.5		

a Target quantities of ponatinib free base shown in table are provided as ponatinib HCl. The amount of ponatinib HCl used for manufacturing is adjusted based on the free base content of each drug substance batch (per the certificate of analysis).

b Amount of lactose monohydrate is adjusted (b) (4) in table to accommodate the quantity of ponatinib HCl used and to maintain target total core tablet weights as shown.

c Contains: talc (USP, Ph. Eur., JP), polyethylene glycol (NF, Ph. Eur., JP), polyvinyl alcohol (USP, Ph. Eur., JP), and titanium dioxide (USP, Ph. Eur., JP)

(b) (4)

Table 2 Ponatinib Batches Used for Toxicology Studies

Manufacturer	Batch Number	Date of Manufacture	Manufacturing Process
(b) (4)	PAK-009-173	Jan 2007	(b) (4)
	PAK-010-013	Feb 2007	
	PAK-010-023	Feb 2007	
	ABL411071	Jun 2008	

Additional ARIAD development lots of AP24534 and (b) (4) were used for pharmacology studies and are listed under the individual studies reviewed below.

2.4 Comments on Novel Excipients

No novel excipients are used in the manufacture of ponatinib tablets.

2.5 Comments on Impurities/Degradants of Concern

There are eight ponatinib-related impurities specified in the ponatinib drug substance.

Table 3 Ponatinib-Related Impurities

ARIAD ID#	Observed Level (%w/w)	Drug Substance Release Specification NMT (%w/w) (b) (4)

Two of these impurities have release specifications that exceed the ICHQ3A guideline, (b) (4)

The nonclinical and clinical use of batches containing levels of these two impurities is the basis of the proposed release specifications that exceed the ICH Q3A limits.

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- The [redacted] lot release specification of [redacted]% w/w is acceptable based on a level of [redacted]% w/w that was measured in Batch F10-00024 which was used in the Phase 1 and 2 clinical trials.
- The [redacted] lot release specification of [redacted]% w/w is acceptable based on the level of [redacted]% w/w that was measured in Batch ABL411071 which was used for the 6-month oral rat toxicology study and the 6-month oral monkey study

ARIAD conducted two independent risk assessments to identify potential genotoxic impurities in the Drug Substance. ARIAD estimated a threshold of toxicological concern for genotoxic impurities to be [redacted]. Their estimate is based on an exposure limit of [redacted] day described in FDA guidance[2] [redacted]

- Three low molecular weight solvent-associated impurities with known genotoxic potential were identified ([redacted]) and the release specification for these are [redacted] or below.
- Six additional process-related impurities were also identified. These compounds were subjected to DEREK screening for mutagenicity and carcinogenicity (DEREK for Windows 13.0.0). One of six compounds analyzed [redacted] using the *in silico* DEREK screening method was assessed by ARIAD as being positive for carcinogenicity[3] based on the presence of an [redacted] group and the remaining 5 did not contain structural features associated with genotoxicity. [redacted] was detected at [redacted] in an intermediate drug substance manufacturing step, but was not detected in the final drug substance. [redacted] is [redacted] of the final drug substance, and ARIAD states that the risk of [redacted] exceeding the [redacted] level is low.

Impurity [redacted] is not included in Drug Substance lot release specifications. Based on the guidance provided in ICH S9, the threshold of toxicological concern approach does not apply to the proposed patient population for ponatinib. Therefore, the potential risk for genotoxicity resulting from possible exposure to [redacted] is not a safety concern at this time.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication for ponatinib is for the treatment of adult patients with chronic phase, accelerated phase, or blast phase CML or Ph+ ALL that is resistant or intolerant to prior tyrosine kinase inhibitor therapy. The recommended starting dose of ponatinib

[2] *Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommend Approaches* (Draft), 2008. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079235.pdf

[3] FDA uses DEREK screening only for Ames Assay results (mutagenicity); the utility of DEREK screening for carcinogenicity has not been evaluated by FDA.

is 45 mg taken orally, with or without food, once daily. In clinical trials of ponatinib, doses were held or reduced to 30 mg or 15 mg to manage adverse reactions.

2.7 Regulatory Background

- FDA received the original IND 78375 for ponatinib on November 20, 2007.
- An End of Phase 1 meeting was held May 14, 2010 to discuss clinical and nonclinical aspects of the development program to support an NDA for treatment of CML and Ph+ ALL patients resistant or intolerant to prior TKI therapy.
- Ponatinib received Fast Track designation for CML and Ph+ ALL patients who have the T315I mutation on Nov. 30, 2010.
- On January 12, 2012, a Type B meeting was held to discuss the details of a phase 3 clinical trial and seek advice related to the nonclinical and clinical aspects of the development program leading to product registration in patients with newly diagnosed CML in chronic phase.
- On July 13, 2012, a grant for rolling review was issued.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology Studies

	Study Number	Title
1	ARP280	Analysis of the In Vitro Kinase Selectivity Profile of AP24534 and its Principal Metabolite AP24600
2	OHSU-001	Effect of AP24534 on Viability of BCR-ABL-Negative Cells and Cells Expressing Native or Mutant BCR-ABL
3	ARP027	Demonstration of Inhibition of BCR-ABL and CrkL Signaling by AP24534 in Cells Expressing Wild-type or T315I Mutant BCR-ABL
4	OHSU-002	Cell-Based In Vitro Mutagenesis Screen for AP24534
5	ARP025	Inhibition of Proliferation of Ba/F3 Cell Lines Expressing Wild-Type or Mutant BCR-ABL by AP24534
6	ARP087	Efficacy Study of AP24534 in a Subcutaneous K562 Tumor Model Dependent on Native BCR-ABL in Mice
7	ARP282	The Effect of AP24600, a Primary Metabolite of AP24534, on Viability of Cells Expressing Native or T315I Mutant BCR-ABL
8	ARP043	Induction of Apoptosis of Cells Expressing Wild-type and T315I Mutant BCR-ABL by AP245
9	ARP033	Efficacy Study of AP24534 in a Leukemia Model Dependent on BCR-ABL T315I Mutant Kinase in Mice (II)
10	ARP035	Oral Efficacy Study of AP24534 in a Subcutaneous Tumor Model Dependent on BCR-ABL T315I Mutant in Mice
11	ARP036	Efficacy Study of AP24534 in a Leukemia Model Dependent on Wild-Type BCR-ABL Kinase in Mice

Toxicology Studies

	Study Number	Title
1	QAA00122	28-Day Oral Toxicity Study of AP24534 with Toxicokinetics in Sprague-Dawley Rats with a 28-Day Recovery Period*
2	QAA00193	A 6-Month Oral Toxicity and Toxicokinetics Study in Sprague-Dawley Rats with a 2-Month Recovery
3	QAA00121	28-Day Oral Toxicity Study of AP24534 with Toxicokinetics in Cynomolgus Monkeys with a 28-Day Recovery Period
4	QAA00194	A 6-Month Oral Toxicity Study in Cynomolgus Monkeys with a 2-Month Recovery
5	6843-152	Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay
6	6843-153	Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes
7	6843-154	In Vivo Mouse Bone Marrow Micronucleus Assay
8	200009232	Oral (Gavage) Embryo-Fetal Development Study of AP24534 in Rats

3.2 Studies Not Reviewed

Pharmacology Studies

	Study Number	Title
1	ARP074	Single Dose PK/PD Study of AP24534 (2.5-30 mg/kg) in a Subcutaneous T315I BcrAbl Tumor Model in Mice
2	ARP075	Single Does PK/PD Study of AP24534 (1-10 mg/kg) in a Subcutaneous K-562 Tumor Model in Mice
3	ARP242	Efficacy Study of AP24534 in a Subcutaneous Xenograft Model Using the MV-4-11 Human AML Cell Line
4	ARP281	Effect of Ponatinib on FLT3-ITD-Mediated Signaling and Cell Viability
5	S07092	Effects of AP24534.HCl on hERG K+ Currents in HEK-293 Cells
6	0200ma53-004	Neuropharmacological Profile (NPP) of AP24534 in Mice
7	1001ma53-006	Effects of AP24534 on Spontaneous Motor Activity (SMA) in Mice
8	0225ma53-006	Effects of AP24534 on Motor Coordination Assay in Mice ((b) (4))
9	0209ra53-006	Effects of AP24534 on Electrolyte Concentrations and Urine Volume in Rats
10	1275ra53-001	Pulmonary Assessment in the Conscious Rat with AP24534

11	0239ra53-001	Assessment of Gastrointestinal Propulsion and Gastric Emptying after Oral Administration of AP24534 in Rats
12	1259da53-004	Cardiovascular (Hemodynamic) Evaluation of AP24534 in the Telemetered Beagle Dog

Pharmacokinetic Studies[a]

	Study Number	Title
Absorption		
1	ARP073	Pharmacokinetics of AP24534 in Mouse after Oral Dosing (Solution) at 2.5, 5, 10, 30 and 100 mg/kg
2	12ARIAP3	Pharmacokinetics of Ponatinib and its Metabolite, AP24600, in Portal Vein and Systemic Plasma Following an Oral or Intravenous Dose of Ponatinib in Male Sprague-Dawley Rats
3	805799	A 14-Day Pharmacokinetic Study of AP24534 by Oral Gavage in Rats
4	ARP289	Pharmacokinetics of Ponatinib in Beagle Dogs
5	ARP263	AP24600 Levels in Human Plasma of Subjects/Patients Orally Dosed with Ponatinib in Clinical Studies AP24534-07-101, AP24534-11-102, and AP24534-11-104
6	ARP049	Oral Bioavailability Study of Two Formulations (Solution and Solid) of AP24534 in Cynomolgus Monkeys
7	ARP050	Bioavailability of AP24534 in Cynomolgus Monkeys after a Single Oral Administration of Capsules
Distribution		
8	ARP053	In Vitro Protein Binding and Blood/Plasma Partitioning of AP24534 in Mouse, Rat, Monkey and Human
9	ARP312	In Vitro Binding of AP24600 to Rat and Human Plasma Proteins
10	280n-1101	Quantitative Tissue Distribution of Drug-Related Material Using Whole-Body Autoradiography Following a Single 10 mg/kg Oral Dose of [^{14}C]AP24534 to Male Long-Evans and Sprague Dawley Rats and a Human Radiation Dosimetry Prediction
Metabolism		
11	ARP265	Metabolism of Ponatinib in Mouse Following a Single Oral Dose
12	ARP259	Mass Balance and Metabolism of [14C]Ponatinib in Male Cynomolgus Monkeys, Single Oral Dose: Metabolite Profiling and Characterization
13	ARP257[b]	A Phase 1, Open-Label, Mass Balance Study to Investigate the Absorption, Metabolism and Excretion of [14C]Ponatinib after a Single Oral Dose in Healthy Male Subjects—Metabolite Profiles in Plasma, Urine and Feces and Characterization of Metabolites
14	ARP266	In Vitro Metabolism of Ponatinib in Aroclor 1254 Induced Rat Liver S9
15	ARP258	In Vitro Biotransformation of [14C]Ponatinib in Liver Microsomes and Hepatocytes of Rat, Monkey and Human, and in Recombinant

		Human CYP Isozymes
16	ARP260	Excretion/Mass Balance, Bile Excretion/Mass Balance, and Pharmacokinetics in Sprague-Dawley Rats after a Single Oral Administration of [14C]Ponatinib: Metabolic Profiling and Metabolite Identification in Plasma, Urine, Bile and Feces
17	ARP261	Investigation of AP24600 Formation in Rats Following Intravenous and Oral Administration of Ponatinib
18	ARP319	In Vitro Investigation of AP24600 Formation During Metabolism of Ponatinib
Drug Interactions		
19	Xt103117	In Vitro Evaluation of AP24534 as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes
20	ARP267	In Vitro Evaluation of Ponatinib and its Metabolite AP24600 as Inhibitors of Human Cytochrome P450 Enzymes
21	11ARIAP5R1	Evaluate the Substrate and Inhibition Potential of Ponatinib for Efflux and Uptake Transporters

[a] Method validation reports are not listed here but were submitted to the NDA.
[b] This study was reviewed by the Clinical Pharmacology team.

Toxicology Studies

	Study Number	Title
1	ARP039	Pilot Acute Oral Toxicity Study of AP24534 in Mice
2	QAA00123	Acute Oral Toxicity Study of AP23434 with Toxicokinetics in CD-1 Mice
3	ARP040	Pilot Acute Oral Toxicity Study of AP24534 in Rats
4	QAA00120	Acute Oral Toxicity Study of AP24534 with Toxicokinetics in Sprague-Dawley Rats
5	QAA00124	Acute Oral Toxicity Study of AP24534 with Toxicokinetics in Cynomolgus Monkeys
6	ARP024	14-Day Pilot Oral Toxicity Study of AP24534 with Toxicokinetics in Mice
7	ARP037	14-Day Pilot Oral Toxicity Study of AP24534 with Toxicokinetics in Rats (I)
8	ARP038	14-Day Pilot Oral Toxicity Study of AP24534 with Toxicokinetics in Rats (II)
9	QAA00113	An Oral Dose-Range Finding Toxicity and Toxicokinetics Study of AP24534 in Cynomolgus Monkeys
10	20009231	Oral (Gavage) Dosage Range-Finding Embryo-Fetal Development Study of AP24534 in Rats
11	20011779	Single-Dosage Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of AP24534 on Eyes and Skin in Pigmented Rats

3.3 Previous Reviews Referenced

Study QAA00122 was reviewed previously by Dr. Doo Y. Lee Ham under IND 078,375

4 Pharmacology

4.1 Primary Pharmacology[4]

1. Study ARP280: Analysis of the In Vitro Kinase Selectivity Profile of AP24534 and its Principal Metabolite AP24600

Report Date: May 16, 2012
Conducting Laboratories: (b) (4)

Introduction

This study evaluated the ability of AP24534 to inhibit a panel of kinases in vitro using two different screening assays. One assay employed a panel of 235 kinases (222 unique kinases plus 13 mutant variants) to test a single AP24534 concentration (1 microM). The second assay screened a subset of 108 kinases that were strongly inhibited in the first screen plus additional kinases using a range of ponatinib concentrations. These results were used to estimate IC50 concentrations.

Methods

- AP24534 development lots # 1, 7 and A24600 #4 were used for these studies.
- ARIAD contracted with (b) (4) to assay the 235 kinase panel, which includes roughly half of the human kinome.
- ARIAD contracted with (b) (4) to perform the second kinase panel screening.
- Both methods measured kinase inhibition by quantifying radioactivity incorporated onto a protein substrate that was transferred from ^{33}P-γ labeled ATP.

Results

- AP24534 inhibited native ABL and all 5 BCR-ABL mutants tested, including T315I, with IC50 values between 0.3 and 2.0 nM.
- AP24534 also inhibited 41 additional kinases, plus 9 mutant variants, with IC_{50} values ≤ 20 nM. These kinases include RET, FLT3, KIT and members of the FGFR, PDGFR, VEGFR, EPH and SRC families of kinases.
- AP24600, a principal metabolite of AP24534, did not inhibit native or T315I mutant ABL (IC50 > 3000 nM). A complete list of kinase results is provided in the Appendix.

[4] Many results submitted to the NDA were published previously in the paper by O'Hare, T. et al., *Cancer Cell* (2009), 16: 401-412.

During the course of this review, a discrepancy was identified for the IC_{50} value for VEGFR2, where the result provided in the NDA (560 nM) was 2-orders of magnitude higher than that published in the O'Hare 2009 Cancer Cell paper[5] (1.5 nM). An Information Request was made on Nov. 5, 2012 to which ARIAD responded on Nov. 8, 2012. ARIAD noted that a mistake was made in reporting the IC_{50} value for VEGFR2, and that the IC_{50} value for ponatinib inhibition of VEGFR2 was 2.9 nM and not 560 nM. They also noted that the results provided in the NDA were from the same analysis as was provided in the O'Hare 2009 paper, with the singular exception for VEGFR2. The O'Hare paper reports a VEGR2 IC_{50} result derived from an earlier set of pilot studies conducted by the (b) (4) *. A request was made to verify the results provided for the IC_{50} values for all 108 kinases tested. This table is provided in the Appendix to this Review.*

2. Study OHSU-001: Effect of AP24534 on Viability of BCR-ABL-Negative Cells and Cells Expressing Native or Mutant BCR-ABL

Report Date: April 13, 2012
Conducting Laboratory: (b) (4)

Introduction

This study evaluated the effect of AP24534 on human leukemia cell viability in vitro. A panel of BCR-ABL-positive and –negative human leukemia cell lines and a panel of Ba/F3 cell transfectants (whose viability is dependent on ectopically expressed BCR-ABL) were used. The Ba/F3 cells expressed BCR-ABL or one of the following BCR-ABL mutants: M244V, G250E, Q252H, Y253F, Y253H, E255K, E255V, T315A, T315I, F317L, F317V, M351T, F359V, and H396P.

Methods

Cell viability was measured using a methanethiosulfonate (MTS)-based colorimetric assay with a 96-well plate reader. IC_{50} values were calculated using the mean of three independent experiments performed in quadruplicate.

Results

AP24534 inhibited proliferation of Ba/F3 cells expressing native BCR-ABL and the BCR-ABL mutants tested, including T315I (Table 4). Growth of parental Ba/F3 cells or non-CML leukemia cells was inhibited only at significantly higher AP24534 concentrations (IC_{50} >1000 nM). Results using non-engineered cell lines also demonstrated AP24534 selective effects on BCR-ABL-driven cell viability.

[5] O'Hare, T. et al., *Cancer Cell* (2009), 16: 401-412.

Table 4 In Vitro Cell Viability of Bcr-Abl Mutants

(Excerpted from the Applicant's Submission)

	AP24534					
	IC_{50} (nM)					
	Exp 1	Exp 2	Exp 3	Mean	SD	SEM
Ba/F3 cells						
Native BCR-ABL	0.8	0.03	0.7	0.5	0.4	0.2
M244V	2.9	0.1	3.6	2.2	1.9	1.1
G250E	2.7	2.2	7.4	4.1	2.8	1.6
Q252H	2.6	0.5	3.6	2.2	1.6	0.9
Y253F	3.4	2.3	2.6	2.8	0.5	0.3
Y253H	3.0	2.8	12.7	6.2	5.7	3.3
E255K	8.0	18.9	14.3	13.7	5.5	3.2
E255V	16.0	67.0	24.1	35.7	27.4	15.8
T315A	2.9	0.03	1.9	1.6	1.4	0.8
T315I	4.7	17.5	12.1	11.4	6.4	3.7
F317L	1.7	0.2	1.5	1.1	0.8	0.5
F317V	5.2	17.0	6.3	9.5	6.5	3.7
M351T	2.0	0.2	2.4	1.5	1.2	0.7
F359V	4.4	16.6	10.2	10.4	6.1	3.5
H396P	0.6	0.1	2.4	1.1	1.2	0.7
Parental	1881.2	1755.4	1503.5	1713.4	192.3	111.0
CML leukemia cells						
K562	5.1	2.3	4.4	3.9	1.5	0.9
KYO1	0.2	0.7	0.3	0.4	0.3	0.2
LAMA	0.1	0.5	0.3	0.3	0.2	0.1
Non-CML leukemia cells						
Marimo	2283.9	1996.3	2365.1	2215.1	193.8	111.9
HEL	2539.3	2353.7	2671.5	2521.5	159.6	92.2
CMK	1331.3	1923.0	1702.2	1652.2	299.0	172.6

In the Pharmacology Written Summary section of the NDA, results using imatinib, nilotinib and dasatinib in Ba/F3 cell lines expressing the BCR-ABL mutants listed above were compiled and compared with the results for ponatinib (Table 5). A side-by-side comparison of ponatinib with the three other TKIs using a subset of these Ba/F3 mutant cell lines are reviewed in Study ARP025 below.

Table 5 Effect of Ponatinib, Imatinib, Nilotinib or Dasatinib on Cell Viability of Native or 14 Mutant Variants of BCR-ABL

Ba/F3 Cell Viability Assay IC_{50} (nM)				
BCR-ABL	**Ponatinib[1]**	**Imatinib[2]**	**Nilotinib[2]**	**Dasatinib[2]**
Native	0.5	260	13	0.8
M244V	2.2	2000	38	1.3
G250E	4.1	1350	48	1.8
Q252H	2.2	1325	70	3.4
Y253F	2.8	3475	125	1.4
Y253H	6.2	>6400	450	1.3
E255K	14	5200	200	5.6
E255V	36	>6400	430	11
T315A	1.6	971	61	125
T315I	11	>6400	>2000	>200
F317L	1.1	1050	50	7.4
F317V	10	350	nd	53
M351T	1.5	880	15	1.1
F359V	10	1825	175	2.2
H396P	1.1	850	41	0.6
Parental	1713	>6400	>2000	>200

1: Report OHSU-001
2: O'Hare T. et al (2005) *Cancer Res*. 65: 4500-4505. O'Hare T. et al (2007) *Blood*. 110: 2242-2249.
nd=not determined

3. Study ARP027: Demonstration of Inhibition of BCR-ABL and CrkL Signaling by AP24534 in Cells Expressing Wild-type or T315I Mutant BCR-ABL

Report Date: October 29, 2007
Conducting Laboratory: Not specified; the source data cited is an ARIAD notebook (#1180).

Introduction
This study examined the effect of AP24534 on BCR-ABL and CrkL phosphorylation using quantitative Western blot methodology.

Methods
- K-562 cells and Ba/F3 cells that expressed either native or BCR-ABL or the T315I mutant were treated with AP24534, dasatinib or nilotinib for 3 hours.
- Antibodies that bind to phosphorylated BCR-ABL (at tyrosine 245) or CrkL (at tyrosine 207) were used,

Results
The phosphorylation of BCR-ABL and CrkL was completely inhibited by AP24534, dasatinib and nilotinib in a dose-dependent manner in K562 cells or Ba/F3 cells expressing native BCR-ABL (Table 6). In Ba/F3 cells expressing the T315I mutant,

AP24534 and not dasatinib nor nilotinib inhibited BCR-ABL and CrkL phosphorylation at nM concentrations.

Table 6 Inhibition of BCR-ABL and CrkL phosphorylation in Cells Expressing Native or T315I BCR-ABL using AP24534, Nilotinib or Dasatinib

(Excerpted from the Applicant's Submission)

IC50 (nM)

	K-562 Native BCR-ABL		Ba/F3 Native BCR-ABL		Ba/F3 T315I BCR-ABL	
	p-BCR-ABL	p-CRKL	p-BCR-ABL	p-CRKL	p-BCR-ABL	p-CRKL
Ponatinib	7	68	25	83	78	580
Nilotinib	21	607	53	125	>1000	>1000
Dasatinib	0.6	16	4	19	>1000	>1000

Source: ARIAD Report ARP027

4. Study OHSU-002: Cell-Based In Vitro Mutagenesis Screen for AP24534

Report Date: April 13, 2012
Conducting Laboratory: (b) (4) [redacted]

Introduction

Ba/F3 cells expressing native BCR-ABL were treated with a mutagen then cultured in AP24534. Resulting clonal outgrowths and alterations in the BCR-ABL kinase sequence were evaluated.

Methods

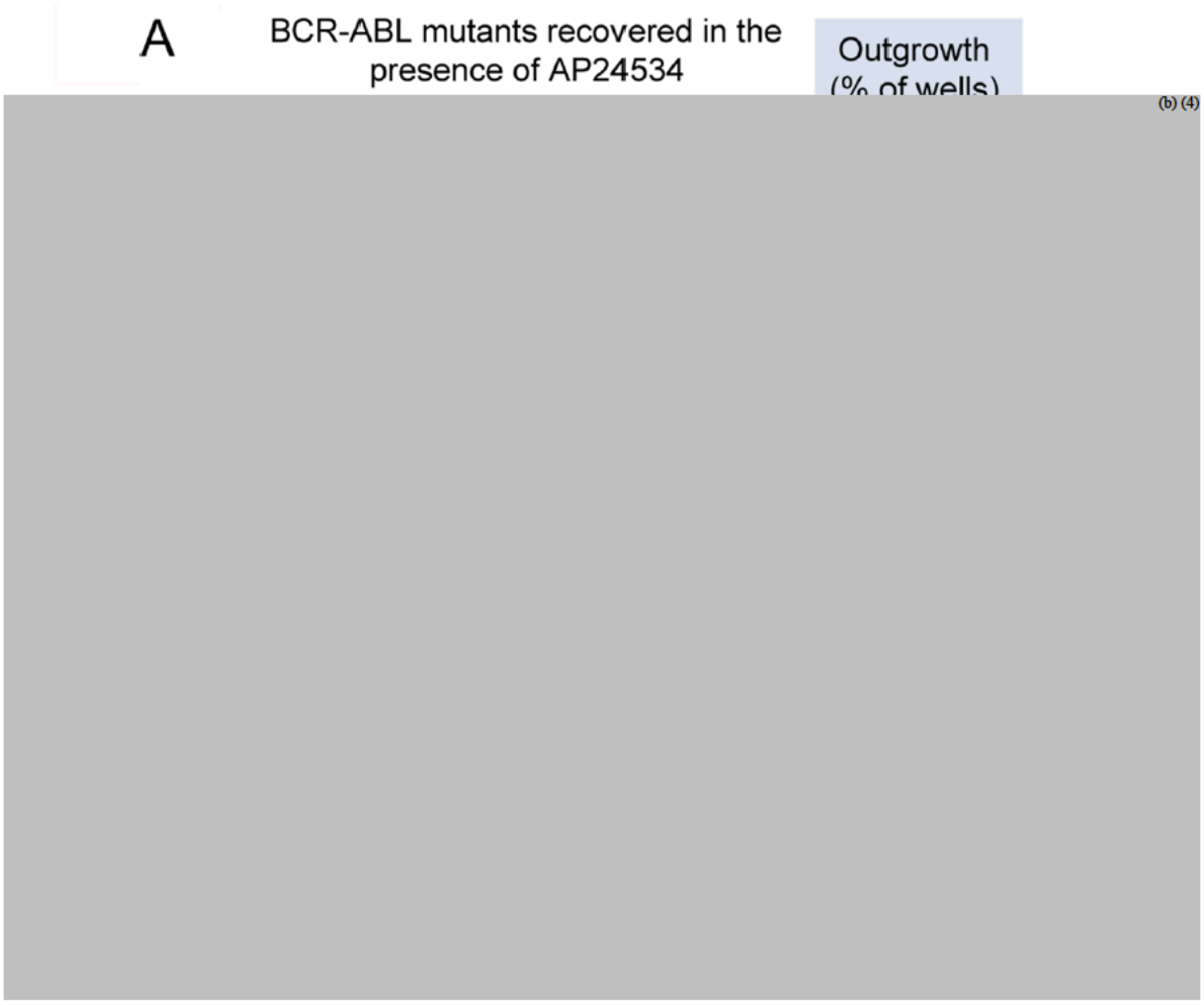
- Cells were treated overnight with N-ethyl-N-nitrosourea (ENU; 50 mcg/ml) and re-plated in media containing 5, 10, 20 or 40 nM AP24534. Cells were maintained and observed for growth for 28 days.
- Genomic DNA was extracted from outgrowths and the BCR-ABL kinase domain was sequenced.

Results

The mutagenesis/AP24534 treatment experiment was performed three times. At 5 nM AP24534, all wells survived and 90% of the sequenced sub-clones expressed native BCR-ABL. At 10 nM, clonal outgrowths contained one of the mutations shown in Figure 1 below. Only the T315I and E255V mutants survived in 20 nM AP24532, and no clones survived in 40 nM AP24534.

Figure 1 Frequency and Scope of BCR-ABL mutants recovered after AP24534 treatment

(Excerpted from the Applicant's Submission)



5. Study ARP025: Inhibition of Proliferation of Ba/F3 Cell Lines Expressing Wild-Type or Mutant BCR-ABL by AP24534

Report Date: October 29, 2007
Conducting Laboratory: ARIAD Pharmaceuticals, Inc.

Summary

This study examined the effect of AP24534 on in vitro proliferation of Ba/F3 cells expressing native or mutant BCR-ABL proteins (one of 5 different mutations: T315I, Y253F, E255K, H396P and M351T). Proliferation was measured indirectly using an MTS assay. Dasatinib, nilotinib and imatinib were also tested. Values of IC_{50} for AP24534 ranged from 0.6 to 7.8 nM (Table 7). AP24534 was found to have similar activity as dasatinib, 10 to 100-fold greater activity than nilotinib, and 1000-fold greater

activity than imatinib in all BCR-ABL cell lines except for the T315I mutant in which only AP24534 demonstrated cell growth inhibition.

Table 7 Inhibition of Cell Proliferation In Vitro by AP24534, Dasatinib, Nilotinib and Imatinib

(Excerpted from Applicant's Submission)

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IC50±SD (nM), or where indicated, IC90±SD (nM)

	AP24534	dasatinib	nilotinib	imatinib
WT Bcr-Abl	0.8±0.2 *IC90= 1.7±0.1*	0.9±0.1	21±2.8	461.5±142.1
T315I	7.8±2.3 *IC90= 28.2±8.3*	>10,000	>10,000	>10,000
Y253F	0.9±0.1 *IC90= 1.6±0.4*	0.6±0.1	65.7±6.4	3111.3 ±661.9
E255K	3.2±0.8 *IC90= 6.4±0.1*	1.5±0.0.4	106.0±8.7	3731.3 ±915.2
H396P	0.6±0.1 *IC90= 0.9±0.1*	0.4±0.1	19.7±5.8	995.5 ±41.6
M351T	1.0±0.2 *IC90= 2.3±0.5*	0.9±0.2	13.0±3.0	1429.0 ±275.1
Parental	1126±282	>10,000	>10,000	>10,000

6. Study ARP087: Efficacy study of AP24534 in a Subcutaneous K562 Tumor Model Dependent on Native BCR-ABL in Mice

Report Date: May 2, 2012
Conducting Laboratory: ARIAD Pharmaceuticals, Inc.

Introduction

This study examined the effect of oral administration of AP24534 to SCID mice harboring subcutaneous tumor xenografts of the K562 human CML cell line expressing native BCR-ABL. Mice were treated with AP24534 daily for 18 days.

Methods

- Tumor volume was measured using calipers at least twice per week and the animal body weight was measured daily. When the average tumor volume

reached approximately 200 mm^3, animals were randomized to the following groups for treatment:

Group	No. of animals/group	Test Article	Dose (mg/kg)	Dosing volume (mL/kg)	Dosing Regimen
1	10	vehicle	0	5	p.o. once daily dosing for 18 days
2	10	AP24534	1	5	p.o. once daily dosing for 18 days
3	10	AP24534	2.5	5	p.o. once daily dosing for 18 days
4	10	AP24534	5	5	p.o. once daily dosing for 18 days
5	10	AP24534	10	5	p.o. once daily dosing for 18 days
6	10	AP24534	30	5	p.o. twice/week dosing (D0, 3, 7, 10, 14 and 17)) during 18 days

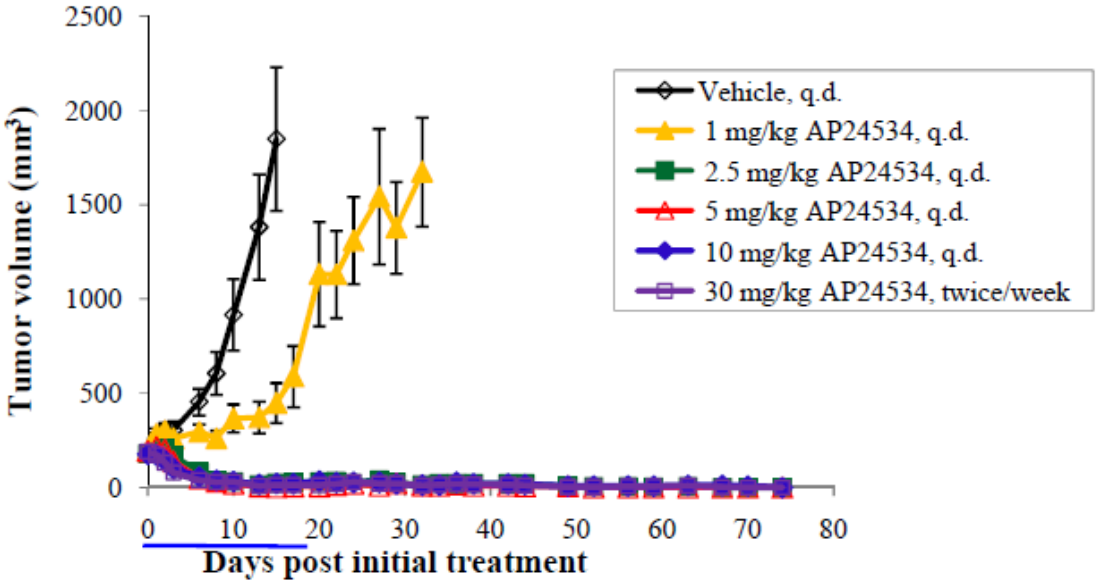
- Following the 18 day treatment cycle, mice were monitored for an additional 8 week period.

Results

AP24534 inhibited tumor growth at all doses tested, and demonstrated tumor regression at doses ≥ 2.5 mg/kg. Half of the mice from the vehicle control group were sacrificed prior to study termination because their tumors grew greater than permitted (>500 mm^3). Mean body weight losses were similar to or less than that observed in the vehicle control group. There were no mortalities resulting from AP24534 treatment or any clinically observable toxicities. Similar tumor regression was observed in mice receiving 30 mg/kg twice a week as in mice that received ≥ 2.5 mg/kg daily, suggesting that intermittent scheduling may be as effective as daily dosing (Figure 2).

Figure 2 K562 Tumor Xenograft Volume Change in SCID Mice

(Excerpted from Applicant's Submission)



(The dosing period is indicated with a bar)

7. Study ARP282: The Effect of AP24600, a Primary Metabolite of AP24534, on Viability of Cells Expressing Native or T315I Mutant BCR-ABL

Report Date: May 3, 2012
Conducting Laboratory: ARIAD Pharmaceuticals, Inc.

Summary
This study examined the cytotoxic effect of AP24534 and its metabolite AP24600 on Ba/F3 cells expressing native or T315I mutant BCR-ABL. Cell viability was measured using a colorimetric MTS assay. The IC_{50} concentrations of AP24534 were 1.3 and 5.0 nM for the native and T315I mutant, respectively. The IC_{50} for AP24600 was >10,000 nM for both cell lines.

8. Study ARP043: Induction of Apoptosis of Cells Expressing Wild-type and T315I Mutant BCR-ABL by AP24534

Report Date: October 29, 2007
Conducting Laboratory: ARIAD Pharmaceuticals, Inc.

Summary
In this study, the apoptotic effects of AP24534, dasatinib, and nilotinib were tested using Ba/F3 or K562 cell lines expressing either native or the T315I mutant BCR-ABL. Caspase 3/7 activity was measured using a commercially available fluorescent substrate (Apo-One Caspase-3/7™; Promega) using a 96-well plate reader. Values of EC_{50} values were determined after 24 hour treatments in Ba/F3 cells and 48 hour treatments in K562 cells. Only AP24534 treatment resulted in apoptosis of the T315I mutant cell line (Table 8). Dasatinib and AP24534 treatment produced similar EC_{50} in the two cell lines harboring native BCR-ABL.

Table 8 Apoptosis in Cells Treated with AP24534, Dasatinib or Nilotinib

(Excerpted from Applicant's Submission)

(EC50±SD, nM)

	Ba/F3 T315I cells 24 hr	Ba/F3 wt cells 24 hr	K-562 cells 48 hr
dasatinib	>5000	17±2	2±0.5
AP24534	57±16	16±3	2±0.4
nilotinib	>5000	332±54	34±13

9. Study ARP033: Efficacy study of AP24534 in a leukemia model dependent on BCR-ABL T315I mutant kinase in mice (II)

Report Date: October 11, 2007
Conducting Laboratory: ARIAD Pharmaceuticals, Inc.

Introduction
This study examined the effect of AP24534 in a mouse model of CML. In this model, the murine pro-B cell line Ba/F3 containing the BCR-ABL T315I mutant was injected intravenously to female SCID mice.

Methods
- Three days after cell inoculation (10^6 cells via tail vein injection), mice were administered AP24534 by oral gavage at doses of 1.25, 2.5, 5, 15 and 25 mg/kg/day (n=10/group) for up to 19 days. Mice were monitored for up to 33 days after cell inoculation.
- The test article used was Lot 4 (synthesized at ARIAD Pharmaceuticals, Inc.). The salt/purity correction factor of 0.786 was used.
- Observations were made at least twice daily during weekdays and once daily on weekends for clinical signs of tumor burden and general health conditions. Body weights were measured daily and spleens and livers (where leukemia cell infiltration was expected) were collected post-mortem and weighed.
- Median survival times for each group was calculated and the Log-rank test method was used to calculate statistical significance ($p<0.05$).

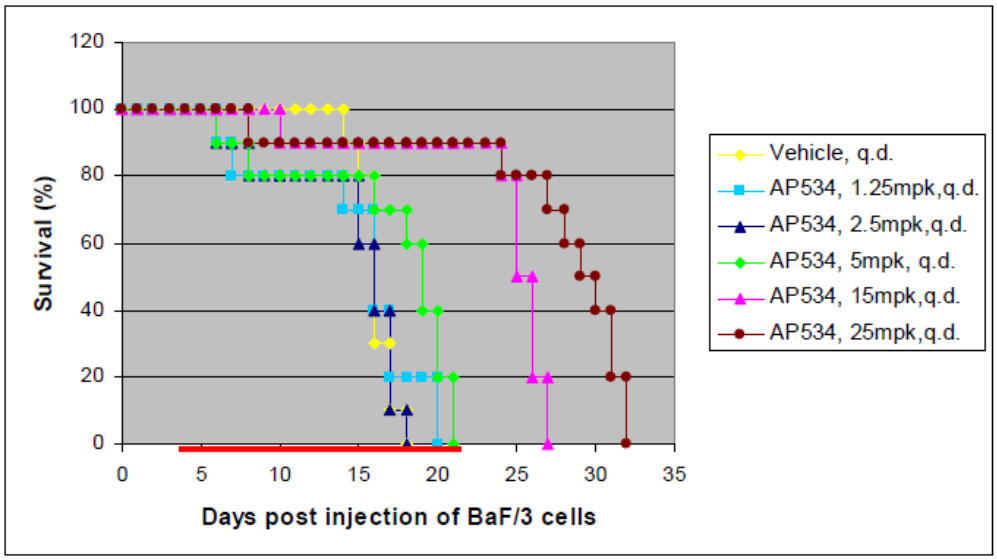
Results
An AP24534 dose-dependent increase in median survival occurred. Tumor regression was not observed as no animals survived past Day 33. Data copied from p.7 of the study report is shown in Figure 3.

Body weight loss occurred that was not clearly attributable to either treatment or tumor burden in liver and spleen. Significant losses occurred in animals (~10-18%) regardless of treatment immediately prior to the day when <50% of animals survived. Clinical signs manifested ~Day 14 for animals treated with ≤5 mg/kg and lasted 3-5 days preceding death. Clinical signs manifested ~Day 17 for animals treated with ≥15 mg/kg and lasted 7-10 days prior to death. Signs included ruffled fur, low level of activity, loose or black stool.

Figure 3 Survival Data of CML Mouse Model with T351I Mutation following AP2435 Treatment

(Excerpted from Applicant's Submission)

(The dosing period is indicated with a red bar)



Survival Data of Vehicle and AP24534 Treatment Groups

Group	Test Article	Dose (mg/kg)	Dosing Regimen	Median Survival (days)	Survival Increase (%)	p Value
1	Vehicle	0	QDx19	16.0	N/A	N/A
2	AP24534	1.25	QDx19	16.5	3.1	> 0.05
3	AP24534	2.5	QDx19	16.5	3.1	> 0.05
4	AP24534	5	QDx19	19.5	21.9	< 0.01
5	AP24534	15	QDx19	26.0	62.5	< 0.01
6	AP24534	25	QDx19	30.0	87.5	< 0.01

10. Study ARP035: Oral efficacy study of AP24534 in a subcutaneous tumor model dependent on BCR-ABL T315I mutant in mice

Report Date: October 11, 2007
Conducting Laboratory: ARIAD Pharmaceuticals, Inc.

Summary

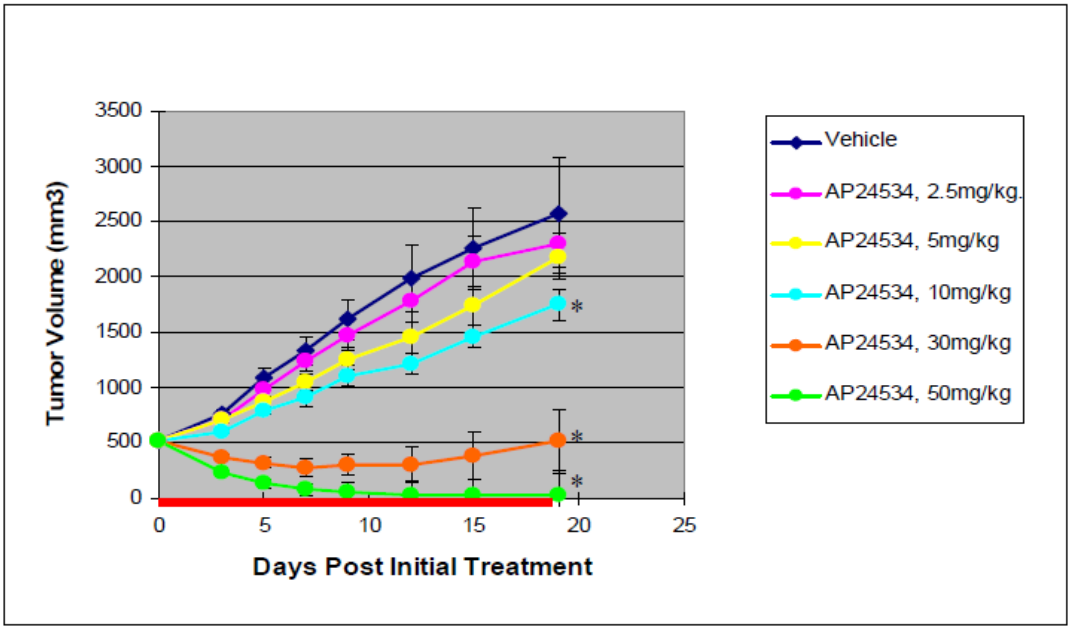
The murine pro-B cell line Ba/F3 harboring the T315I mutant isoform of BCR-ABL was subcutaneously injected into nude mice (*nu/nu).* Mice were treated with AP24534 at doses of 2.5, 5, 10, 30 or 50 mg/kg/day for 19 days. Tumor volume was measured in two dimensions using a caliper at least twice a week (volume= L x W^2 x 0.5).

Tumor growth inhibition was observed at doses ≥10 mg/kg and tumor stasis and tumor regression occurred at dose levels of 30 and 50 mg/kg/day (Figure 4). Skin rash and >10% body weight loss was observed at 50 mg/kg/day but not at lower levels.

Figure 4 Tumor Volume Change in Xenografts with T351I Mutation

(Excerpted from Applicant's Submission)

(The dosing period is indicated with a red bar. * indicates p< 0.01)



Mean Percentage Body Weight Loss of Vehicle and AP24534 Treatment Groups

Group	Test Article	Dose (mg/kg)	Dosing Regimen	Mean Max % Body Weight Loss
1	vehicle	0	QDx19	0
2	AP24534	2.5	QDx19	0
3	AP24534	5	QDx19	0
4	AP24534	10	QDx19	-3.9
5	AP24534	30	QDx19	-4.2
6	AP24534	50	QDx19	-11.8

11. Report ARP036: Efficacy study of AP24534 in a leukemia model dependent on wild-type BCR-ABL kinase in mice

Report Date: October 11, 2007
Conducting Laboratory: ARIAD Pharmaceuticals, Inc.

Introduction

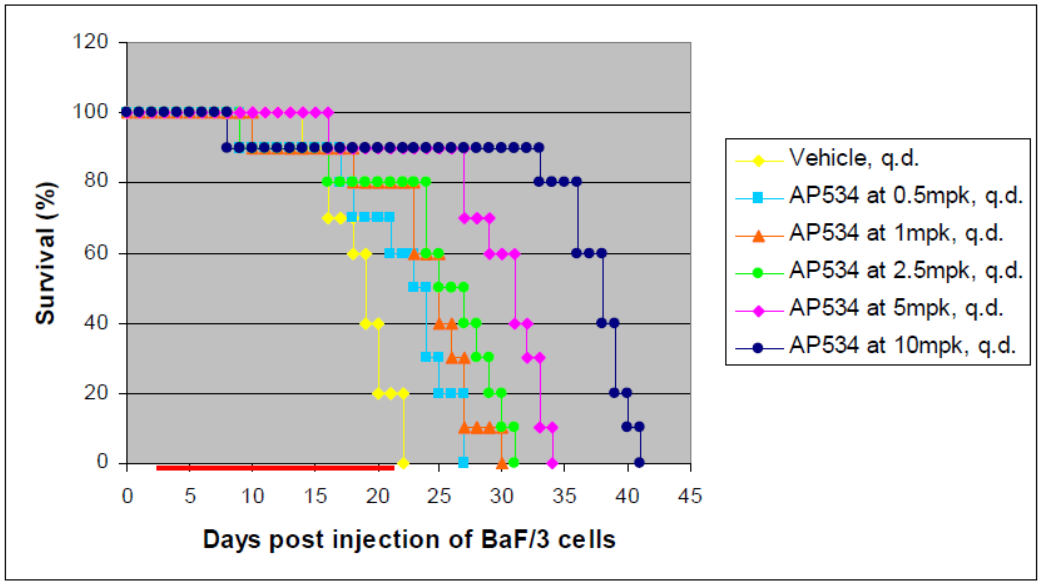
Ba/F3 cells expressing BCR-ABL were injected into the tail vein of female SCID mice. Three days later, treatment began with AP24534 by oral gavage (0.5, 1, 2.5, 5, or 10 mg/kg/day) for 19 days.

Results
Survival was prolonged in a dose-dependent manner (Figure 5). Body weight losses were ~3-6% in all groups, including the vehicle control.

Figure 5 Survival Data of CML Mouse Model with Native BCR-ABL Following AP2435 Treatment

(Excerpted from Applicant's Submission)

(The dosing period is indicated with a red bar)



4.2 Secondary Pharmacology

Secondary Pharmacology studies were not reviewed. The study summaries described in the Pharmacology Written Summary section of the NDA state that the in vitro kinase assay results further evaluated the ability of ponatinib to inhibit the activity of RET, FLT3, KIT and members of the FGFR, PDGFR, VEGFR, EPH and SRC families of kinases with IC50s ≤20 nM. Additional data using cell-based assays confirmed that ponatinib inhibits RET, KIT and PDGFR-α and also FLT3 and FGFRs in cell lines and in vivo models. ARIAD states these data are provided in Study Reports ARP281, ARP242, ARP087 and in publications containing experimental results using ponatinib.[6] There were no pharmacology studies that specifically examined platelet function.

[6] Gozgit JM, et al., *Mol Cancer Ther*, 2012, 11(3):690-9; Zirm E. Et al., *Br J Hematol, 2012*. 157(4):483-92; Gozgit JM et al., *Mol Cancer Ther,* 2011, 10(6):1028-35.

4.3 Safety Pharmacology

Safety Pharmacology studies were not reviewed but the following summary information was compiled from the Pharmacology Written and Tabulated Sections of the NDA. All Safety Pharmacology studies were described as being GLP Compliant; all animals were administered one dose of AP24534 by oral gavage.

hERG Channel Activity (Study S07092)

- Doses used were 0.1, 0.3, 1, 3 and 9 microM
- Ponatinib inhibited hERG current in a dose dependent manner with an IC_{50}=2.33 microM. Percent inhibition at each test concentration (lowest to highest) were: -7, 3, 14, 57, and 91%.

Cardiovascular (Hemodynamic) Evaluation (Study 1259DA53.004)

- Telemeterized Beagle Dogs
- 2/sex/group
- 0, 2, 5, 10 mg/kg
- No effects on cardiac, circulatory functions or ECGs.

CNS Neuropharmacologic Profile (Study 0200MA53.004)

- 10 Female CD-1 Mice/group
- 0, 10, 30 or 100 mg/kg
- No neuropharmacological or toxicological signs observed up to 72-hours post dosing.

CNS Motor Activity (Study 1001MA53.006)

- 10 Female CD-1 Mice/group
- 0, 10, 30 or 100 mg/kg
- No effects at 1 hour post-dose; a decrease in spontaneous activity was observed in the 100 mg/kg dose group 24 hours post-dose but reverted to control group behavior.

CNS Motor Coordination (Study 0225MA53.006)

- 10 Female CD-1 Mice/group
- 0, 10, 30 or 100 mg/kg
- No effect observed up to 72-hours post-dosing.

Pulmonary (Study 1275RA53.001)

- 4 Male Sprague-Dawley rats per group
- 0, 3, 10, 30 mg/kg
- No treatment effects noted on respiratory rate, tidal volume or minute volume in conscious rats.

Renal: Electrolyte Concentrations and Volume Diuresis (Study 0209RA53.006)

- 10 male Sprague-Dawley rats per group
- 0, 3, 10, 30 mg/kg

- Increased urine volume of 42, 51 and 46% over a 4-hour period following administration of 3, 10 and 30 mg/kg, respectively.

Gastrointestinal Propulsion and Gastric Emptying (Study 0239RA53.001)

- 10 Male Sprague-Dawley rats per group
- 0, 3, 10, 30 mg/kg
- No effect on GI motility. Decrease in gastric emptying at all AP24534 doses.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The following summary information was compiled from the NDA but the primary data were not reviewed:

The absorption, distribution, metabolism and excretion (ADME) of ponatinib were studied in mice (CD-1), rats (Sprague Dawley (SD), Long-Evans (LE)), dogs (Beagle), monkeys (Cynomolgus) and humans after oral and/or intravenous administration. All ADME studies used ponatinib formulated in an aqueous buffer (25 mM citrate buffer, pH 2.75) for both oral and intravenous administration. In one monkey study a capsule formulation was also used. Toxicology studies also used the citrate buffer formulation. All ADME and toxicology studies were conducted using ponatinib mono-hydrochloride salt but the administered doses were calculated on the basis of ponatinib-free-base equivalents.

Pharmacokinetic parameters were measured in single dose (intravenous or oral) PK studies using rats and monkeys and as part of the repeat dose toxicology studies using rats and monkeys (oral). Ponatinib was absorbed slowly with a T_{max} of 6 and 4 hours, respectively, in the rat and monkey following an oral dose. The oral bioavailability in rats and monkeys was 54.0 % and 20.6%, respectively. The terminal half-life of ponatinib in plasma after an intravenous dose was 9.7 hours in rats and 5.3 hours in monkeys. Blood clearance was moderate in rats but was slow in monkeys. In vitro plasma protein binding was high (>99.7%) in all species tested (mouse, rat, monkey and human).

Table 9 Summary of Ponatinib PK Parameters in Rat and Monkey Plasma Following a Single Dose

Route and Dose	PK Parameters	Rat	Monkey
Intravenous (3 mg/kg to rats) 1 mg/kg to monkeys	C_0 (ng/mL)	427 ± 298	1219 ± 525
	$t_{1/2}$ (hr)	9.72 ± 1.73	5.3 ± 2.6
	CL (mL/min/kg)	26.5 ± 24.2	8.7 ± 2.4
	V_{ss} (L/kg)	17.7 ± 12.4	2.6 ±0.9
	AUC_∞ (hr•ng/mL)	3088 ± 2090	2096 ± 772
Oral (15 mg/kg to rats) 2-3 mg/kg in a capsule to monkeys	C_{max} (ng/mL)	453 ± 68.7	96 ± 57
	t_{max} (hr)	6.0 ± 0	4
	$t_{1/2}$ (hr)	9.95 ± 2.17	7.1 ± 2.3
	AUC_∞ (hr•ng/mL)	8320 ± 1786	942 ± 438
	Oral Bioavailability (%)	54 ± 12	20.6

Excerpted from the Pharmacokinetics Written Summary.

Based on results from liver microsome analysis and the plasma samples from rat and monkey studies, the ponatinib metabolite, N-desmethyl ponatinib (AP24567) was monitored in initial clinical studies. Later studies using radiolabeled ponatinib identified metabolite AP24600 as a major metabolite in rat and human plasma, but only at trace levels in monkeys. Qualitatively, all metabolites observed in human plasma were also detected in either rat or monkey. AP24600 was the major metabolite in plasma of humans and rats but not monkeys. AP24600 had no effect on cells expressing native or T315I mutant BCR-ABL. Orally administered ponatinib was eliminated predominantly by metabolism in rats, monkeys and humans.

A tissue distribution study (Report No. 280N-1101) using radiolabeled ponatinib [^{14}C]AP24534 in pigmented (Long Evans) and albino (Sprague-Dawley) rats demonstrated that ponatinib is widely distributed throughout the body with maximum tissue concentration observed by 8h post-dose. Quantitative whole body autoradiography was conducted following a single 10 mg/kg oral (PO) administration of [^{14}C] AP24534. Selected sagittal sections were exposed to phosphor image screens, and tissue radioactivity concentrations were quantified from the whole-body autoradiograms using a validated image analysis system. Concentrations of radioactivity were expressed as mcg equivalents of [^{14}C] AP24534 per gram of matrix (mcg equiv/g). The tissues of pigmented and albino rats with the highest relative tissue concentrations (range: 10.308 and 691.179 mcg equiv/g) were: small intestine, uveal tract of the eye, brain (meninges), lung, liver, pituitary gland, adrenal gland, white pulp of spleen, red pulp of spleen, Harderian gland, kidney cortex, and thyroid. Concentrations in the central nervous system tissues in male albino and pigmented rats were < 1.000 mcg equiv/g for the entire study, except for the brain (meninges) in pigmented rats (C_{max} of 71.284 mcg equiv/g at 48 h). Distribution in pigmented vs. albino rats was essentially similar but minor differences were observed. Tissue concentrations in the pigmented uveal tract of the eye of the albino rats (C_{max} of 86.632 mcg equiv/g at 96 hours) was notably higher than that observed in the same tissue of albino rats (C_{max} of 2.099 mcg equiv/g at 24 hours), suggesting a possible association of ponatinib-derived radioactivity with melanin.

5.2 Toxicokinetics

Refer to TK results from the repeat-dose studies reviewed below.

6 General Toxicology

6.1 Single-Dose Toxicity

The single-dose toxicology study reports were not reviewed individually.

6.2 Repeat-Dose Toxicity

Study title: 28-Day Oral Toxicity Study of AP24534 with Toxicokinetics in Sprague-Dawley Rats with a 28-Day Recovery Period

Study no.:	QAA00122
Study report location:	NDA Section 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 12, 2007
GLP compliance:	FDA, OECD, MHLW
QA statement:	Statement included and signed
Drug, lot #, and % purity:	AP24534 (Ponatinib), PAK-009-173, 99.80% by HPLC (91.3% as free base). Correction factor of 0.913 used for dose formulation preparations.

Key Study Findings

- Mortality occurred in 11/46 animals receiving the high dose (6 mg/kg/day) in the main and TK groups on Days 5-10; 6/46 animals receiving 3 mg/kg/day on days 12-28, and 1/46 animals receiving 1.5 mg/kg/day on day 28. Because of early high mortality in the 6 mg/kg/day group, dosing of this group was stopped and necropsies performed prior to study termination.
- Dose-related clinical signs included hunched posture, rough hair coat, lethargy, cold to touch, decreased activity, eye squint, scant feces, dry, flaky skin, labored breathing and weight loss.
- Target organs affected were bone marrow, epiphyseal plate of the femur, GI tract, adrenal and thymus.
- Histopathological lesions included variable changes in the stomach (hyperkeratosis of the epithelium, submucosal edema, necrosis of the glandular and non-glandular mucosa), necrosis of the thymus and hyperplasia of bone marrow and the epiphyseal plate of the femur.

Methods

Doses:	0, 1.5, 3, and 6.0 mg/kg/day (free base) 0, 9, 18, and 36 mg/m^2/day
Frequency of dosing:	Once daily
Route of administration:	Orogastric gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	25 mM Citrate Buffer, pH 2.75
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	15/sex/group
Age:	7 to 11 weeks
Weight:	180.3 to 199.8 g
Unique study design:	4 rats/sex in control and 8 rats/sex in treatment groups for toxicokinetic analysis; 5 rats/sex/group assigned for a 28-days recovery period
Deviation from study protocol:	Initial weight of rats was outside the protocol range. This protocol deviation was reported.

Results

Mortality

Early mortality occurred in all AP24534 dose groups and was most severe at the highest dose. At the time of necropsy, many early decedents had dilated gastrointestinal tracts without microscopic correlates. Several 6 mg/kg early decedents had moderate cortical necrosis of the adrenal gland and/or mild to marked necrosis of the thymus. A consistent microscopic change was not established as the common cause of death. Dosing of 6 mg/kg was terminated early because of the severe toxicity/early mortalities observed. Early necropsy was performed on remaining main study group animals on Day 9 (females) or Day 10 (males), and dosing was terminated for remaining recovery animals.

Table 10 Early Mortality in the 28-Day Rat Study

(*Excerpted from Dr. Lee Ham's review*)

Group	# Animal test group	Dose, mg/kg (mg/m2)	Mortality
1	15/sex/group for main study (5/sex/group for recovery) 8/sex/group for TK study	0	0
2		1.5 (9)	Main: 1 male/30 died on D28; 2 died due to dosing D14 and blood collection procedure on D29. TK: 1/30 died after blood collection D14. Overall mortality was 1/46 (2%) of all animals dosed.
3		3 (18)	Main: 3 (1M, 2F)/30 died/sacrificed on D9-13; 1F died on D8 after blood collection. TK: 3(2M, 1F)/16 died on D12, 24, and 28. Overall mortality was 6/46 (13%).
4		6 (36)	Main: 7 (2M 5F)/30 died/sacrificed on D5 and 9 Recovery: 1 recovery animal was sacrificed on D13. TK: 2(1F 1M)/16 sacrificed on D7 Overall mortality was 10/46 deaths (22%).

Clinical signs

Prior to early deaths, clinical signs of these rats included rough hair coats, inappetence, thinness, lethargy, hunched posture, cold to touch, yellow discolored urine, dry and red material on the eye, nose, face, and forepaws, scant feces, urine stain, eye squint and labored breathing. Dry, flaky skin of the forepaws was observed in many of female early decedents, and also present in one 6 mg/kg male during the recovery period. In surviving rats, rough hair coat, dry, flaky skin of the fore- and hind limbs were noted at 3 and 6 mg/kg/day.

Body Weight

Daily administration of 3 or 6 mg/kg/day AP24534 resulted in reduced body weight gain during the dosing phase in comparison to the vehicle control. Significantly lower mean body weights, relative to controls, started on Day 14 in the 3 mg/kg group (Table 11). In the 6 mg/kg/day, lower mean body weights occurred on Day 7 (note that dosing was terminated for Group 4 animals beginning on Day 9/10).

Table 11 Body Weight Changes in the 28-Day Rat Study

(*Excerpted from Dr. Lee Ham's review)*

Group	Dose mg/kg	Sex	Dosing Phase (n=15) Day					Recovery Phase (n=5) Day			
			-1	7	14	21	28	35	42	49	56
1	0	M									
		F									
2	1.5	M									
		F									
3	3	M			-9%	-13%	-14%	-17%	-15%	-14%	-13%
		F			-6%	-8%	-8%				
4	6	M		-9%	-11%						
		F		-6%							

Food Consumption

Food consumption was decreased in the 6 mg/kg/day males (-15% at week 2) and females between days -1 and 7 (-21% week 1). Decreased food consumption was observed in the 3 mg/kg/day males between week 2 (-17%) and week 4 (-12%) and in females at week 2 (-14%) and corresponded with decreased mean body weight.

Ophthalmic Examinations

Not remarkable

Hematology

Sample collection for clinical pathology is shown below (excerpted from the study report):

Clinical Pathology Sample Collection Schedule

Group Number	Time Point	Hematology	Coagulation	Serum Chemistry	Thyroid Hormones	Urinalysis (recovery animals only)
1, 2, 3, 4	Week -1[a]		X		X	
1, 2, 3	Day 8 (predose)	X	X			
4	Day 10 (males) and Day 9 (females) (before necropsy)	X X X			X	
4	Day 10 (males) and Day 9 (females) (recovery animals)	X	X		X	X
1, 2, 3, 4	Day 15 (predose)[b]	X	X			
1, 2, 3, 4	Day 29 (recovery animals)	X	X		X	X
1, 2, 3, 4	Day 29 (before necropsy only)	X X X			X	
1, 2, 3, 4	Day 57	X X X			X	X

[a] Week -1 (Day -6) clinical pathology samples were obtained from five alternate males, five alternate females, and from all main study animals assigned to the study.

[b] Beginning on Day 15, clinical pathology samples were collected from Group 4 female Animal No. 174. Because dosing was suspended on Day 8 or 9, the Day 15 sample for Group 4 was not a predose sample.

Results from the 6 mg/kg main study group animals on Day 9/10 were compared with either Day 8 controls or to group baseline values. Statistically significant increases occurred in mean absolute values of neutrophils (4.4-8.5-fold), monocytes (5.6-6.6-fold), and eosinophils (2.7-fold) and significant decreases in mean lymphocytes (0.53-0.67-fold) in this group. Statistically significant changes in these parameters were also seen in the 1.5 and 3 mg/kg treated animals on Days 8, 15 and 29 but were of a lesser magnitude.

On Day 29, remaining 6 mg/kg males had reduced mean white blood cells (WBCs) (0.63x control) and reduced mean absolute lymphocytes (0.53x-0.71x control). Mean absolute neutrophils and monocytes were significantly elevated in the 3 mg/kg females (3.2-fold and 2.3-fold, respectively). Mean absolute lymphocyte counts were lower (0.61x-0.71x) and mean absolute eosinophils were higher (1.6-2.2-fold) than controls in the 1.5 and 3.0 mg/kg females.

By the end of the recovery period, mean hematological parameters were similar among controls and AP24534-treated males. Significant elevations in mean WBC (1.46x control), absolute neutrophils (2.0x control), and absolute lymphocytes (1.38x) were present in the 6-mg/kg females on Day 57.

Clinical Chemistry and Coagulation

Not remarkable

Thyroid Hormones

Histopathological findings in the thyroid were observed in the single dose toxicity study in monkeys (QAA00124) and may be the reason for monitoring of hormone levels: follicular atrophy of the thyroid gland in one of two females dosed with 15 mg/kg of ponatinib and in one of two males and two of two females dosed with 45 mg/kg of ponatinib. This change was graded as minimal to mild, with the higher intensity noted in females in the 45 mg/kg dose group.

Decreases in T_3 and T_4 occurred on Day 29 (Table 12). No histological correlates in the thyroid gland observed and these alterations reversed by Day 57.

Table 12 Changes in Thyroid Hormone Levels (28-Day Rat Study)

(*Excerpted from Dr. Lee Ham's review*)

Group	Dose	Day	Gender	TSH (ng/Ml)	T3 (ng/Ml)	T4 (ng/Ml)
1	0	29	M	15.98	67.6	5.21
			F	10.82	62.7	2.89
		57	M	11.96	54.4	4.32
			F	6.44	74.0	2.80
2	1.5	29	M	17.02	**55.0**	4.34
			F	12.41	67.3	3.13
		57	M	8.60	63.8	4.66
			F	7.88	66.0	2.35
3	3	29	M	16.06	**53.2**	4.29
			F	10.35	49.9	2.63
		57	M	9.88	63.6	4.48
			F	7.54	74.0	2.70
4	6	10	M	15.02	34.5	**2.06**
		29	M	9.90	62.4	5.16
			F	7.75	68.0	3.15
		57	M	12.94	71.4	4.90
			F	6.78	63.5	2.63

Bold= Statistically significant difference in comparison to control group 1 ($p \leq 0.05$

Urinalysis

Urine samples were collected at necropsy. Two of five males on day 10 and 2/5 females on day 9 at 6 mg/kg/day had urinary blood. All 6 mg/kg males on Day 10 and 2/5 females on Day 9 had positive leukocyte esterase activity in their urine which appeared AP24534-related. Minimal chronic progressive nephropathy was noted in one 6 mg/kg/day female (#111) on day 9; there were no microscopic correlates in the other rats.

Gross Pathology

Gross pathology findings from all study animals were compiled in Table 13. Many of the early decedents had dilated gastrointestinal tracts. Several of the high dose early decedents had moderate cortical necrosis of the adrenal gland and/or mild to marked necrosis of the thymus. Additional findings in early decedents were for one 1.5 mg/kg

male found dead on Day 28, that had dark discolored lung lobes (moderate hemorrhage and inflammation) and dark, diffuse, discolored skin.

Table 13 Macroscopic Findings in the 28-Day Rat Study

Dose, mg/kg/day	0		1.5		3		6	
Sex of animals	M	F	M	F	M	F	M	F
Number of animals	15	15	15	15	15	15	15	15
Adrenal gland discoloration / congestion						1		7
All tissues dark								1
Heart enlarged					1			
Intestine, cecum dilation / small / inflammation					1	3		3
Intestine, colon inflammation						1		
Intestine, duodenum discoloration					1			
Intestine, ileum dilation			1		1	3		4
Intestine, jejunum dilation / discoloration					1	3		4
Liver, dark /congestion						2		
Lung, dark RL/R/L	2		2					
Lymph node, dark mesenteric / mandibular / enlarged	3		3		1	1	3	1
Kidney, pelvis dilation / inflammation	1					1		
Kidney, discoloration / pale				1	2		1	
Ovary, discoloration								1
Skin, discoloration			1					
Skin, hyperkeratosis						1		2
Skin, dry flaky						1		2
Spleen, tan parenchyma / small					1	1	1	4
Stomach, dilation / discoloration					1			1
Thoracic cavity, fluid								1
Thymus discoloration / dark / small					1	2		6
Urinary bladder, inflammation						1		
Uterus, dilation						1		

Empty cell = no findings

During the scheduled necropsies, intestinal dilation and/or color changes (dark, discolored, or pale) were noted in various organs and was usually associated with hemorrhage or congestion. However, these observations were sporadic and incidental.

Organ Weights

On Day 29, low and mid dose group males had reduced mean liver, spleen, thymus and prostate weights compared to controls. Females had lower thymus and higher ovary weights. Statistically significant changes are shown in bold in the table below.

Table 14 Organ Weight Changes in the 28-Day Rat Study

(*Excerpted from Dr. Lee Ham's review*)

	Absolute Organ Weights (g) Following 28-Day Oral Gavage Toxicity Study with AP24534 in Rats with 4-Week Recovery Period							
Dose, mg/kg/day	0		1.5		3		6	
Sex of Animals	M	F	M	F	M	F	M	F
Number of Animals	10	10	10	10	10	10	10	10
Organ Weights (Day 29)								
Liver	10.59	6.47	**8.81**	6.36	**8.84**	6.29	6.95	5.57
Lung	1.229	1.104	1.245	1.042	**1.197**	1.066	1.024	0.933
Pituitary gland	0.011	0.017	0.011	0.015	**0.010**	0.016	0.008	0.010
Prostate	1.147		1.043		**0.899**		0.625	
Spleen	0.840	0.496	**0.660**	0.460	0.594	0.409	0.421	0.380
Thymus	0.470	0.403	0.433	0.314	0.381	0.383	**0.220**	0.126
Seminal vesicle	1.098		0.892		0.949		**0.587**	
Testes	3.158		3.148		2.954		2.794	
Ovary		0.087		0.100		**0.113**		**0.063**

Bold=Statistically significant difference in comparison to control group ($p \leq 0.05$)

Histopathology

Microscopic findings were graded by the pathologist on a scale 1 to 4, minimal<mild<moderate<marked, according to the intensity and extent of change.

Adequate Battery: ***Yes***

Peer Review: ***No***

The high dose group early decedents had findings in bone marrow, epiphyseal plate of the femur, GI and thymus. Most of the remaining high dose animals from the main study exhibited minimal to moderate myeloid hyperplasia of the bone marrow, minimal to mild cartilaginous hyperplasia of the epiphyseal plate, and minimal to mild hyperkeratosis, edema and/or sporadic necrosis of stomach tissues. Minimal to marked necrosis of the thymus was also observed in most animals.

The only clearly dose-related findings in the recovery groups were: minimal to moderate hemorrhage of the mandibular lymph node of males, and minimal to mild inflammation of the prostate gland.

Table 15 Histopathological Findings in the 28-Day Rat Study

Dose, mg/kg/day	0		1.5		3		6*	
Sex of animals	M	F	M	F	M	F	M	F
Number of animals	10	10	8	10	9	7	8	6
Adrenal gland, congestion cortical						1b		1c
Adrenal gland, hemorrhage		1b						
Bone, femur hyperplasia			1b			3a	1a, 6b, 1c	1b, 1c
Bone, femur necrosis			1b					
Esophagus, inflammation	1b	1a, 1b	1b			1a	1a,2b	
Esophagus, necrosis							1a	1b
Heart, inflammation		1a	1a	1a			1b	
Intestine, colon inflammation			2b			2a		2b
Intestine, duodenum inflammation					1b		1b	1b
Intestine, ileum inflammation								
Intestine, ileum intussusception				1b				
Intestine, jejunum inflammation								1b
Kidney, congestion			1b	1b				
Kidney, inflammation pelvis			1b					
Kidney, chronic progressive nephropathy		1b	2a, 1b	3a	2a,1b			1a
Kidney, cyst					1			
Liver, congestion			1a	1a				
Liver inflammation mixed	6a	5a		1a	1b			
Liver, , proliferative fibrosis					1b			
Lung, inflammation mixed	1a		1c		2a		1a	1b
Lung, hemorrhage			1c					
Lung, infiltration histiocytic				1a		1a		1a
Lymph node, mesenteric inflammation			1a					
Lymph node, mandibular, infiltration, neutrophilic							2b	
Lymph node, mandibular hemorrhage			1a,1b, 1c					
Pancreas, inflammation			1a					1a
Pancreas, focal atrophy								
Prostate gland, inflammation	1b		2a, 1b		1b			
Skin, hindfoot hyperkeratosis						1b		
Skin, tail hyperkeratosis						1c		
Spleen, inflammation	1a		1b					
Spleen, fibrosis							1c	
Spleen, hyperplasia, myeloid								2b,1c
Thymus, atrophy				1b				
Thymus, hemorrhage	1b	1a	1b			1c		
Thymus necrosis			1c, 1d				2b,3c	1a,3c,1d
Urinary bladder, inflammation			1b					
Uterus, dilation		1b		1b		2b		

Empty cell = no findings; a, minimal; b, mild; c, moderate; d, marked
*Necropsy on Day 9 and 10

Toxicokinetics

Samples for TK analysis were collected before dosing on Days 15 and 28, at 30 min and 1, 2, 4, 8, 16 and 24 hours after dosing on Days 1, 8, 15 and 28, and at 48 hours after dosing on Day 28. After oral administration, mean AP24534 plasma concentration profiles over time were generally similar in male and female monkeys on Days 1, 15 and 28. The figure below shows plasma concentration over time on Day 28 for the low and mid dose groups.

Systemic exposure to AP24534, as measured by C_{max} and AUC_{last}, generally increased with increasing dose from 1.5 to 3 mg/kg/day (

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Table 16). The increases in C_{max} were approximately dose proportional except for mean C_{max} in 3 mg/kg/day females on Day 28. The increases in AUC_{last} were dose proportional on Day 1 and lower than dose proportional for both males and females on Days 15 and 28. There were no signs of accumulation after repeated dosing for 28 days.

Figure 6 Plasma Concentration vs. Time Profiles of AP24534 in Male and Female Rats after 28 Days Dosing

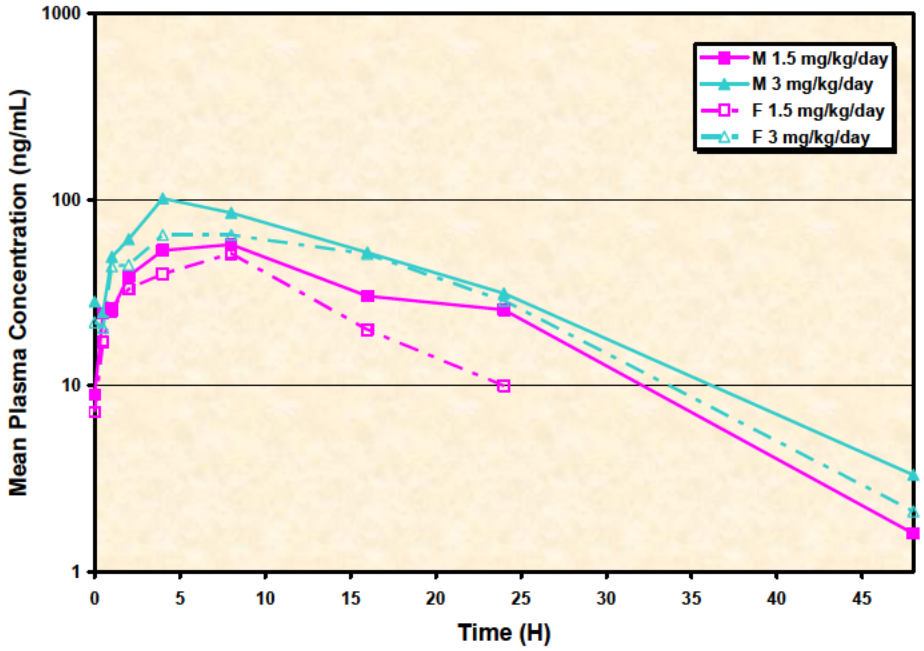


Table 16 Toxicokinetic Parameters for AP24534 in the 28 Day Rat Study

(Excerpted from Applicant's Submission)

Group	Dosage (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{½}$ (h)
Day 1								
2	1.5	M	30.2	8	24	430	NE	NE
		F	38.4	8	24	541	NE	NE
3	3	M	61.1	8	24	855	NE	NE
		F	83.4	8	24	1240	NE	NE
4	6	M	125	8	24	2020	NE	NE
		F	155	8	24	2410	NE	NE
Day 15								
2	1.5	M	40.9	4	24	596	681	7.2
		F	57.5	8	24	851	NE	NE
3	3	M	71.5	8	24	1160	NE	NE
		F	90.8	4	24	1230	NE	NE
Day 8								
4	6	M	170	8	24	2970	NE	NE
		F	162	8	24	2510	NE	NE
Day 28								
2	1.5	M	57.2	8	48	1260 (938)[a]	1280	7.1
		F	51.1	8	24	704	NE	NE
3	3	M	101	4	48	1920 (1500)[a]	1950	7.9
		F	64.5	8	48	1580 (1220)[a]	1610	6.8

C_{max} = maximum concentration; t_{max} = time of maximum concentration; t_{last} = time of last observable concentration; AUC_{last} = area under the plasma mean concentration-time curve through t_{last}; AUC = area under the plasma mean concentration-time curve; $t_{½}$ = terminal elimination half-life; M = male; NE = not estimated due to insufficient characterization of the terminal phase of the mean concentration-time curve; F = female.

[a] For comparison purposes, the AUC_{0-24} (value in parentheses) was also calculated.

The metabolite AP24567 C_{max} and AUC_{last} appeared to be independent of the duration of dosing, as values on Day 15/8 at 3 and 6 mg/kg/day for males and at 6 mg/kg/day for females were roughly comparable to those observed on Day 1, and values on Day 28 for males at 3 mg/kg/day were similar to the those on Day 1. C_{max} and AUC_{last} for

AP24534 were substantially higher than corresponding values for AP24567. T_{max} occurred most often in the range from 4 to 8 hours at this dose range.

Table 17 Toxicokinetic Parameters for the AP24567 Metabolite in the 28 Day Rat Study

(Excerpted from Applicant's Submission)

Group	Dosage (mg/kg/day)[a]	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{½}$ (h)
Day 1								
2	1.5	M	1.47	8	8	2.94	NE	NE
		F	0[b]	NA	NA	0[b]	NE	NE
3	3	M	5.14	8	24	71.2	NE	NE
		F	2.28	8	16	21.7	NE	NE
4	6	M	14.0	16	24	231	NE	NE
		F	6.89	8	24	105	NE	NE
Day 15								
2	1.5	M	2.33	8	16	24.9	NE	NE
		F	1.25	8	8	2.50	NE	NE
3	3	M	5.69	8	24	89.4	NE	NE
		F	7.22	4	16	56.9	NE	NE
Day 8								
4	6	M	10.5	16	24	191	NE	NE
		F	5.49	8	16	67.5	NE	NE
Day 28								
2	1.5	M	2.80	4	16	34.1	NE	NE
		F	0[b]	NA	NA	0[b]	NE	NE
3	3	M	7.13	8	24	101	NE	NE
		F	1.18	4	4	1.18	NE	NE

C_{max} = maximum concentration; t_{max} = time of maximum concentration; t_{last} = time of last observable concentration; AUC_{last} = area under the plasma mean concentration-time curve through t_{last}; AUC = area under the plasma mean concentration-time curve; $t_{½}$ = terminal elimination half-life; M = male; NE = not estimated due to insufficient characterization of the terminal phase of the mean concentration-time curve; F = female; NA = not applicable.

[a] Dosage of AP24534.
[b] All mean concentrations were below the limit of quantification (BQL).

Study title: AP24534: A 6-Month Oral Toxicity Study and Toxicokinetics Study in Sprague-Dawley Rats with a 2-Month Recovery Period

Study no.:	QAA00193
Study report location:	NDA Section 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 26, 2008
GLP compliance:	FDA, OECD, MHLW
QA statement:	Statement included and signed
Drug, lot #, and % purity:	AP24534 (ponatinib) ABL411071, 99.6% by HPLC (88% as free base). Correction factor of 0.88 used for dose formulation preparations.

Key Study Findings

- AP24534-related mortalities (n=20/68) occurred in the high dose group (2 mg/kg/day) and 6 in the mid dose group (0.75 mg/kg/day). Specific causes of the majority of these deaths were not determined, but lymphoid depletion in the thymus, spleen and lymph nodes and hypocellularity of the bone marrow were observed in early decedents. Dose-related clinical signs in early decedents included labored breathing and/or in irregular pattern, cold to touch, lethargy, hunched posture, dehydration, rough hair coat, thin, and scant or soft feces.
- Dose-dependent decreases in mean body weight, body weight gain and food consumption occurred in the 0.75 and 2 mg/kg/day rats.
- Increases in neutrophils, monocytes and eosinophils were seen at 0.75 and 2 mg/kg/day that were not present at the end of recovery.
- Target organs affected in animals at terminal necropsy included, femoral bone, kidneys and thymus with corresponding hematological, coagulation and/or serum chemistry findings.

Methods

Doses:	0, 0.25, 0.75, and 2 mg/kg/day 0, 1.5, 4.5, and 12 mg/m^2/day
Frequency of dosing:	Once daily for 182 days
Route of administration:	Orogastric gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	25 mM Citrate Buffer, pH 2.75
Species/Strain:	Sprague-Dawley rats
Number/Sex/Group:	25/sex/group, 10 rats/sex for 2-month recovery
Age:	7 to 10 weeks
Weight:	200 to 350 g
Unique study design:	3 rats/sex in control and 9 rats/sex in groups 2-4 for toxicokinetic analysis

Results

Mortality

A total of 31 unscheduled deaths occurred during the study (Table 18). The majority of these deaths were probably- or possibly-related to AP24534 administration: 20 occurred in the 2 mg/kg group, 6 in the 0.75 mg/kg group and 1 in the 0.25 mg/kg group; the 4 remaining deaths were the result of blood collection procedures or other causes unrelated to AP24534 administration.

Clinical signs in early decedents at 2 mg/kg/day included labored breathing and/or in irregular pattern, cold to touch, lethargy, hunched posture, dehydration, rough hair coat, thin, and scant or soft feces. AP24534-related macroscopic findings included small thymus and/or spleen that corresponded with moderate to marked lymphoid depletion. Additional histopathological findings included reduced chondrocytes in the physis with and without reduced trabecular bone of the femur, lymphoid depletion in the thymus, spleen, and mesenteric and mandibular lymph nodes.

Clinical Signs

Clinical signs observed in surviving animals were described as sporadic and not exhibiting a dose-dependent pattern.

Body Weight

Statistically significant differences in weight gain occurred in males and females receiving 0.75 and 2 mg/kg that persisted through the recovery period (see figures and table below). Male rats receiving AP24534 at 0.25 mg/kg/day had a significant reduction in mean body weight during recovery days 189 to 217 with a 12.2% lower mean body weight at the end of the recovery period. The lower mean body weight (≥10%) resulted from some males having a lower body weight compared to the rest of males in this group. The calculated mean excluding those lower weight males (612.2 g; n=7) was comparable to control mean (659.6 g, n=9) on Day 189.

Ap24534-related decreases in male and female body weights in all dose groups were observed during the dosing phase and persisted throughout the recovery period for both sexes at 2 mg/kg and for males at 0.75 mg/kg.

Food Consumption

See Table 19 for mean food consumption values at the end of the dosing and recovery periods. The AP24534-related effects on mean food consumption in the 0.25 mg/kg group did not reach statistical significance at any interval. Decreases in mean food consumption were observed in the 0.75 mg/kg/day males during several intervals starting on Day 77, in the 2 mg/kg/day males starting on Day 14, and sporadically in the 2 mg/kg/day females. The percent cumulative mean food consumption was ≥10% lower than control in the 2 mg/kg/day males at both the end of the dosing period Day 182 and recovery period Day 238, and in 0.75 mg/kg/day males at the end of the dosing period

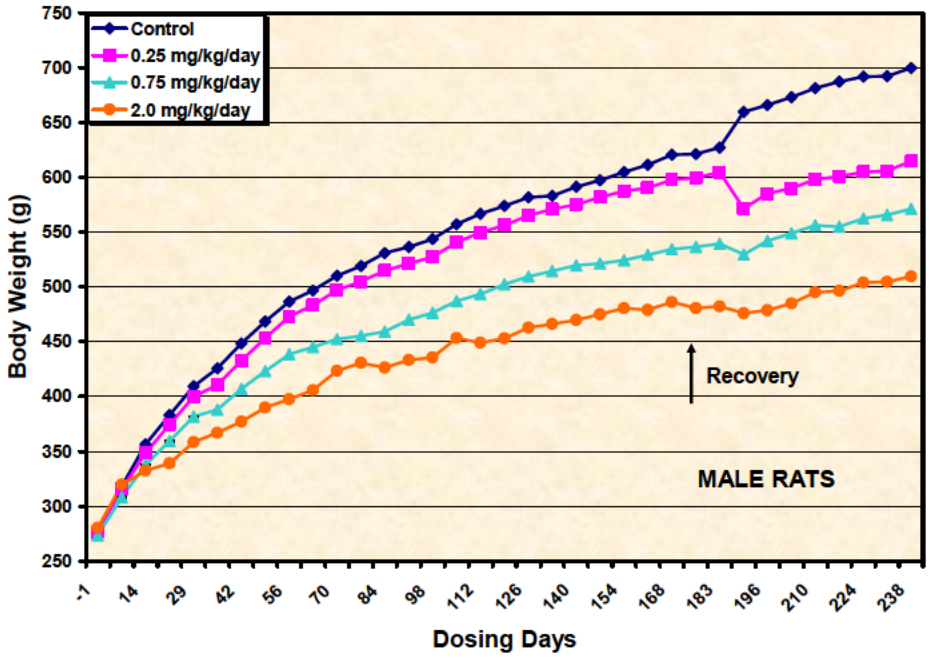
Table 18 Summary of Early Mortality in the 6-Month Rat Study

(*Excerpted from Applicant's submission*)

Group No. (Dose)	Animal No.	Sex	Disposition	Potential Cause of Moribund Condition/Death	Day
Main Study Animals					
1 (Vehicle)	110	Female	Moribund euthanasia	Acute trauma, neck	9
1 (Vehicle)	115	Female	Observed death	Lymphoma seen in thymus, heart, lungs, and liver	32
2 (0.25mg/kg/day)	28	Male	Moribund euthanasia	ND	50
3 (0.75mg/kg/day)	60	Male	Moribund euthanasia	Bacterial infection in urinary bladder and kidneys	106
3 (0.75mg/kg/day)	61	Male	Observed death	NA	29
3 (0.75mg/kg/day)	62	Male	Moribund euthanasia	Urolithiasis	114
4 (2.0mg/kg/day)	79	Male	Moribund euthanasia	ND	25
4 (2.0mg/kg/day)	80	Male	Found dead	NA	21
4 (2.0mg/kg/day)	81	Male	Found dead	NA	128
4 (2.0mg/kg/day)	84	Male	Moribund euthanasia	ND	54
4 (2.0mg/kg/day)	90	Male	Moribund euthanasia	ND	43
4 (2.0mg/kg/day)	176	Female	Observed death	NA	29
4 (2.0mg/kg/day)	183	Female	Found dead	NA	29
4 (2.0mg/kg/day)	184	Female	Observed death	NA	29
4 (2.0mg/kg/day)	185	Female	Found dead	NA	158
Recovery Animals					
1 (Vehicle)	263	Male	Found dead	NA	45
2 (0.25mg/kg/day)	141	Female	Found dead	Blood collection procedures	183
3 (0.75mg/kg/day)	75	Male	Moribund euthanasia	Inflammatory lesion of preputial gland – uncertain relation to AP24534	216
3 (0.75mg/kg/day)	171	Female	Moribund euthanasia	Acute trauma, eye	212
4 (2.0mg/kg/day)	92	Male	Moribund euthanasia	ND	35
4 (2.0mg/kg/day)	93	Male	Moribund euthanasia	Inflammatory skin lesion – uncertain relation to AP24534	185
4 (2.0mg/kg/day)	95	Male	Moribund euthanasia	ND	110
4 (2.0mg/kg/day)	98	Male	Moribund euthanasia	ND	28
4 (2.0mg/kg/day)	100	Male	Moribund euthanasia	ND	115
4 (2.0mg/kg/day)	194	Female	Found dead	NA	23
Satellite Animals					
3 (0.75mg/kg/day)	269	Male	Moribund euthanasia	-	36
4 (2.0mg/kg/day)	222	Male	Moribund euthanasia	-	176
4 (2.0mg/kg/day)	223	Male	Found dead	-	22
4 (2.0mg/kg/day)	224	Male	Found dead	-	35
4 (2.0mg/kg/day)	256	Female	Moribund euthanasia	-	71
4 (2.0mg/kg/day)	260	Female	Found dead	-	90

ND = not determined grossly and/or microscopically; NA = not readily apparent grossly and/or microscopically; - = no findings related to procedural error.

Figure 7 Mean Body Weight of Male and Female Rats Dosed with AP24534 during 182 Days followed by 56 Days of Recovery



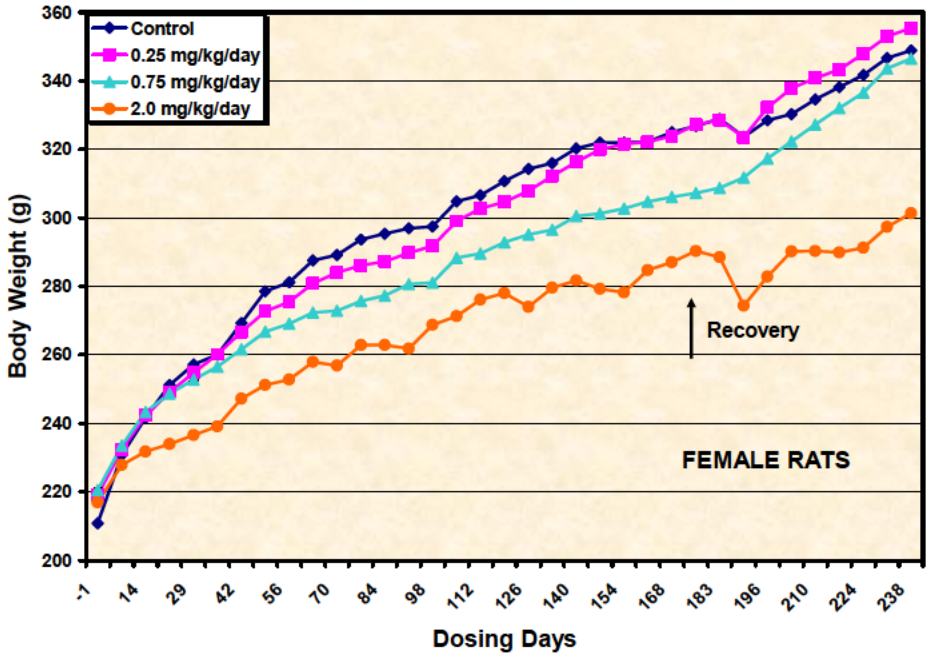


Table 19 Body Weight and Food Consumption Summary for Rats Dosed with AP24534 during 182 Days followed by 56 Days of Recovery*

	AP24534 Dose mg/kg/day					
	0.25		0.75		2	
	M	F	M	F	M	F
% Mean body weight vs. control, Day 30	-2.3	-0.9	-6.7	-2.7	**-12.4**	**-8.0**
% Mean body weight vs. control, Day 182	-3.6	-0.1	**-14.0**	-6.1	**-23.1**	**-12.3**
% Mean body weight vs. control, Day 238	**-12.2**	+1.9	**-18.4**	-0.7	**-27.2**	**-13.6**
% Mean body weight gain vs. control, Day 182	-6.2	-7.3	**-23.9**	**-25.3**	**-42.2**	**-39.4**
% Mean body weight gain vs. control, Day 238	+7.0	+26.1	+3.5	+37.2	**-16.4**	+6.7
% Cumulative mean food consumption vs. control, Day 182	-1.9	+2.7	-7.1	+2.0	**-12.4**	-1.3
% Cumulative mean food consumption vs. control, Day 238	-9.0	+6.2	**-10.0**	+4.8	**-12.0**	-0.2

*Values in bold were significantly different from control values ($p \leq 0.05$).

(see table above). The percent cumulative mean food consumption was reduced (9%) compared to control in the 0.25 mg/kg/day males. The reductions in food consumption corresponded with reductions in body weight observed in males only.

Ophthalmic Evaluations

Ophthalmic evaluations were conducted before treatment, and during Weeks 4, 13 and 26 for the main study and recovery animals and during Week 34 for recovery animals. No AP24534-related ophthalmic abnormalities were observed.

Hematology and Coagulation

Hematology and serum chemistry samples were collected Week -1 and on Days 29, 92 and 183 for main study and recovery rats and on Day 239 for recovery rats. Samples for coagulation were collected on Day 183 for the main study group and on Day 239 for recovery groups.

AP24534 administration resulted in dose-dependent increases in mean absolute neutrophil, eosinophils and monocyte counts (Table 20). By Day 183, mean absolute neutrophils and eosinophils were 1.54- and 1.52-fold above control values, respectively, for males at 0.75 mg/kg/day and 3.15–2.18-fold and 2.69–1.80-fold above control values in the males and females at 2 mg/kg/day, respectively. Increases in mean absolute monocytes were observed beginning on Day 92 in the females at 2 mg/kg/day, and were 1.95–1.79-fold above control values in the males and females on Day 183. Statistically significant increases in platelets were observed in males and females of the

high dose group on Day 183. Changes in these parameters were reversible by the end of recovery Day 239 except for increased absolute monocytes in males that were ~1.3-fold control values in the mid and high dose groups.

Table 20 Fold Changes in Hematology Values in the 6-Month Rat Toxicology Study

Dose (mg/kg/day)	0.75		2	
Number of Animals	M=22	F=21	M=16	F=20
RBC ($x10^6$ µL)	1.01	1.00	0.95	1.08*
Hb (g/dL)	1	1	0.93	1.07*
NEU ($x10^3$ µL)	1.54*	1.42	3.15*	2.18*
MON ($x10^3$ µL)	1.24	1.18	1.95*	1.79*
EOS ($x10^3$ µL)	1.52*	1.45	2.69*	1.80*
Platelet ($x10^3$ µL)	1.01	1.05	1.20*	1.14*

*Parameter values were significantly different from control values, p≤0.05.

AP24534-related dose-dependent changes in coagulation parameters included increases in fibrinogen values in males and females on Day 183 at 2 mg/kg/day. Increases in fibrinogen corresponded with increases in neutrophils and monocytes and are suggestive of inflammation processes and correlated microscopically with abscesses or granulomatous inflammation observed in the preputial or clitoral glands in the high dose groups. Fibrinogen values were still elevated in males on Day 239.

Clinical Chemistry

AP24534-related serum chemistry findings were observed at 2 mg/kg/day and included increases in blood urea nitrogen, creatinine and cholesterol values and decreases in alkaline phosphatase, total protein, and albumin and globulin values, Table 21. AP24534-related dose- and time-dependent increases in blood urea nitrogen and creatinine in males at 2 mg/kg/day were not reversible during the recovery period.

Decreases in total protein, albumin and globulin were seen in males and/or females, and these findings could be correlated with the chronic progressive nephropathy observed in 2 mg/kg/day males. Additionally, glucose values significantly decreased in males at all dose levels by the end of recovery. A significant increase in mean cholesterol observed in 2 mg/kg/day males was not reversible and increased between Days 183 and 239.

Thyroid Hormones

Samples for thyroid hormone analysis were collected on Day 183 for main study groups and on Day 239 for recovery groups. No clear AP24534 dose-dependent effects were observed at these two time points.

Table 21 Fold Changes in Serum Chemistry Values in the 6-Month Rat Toxicology Study (2 mg/kg Group)

Study Phase	End of Dosing Day 183		Recovery Day 239	
Control No. Animals	M=24	F=23	M=9	F=10
Number of Animals	M=16	F=20	M-5	F=9
ALP (U/L)	0.65*	0.73	0.66	0.88
BUN (mg/dL)	1.50*	0.96	2.03*	1.0
CRE (mg/dL)	1.17*	0.91	1.56*	0.94
Total Protein (g/dL)	0.85*	0.84*	0.93*	0.95
ALB (g/dL)	0.82*	0.83*	0.85*	0.96
GLOB (g/dL)	0.88	0.85*	1.0	0.93
CHOL (mg/dL)	1.94*	0.95	2.19	1.0

*Parameter values were significantly different from control values, p≤0.05.

Urinalysis

Urine samples were collected from recovery groups pre-study and on Days 29, 92, 183 and 239. AP24534-related increases in urine protein levels at 2.0 mg/kg/day were observed on Days 29, 92, and 183. At the end of the dosing period, 6/6 males and 4/9 females at 2 mg/kg/day had urine protein levels ≥300 mg/dL. These increases correlated to a decrease in total serum protein levels and microscopically to findings of chronic progressive nephropathy and increases in relative mean kidney weights.

Gross Pathology

Gross finding seen in early decedents without microscopic correlates included: intestinal findings of accumulation or dilation; and accumulation of firm and discolored material in the lumen of the small and large intestines. AP24534-related findings at 0.75 and 2 mg/kg/day from early decedents and scheduled necropsies included non-reversible skin accumulations/crusts in males and females and accumulation of firm nodules in the inguinal skin at an increased incidence in males and females at 0.75 and 2 mg/kg/day, with females exhibiting a higher incidence than males. These findings corresponded microscopically with abscesses or granulomatous inflammation associated with the preputial or clitoral glands. No AP24534-related macroscopic findings were noted in the 0.25 mg/kg/day groups.

Table 22 Macroscopic Findings in the 6-Month Rat Study

Dose, mg/kg/day	0		0.25		0.75		2	
Sex of animals	M	F	M	F	M	F	M	F
Number of animals	15	13	14	15	12	15	10	11
Adrenal gland dark / enlarged							1	1
Epididymis, small	1				1			
Esophagus, dark		1						
Eye, white / dark						1		1
Heart, dark		1						
Heart, pale							1	
Intestine, cecum dark / dilation							5	3
Intestine, colon dark / dilation							4	1
Intestine, duodenum dilation							1	
Intestine, ileum dark / dilation							5	
Intestine, jejunum dark / dilation	1						5	
Kidney, pelvis dilation / enlarged		3		1		1	2	
Kidney, tan / depressed				1	1			
Liver, small								1
Lung, adhesion								1
Lymph node, dark mesenteric								1
Muscle, skeletal hematoma		1						
Pancreas, small							1	
Preputial gland, mass					1		1	
Prostate gland, pale							1	
Salivary gland, tan							1	
Seminal vesicle, white / small							2	
Skin, abdominal / inguinal crust	2	1			4	4	3	10
Spleen, cyst / small					1		4	1
Testis, small	1				2			
Thoracic cavity, fluid	1			1				
Thymus small / enlarged / adhesion		1			1		6	1
Urinary bladder, uroliths		1		1	1			
Urinary bladder, dark					2			
Uterus, fluid						4		4

Empty cell = no findings

Organ Weights

AP24534-related effects on organ weight at 2 mg/kg/day included decreased mean thymus weight in males and increased mean ovarian weights in females (see table below). The decreased mean thymus weight in males correlated with lymphoid depletion in the cortex and medulla of the thymus. Similar microscopic findings were observed in females at 2 mg/kg/day. At the end of recovery, 0.75 and 2 mg/kg/day males had a statistically significant increase in thymus weight relative to body weight and a non-statistically significant increase in absolute thymus weight.

The increased mean absolute and relative ovarian weight could be related to a slight increase in the number of corpora lutea and follicles but microscopic evaluation displayed no appreciable differences. This finding was of uncertain relation to AP24534 administration. Increased mean absolute and relative ovarian weight was also seen in females at 0.75 mg/kg/day (Table 23) but microscopic evaluation was not conducted.

Table 23 AP24534-Related Thymus and Ovaries Weight Changes at the end of Dosing Period Day 183

(*Excerpted from Applicant's submission*)

Group/Dose	N	Male					
		Absolute Weight (g) with % change[a]		Organ to Body Weight Percentage with % change[a]		Organ to Brain Weight Ratio with % change[a]	
Thymus							
1 (Vehicle)	15	0.1765		0.0305		0.0806	
2 (0.25mg/kg)	14	0.1835	4	0.0302	-1	0.0850	5
3 (0.75mg/kg)	12	0.1460	-17	0.0279	-9	0.0675	-16
4 (2.0mg/kg)	10	0.1230*	-30	0.0267	-12	0.0584**	-28

N: Number of animals.
* indicates a significant difference from group 1 controls ($p \leq 0.05$; ANOVA with Dunnett's t-Test)
** indicates a significant difference from group 1 controls ($p \leq 0.05$; Kruskal-Wallis with Dunn's procedure).
[a] = % change from control

Group/Dose	N	Female					
		Absolute Weight (g) with % change[a]		Organ to Body Weight Percentage with % change[a]		Organ to Brain Weight Ratio with % change[a]	
Ovaries							
1 (Vehicle)	13	0.0644		0.0206		0.0326	
2 (0.25mg/kg)	15	0.0645	0	0.0210	2	0.0327	0
3 (0.75mg/kg)	15	0.0831	29	0.0289*	40	0.0419	29
4 (2.0mg/kg)	11	0.1027**	59	0.0367*	78	0.0521**	60

N: Number of animals.
* indicates a significant difference from group 1 controls ($p \leq 0.05$; ANOVA with Dunnett's t-Test)
** indicates a significant difference from group 1 controls ($p \leq 0.05$; Kruskal-Wallis with Dunn's procedure).
[a] = % change from control

Histopathology

Microscopic findings for early decedents were reviewed in the mortality section. Microscopic findings were graded by the pathologist on a scale 1 to 4, minimal<mild<moderate<marked, according to the intensity and extent of change. Tissues from the control and high dose groups were evaluated microscopically. Based on pathologic findings considered to be test-article related, the kidneys, thymus, bone

(femur) and gross lesions/masses from the low and mid dose groups were evaluated microscopically.

Adequate Battery: ***Yes***
Peer Review: ***No***

Table 24 AP24534-Related Microscopic Findings (6 Month Rat Study)

	Males				Females			
Dose (mg/kg)	**0**	**0.25**	**0.75**	**2.0**	**0**	**0.25**	**0.75**	**2.0**
Number of Animals in Group	**15**	**14**	**12**	**10**	**13**	**15**	**15**	**11**
Terminal Groups								
Adrenal Gland -cortex, focal necrosis	0	--	--	0	0	--	--	1b 1c
Bone, femur -physis, reduced chondrocytes	2a	0	 5b 6c	 1b 9c	0	0	3a 6b 1c	 1b 10c
Bone -femur, reduced trabeular	0	0	3a 8b	 7b	0	0	 1b	0
Eye -lens, bilateral lenticular degeneration	0	--	--	0	0	--	--	1a
Eye -retinal dysplasia, focal	0	--	--	1b	0	--	--	0
Kidney -chronic progressive nephropathy	4a 2b	6a	9a 2b	2a 3b 5c	1a	1a 1c	1a	4a 3b 1c
Thymus -lymphoid depletion	1a 3b	0	 3b	 8b	1a	0	0	 5b
Recovery Groups								
Number of Animals in Group	**9**	**10**	**9**	**5**	**10**	**9**	**9**	**9**
Adrenal Gland -cortex, focal necrosis	0	--	--	0	1b	--	--	1b
Bone, femur -physis, reduced chondrocytes	 1b	1a	 9c	 5c	0	0	1a 4b 1c	 9c
Bone, femur -reduced trabecular bone	 1b	0	 6b 1c	 5b	0	0	1a 1b	 1b
Kidney Chronic Progressive Nephropathy	5a 3b	4a 3b	6a 2b	1a 4c	1a	1a	3a	3a 2b

--: not evaluated
a, minimal; b, mild; c, moderate; d, marked

AP24534-related findings in males and females that survived to terminal and recovery necropsy included nonreversible microscopic findings in the femoral bone and kidneys and reversible findings in the thymus, see table below. Additional sporadic findings that may be AP24534-related included focal necrosis in the adrenal gland and abnormal findings in the eye. Microscopic findings in the early decedents that were consistent with those seen at terminal necropsy included reduced chondrocytes in the physis with and without reduced trabecular bone of the femur, findings related to chronic progressive nephropathy in the kidneys, and lymphoid depletion in the thymus.

Additional findings that were not observed at terminal necropsy included lymphoid depletion of the spleen, mesenteric and mandibular lymph nodes and hypocellularity of the femoral bone marrow.

Findings in the thymus in male and female rats at 2 mg/kg/day included increased incidence of lymphoid depletion. Although lymphoid depletion was seen in the controls, the 2 mg/kg/day rats had decreased lymphocytes in the cortex and medulla, whereas the decrease for all control animals was seen in the cortex only. Also, the dose-dependency of lymphoid depletion of the thymus was further confirmed by the marked findings observed in early decedents and macroscopic correlation of small thymus.

Findings in the femur included reduced number of chondrocytes along the physis (growth plate), and in the proliferation and hypertrophy zones. Islands of residual cartilage were present, but also at reduced numbers. There was reduced trabecular bone in several males.

Kidney findings of chronic progressive nephropathy in male and female rats at 2 mg/kg/day included increased basophilia of tubules in the cortex, thickening of tubule basement membranes, variable infiltrates of mononuclear cells in the interstitium, occasional interstitial fibrosis, and variable accumulations of hyaline droplets in renal tubule epithelium. These microscopic findings correlated with significantly increased creatinine and BUN values in males at 2 mg/kg/day.

At the end of recovery, the findings in the femur and kidneys were still present at roughly equivalent incidence and severity. Kidney findings corresponded with statistically significant increases in renal serum chemistry values (BUN and creatinine) in the 2 mg/kg/day males.

Toxicokinetics

Toxicokinetic samples were collected on Days 1, 91 and 182 (predose, and 15 min and 1, 2, 4, 8, 24 and 48 hours postdose) and both AP24534 and the metabolite, AP24567, were measured.

Systemic exposure of AP24534 was greater than dose proportional between 0.25 and 0.75 mg/kg/day, and was nearly proportional between 0.75 and 2 mg/kg/day (Table 25). After 182 days of dosing, a greater than 2-fold increase in exposure compared to Day 1 was observed in the male rats, suggesting accumulation of the test article in this gender only, as a less than 2-fold increase was observed in the female rats. Accumulation ratios decreased in a dose-dependent manner in both genders. The slight gender difference (approximately 1.5- to 2-fold) that was observed in the exposure of AP24534 after single administration evidently decreased with repeated, multiple dosing. Maximum plasma concentrations were generally observed 4 or 8 hours after dosing, and the terminal elimination half-life ($T_{1/2}$) of AP24534 was estimated to be between 4.91 and 12.1 hours.

The plasma concentrations of the AP24567 metabolite were quantifiable only in Group 3 males and Group 4 males and females; the metabolite exposure in these groups was approximately 2% to 7% of the test article exposure. Due to the limited amount of metabolite data, the toxicokinetic parameters of AP24567 were not determined.

Table 25 Toxicokinetic Parameters for AP24534 (6 Month Rat Study)

(*Excerpted from Applicant's submission*)

Group No.	Dose	Sex	C_{max} (ng/mL)	T_{max} (h)	T_{last} (h)	AUC_{0-24} (ng*hr/mL)	AUC_{last} (ng*hr/mL)	AUC_{inf} (ng*hr/mL)	$T_{1/2}$ (h)
Day 1									
2	0.25	M	1.64	8.00	8.00	[a]	[a]	[a]	[b]
3	0.75	M	8.63	4.00	24.0	134	134	149	7.16
4	2.0	M	37.7	8.00	24.0	550	550	618	7.70
2	0.25	F	2.66	4.00	8.00	[b]	15.9	[b]	[b]
3	0.75	F	16.5	4.00	24.0	201	201	209	4.91
4	2.0	F	58.3	8.00	24.0	810	810	891	7.29
Day 28									
2	0.25	M	2.78	8.00	8.00	[b]	16.2	[b]	[b]
3	0.75	M	17.9	4.00	24.0	257	257	288	7.32
4	2.0	M	54.8	2.00	24.0	936	936	[c]	[c]
2	0.25	F	3.67	4.00	8.00	[b]	21.2	[b]	[b]
3	0.75	F	22.2	4.00	24.0	277	277	288	4.96
4	2.0	F	71.9	8.00	24.0	999	999	[c]	[c]
Day 91									
2	0.25	M	5.24	8.00	24.0	84.2	84.2	[c]	[c]
3	0.75	M	25.8	4.00	24.0	411	411	502	9.63
4	2.0	M	53.6	8.00	24.0	974	974	[c]	[c]
2	0.25	F	4.43	4.00	8.00	[b]	26.8	[b]	[b]
3	0.75	F	23.8	4.00	24.0	360	360	397	7.03
4	2.0	F	68.3	8.00	24.0	1081	1081	1341	10.4
Day 182									
2	0.25	M	5.65	8.00	24.0	91.9	91.9	[c]	[c]
3	0.75	M	27.8	8.00	48.0	445	559	583	9.93
4	2.0	M	85.5	8.00	48.0	1434	1924	2074	12.1
2	0.25	F	4.28	8.00	8.00	[b]	23.9	[b]	[b]
3	0.75	F	23.2	4.00	24.0	374	374	411	6.99
4	2.0	F	61.0	8.00	48.0	1064	1375	1438	10.4

C_{max} = maximum plasma concentration; T_{max} = time of maximum plasma concentration; T_{last} = time of last quantifiable plasma test article concentration; $AUC_{(0-24)}$ = area under the plasma concentration-time curve from time zero to 24 hours after dose; $AUC_{(last)}$ = area under the plasma concentration-time curve from time zero to the time of the last quantifiable plasma test article concentration; $AUC_{(inf)}$ = area under the plasma concentration-time curve from time zero to infinity; $T_{1/2}$ = terminal elimination half-life; M = male; F = female.

[a] Value not considered reportable when the number of quantifiable plasma concentration time points are less than 3.

[b] No reportable results because the terminal phase could not be identified.

[c] Values are not reported because the $AUC_{(0-\infty)}$ was extrapolated by more than 20% or R^2 was < 0.8.

Dose Formulation Analysis

Samples were analyzed by HPLC with UV detection by a validated method within a dynamic range of 0.03 to 5 mg/ml in 25 mM citrate buffer, pH 2.75. Mean AP24534 concentrations in the dosing solutions ranged between 97% and 108% of intended, which was within acceptable limits (≤10% difference from nominal concentration).

Study title: 28-Day Oral Toxicity Study of AP24534 with Toxicokinetics in Cynomolgus Monkeys with a 28-Day Recovery Period

Study no.:	QAA00121
Study report location:	NDA Section 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 2, 2007
GLP compliance:	FDA, OECD, MHLW
QA statement:	Statement included and signed
Drug, lot #, and % purity:	AP24534 (ponatinib), lot PAK-010-013, 99.70% by HPLC (93.2% as free base). Correction factor 0.932 used for dose formulation preparations.

Key Study Findings

- AP24534-related mortality of 2/5 males and 1/5 females at 5 mg/kg/day.
- Early decedents exhibited abnormal thyroid hormone levels, lymphoid depletion in different organs, multifocal follicular atrophy in the thyroid gland, minimal acinar cell necrosis in the pancreas, and hyperkeratosis of multiple skin sites.
- Surviving monkeys at 5 mg/kg/day presented adverse clinical signs, systolic heart murmurs (Grade I/VI or II/VI), harsh lung sounds, and/or abdominal bloat or gassy intestines, decreases in hemoglobin, hematocrit, corpuscular volume, and corpuscular hemoglobin, thyroid hormone levels, and microscopic findings in the pancreas, thymus and thyroid glands. Similar microscopic findings were observed in early decedents.
- Severe clinical signs at 2.5 mg/kg/day including systolic heart murmurs (Grade III/VI), harsh lung sounds, and/or abdominal bloat or gassy intestines, high T_4 levels, and microscopic findings in the pancreas, thymus and thyroid glands.
- Less severe clinical signs at 1 mg/kg/day including systolic heart murmurs (Grade I/VI), harsh lung sounds, and microscopic changes in the thyroid.

Methods

Doses:	0, 1.0, 2.5, and 5.0 mg/kg/day (free base) 0, 12, 30, and 60 mg/m^2/day
Frequency of dosing:	Once daily
Route of administration:	Orogastric gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	25 mM Citrate Buffer, pH 2.75
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	5/sex/group; 2/sex/group for recovery
Age:	Young adult; ages not provided.
Weight:	2 to 5 kg
Unique study design:	Due to early mortality, 1 monkey/sex at 5 mg/kg for recovery.

Results

Mortality

Three 5 mg/kg/day monkeys were euthanized in moribund condition, one female on Day 19 and one male each on Days 21 and 22. Common clinical signs recorded before euthanasia included hunched/recumbent posture, lethargy, dehydration, marked soft feces, bruise, dry flaky skin and skin erythema at different body parts. The female monkey also presented with green eye discharge and scratching in neck and face and the male euthanized on Day 22 presented with clear eye discharge, pale mucus membranes, mouth ulceration, thinness and lack of eating. These unscheduled deaths and clinical signs were attributed to AP24534 administration.

Several clinical chemistry parameters were affected in early decedents; however, the cause of these changes can not be explained alone by AP24534 administration given the poor nutritional status and cachexia of these monkeys. Changes included increases in blood urea nitrogen and creatinine and decreases in albumin and globulin levels and changes in serum electrolytes such as phosphorus, calcium, sodium, potassium, and chloride.

Thyroid hormones values for early decedents were abnormal (Table 26) and corresponded with microscopic findings of follicular atrophy of the thyroid.

Table 26 Fold Changes in Thyroid Hormone Levels in Early Decedents

Animal Number	Thyroid Stimulating Hormone*	T_3*	T_4*
M 4001	1.9x	0.24x	1.8x
M 4004	7.1x	0.07x	0.32x
F 4104	0.4x	0.30x	2.6x

*Compared to predose levels.

AP24534-related histopathological findings in early decedents included lymphoid depletion in thymus, spleen, mandibular lymph nodes, and/or gut-associated lymphoid tissues (GALT); mild, multifocal follicular atrophy in the thyroid gland, minimal acinar cell necrosis in the pancreas, and hyperkeratosis of multiple skin sites (face, inguinal, forelimb, hindlimb, and shoulder). Possible AP24534-related findings in early decedent males was mild degeneration of germ cell epithelium in the testes, decreased number of spermatids in seminiferous tubules in association with numerous spermatid giant cells although a secondary effect related to stress/cachexia may also be possible.

Findings secondary to AP24534 administration included fat atrophy and/or edema in the coronary groove; hemorrhage; necrosis; and decreased vacuolation of the adrenal cortex; zymogen granule depletion of acinar cells of pancreas and salivary gland; interstitial hemorrhage involving the testes; hemorrhage, necrosis, and thrombosis involving the thyroid gland; and erosion/ulceration of the tongue.

Findings of uncertain relation to AP24534 administration included granulomatous inflammation involving the lungs present in the female and one male early decedent.

Clinical Signs and Physical Examinations

Clinical observations were conducted daily; physical exams were conducted within 6 days before the Day 30 necropsy and within 3 days of the Day 57 necropsy. In addition to the clinical signs observed in early decedents, AP24534-related clinical signs in the 5 mg/kg/day monkeys included dry flaky skin at different anatomic locations (legs, thorax, face, inguinal region, arms, neck, tail, scrotum, axillae, mouth, and/or shoulders), mild to moderate or marked skin erythema (shoulders, neck, face, inguinal region, arms, axillae, and abdomen), scratching (arms, head, neck, face, and legs), and soft feces.

The time of onset for dry flaky skin started as early as Day 6 and affected all males and females at 5 mg/kg/day. The dry flaky skin clinical sign disappear in the 5 mg/kg/day male assigned for recovery on Day 35 and in the female on Day 42.

The time of onset for skin erythema ranged from Day 6 to 18 and affected four of five males and four of five females in the 5-mg/kg dose group at diverse anatomical locations. The reversibility of skin erythema could not be assessed because the study report lacks information about this clinical sign during the dosing and recovery periods in both the male and female monkeys assigned for recovery.

The time of onset for scratching began as early as Day 11 and affected three out of five males and two out of five females in the 5 mg/kg dose group. This clinical signs was observed only in one 5 mg/kg/day female during Days 35-36 of the recovery period.

The time of onset for fecal abnormalities ranged from Day 7 to 24 and was present in monkeys of all groups including control with limited duration and with no patterns that suggested a relationship to AP24534; however, soft feces were attributed to AP24534 administration in the 5 mg/kg/day group based on the severity, frequency and prolonged duration of fecal abnormalities.

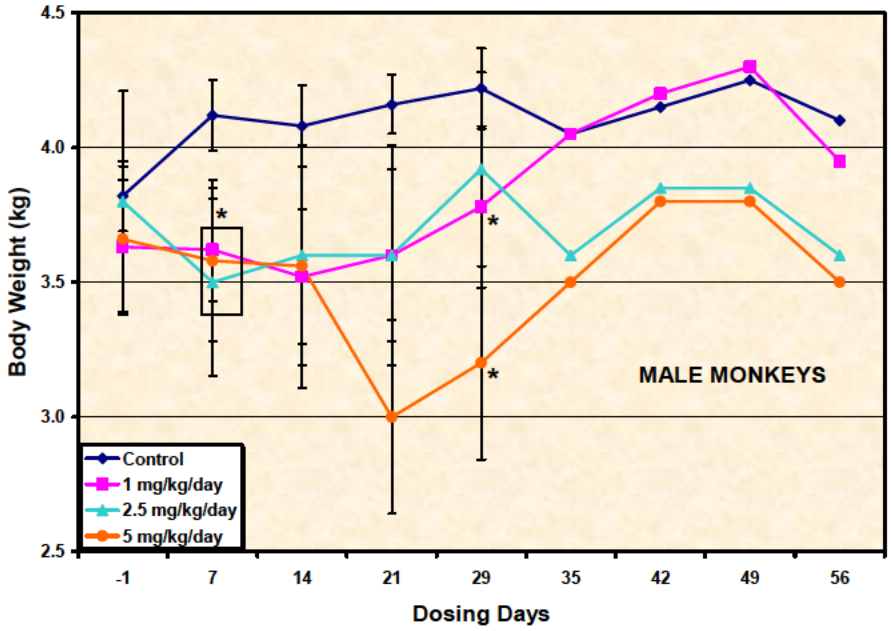
In addition to the clinical signs described above, AP24534-related findings during physical examinations included systolic heart murmurs, harsh lung sounds, mild dehydration and/or abdominal bloat and /or gassy intestines, which were also noted for the 1 and 2.5 mg/kg/day monkeys. These findings were not observed at physical examinations during the recovery period. Heart murmurs were present in one 1 mg/kg/day male (Grade I/VI), one 2.5 mg/kg/day female (Grade III/VI) and one each 5 mg/kg/day male (Grade II/VI) and female (Grade I/VI) on Days 24/25 but the relation to AP24534 administration was uncertain. No macroscopic or microscopic correlates were found in the heart of these animal euthanized on Day 30.

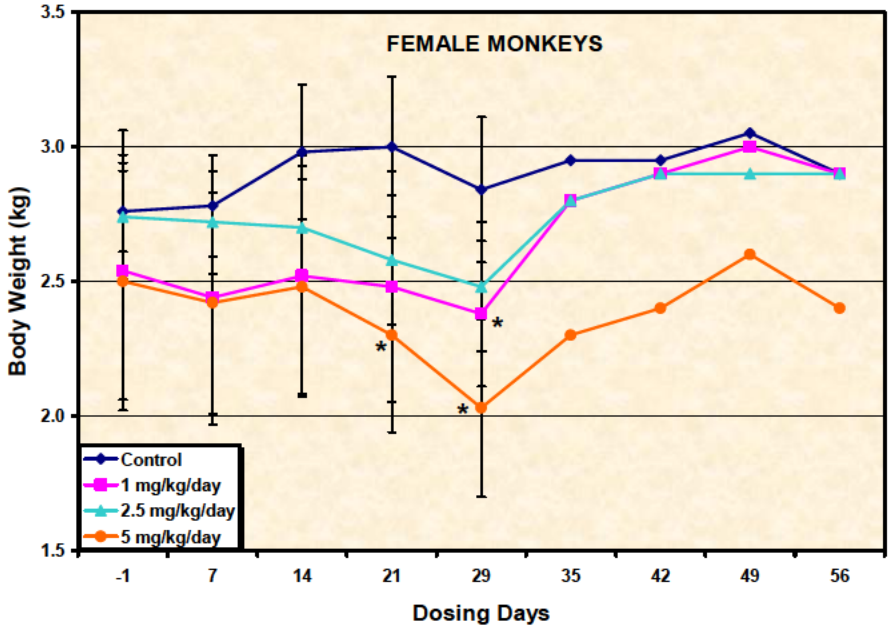
Body Weights (measured weekly)

Administration of AP24534 at 1, 2.5 or 5 mg/kg/day resulted in significant decreases in body weight at different intervals during the dosing period (Figure 8). The decrease in

mean body weight reached its maximum difference compared to control on Day 21 in males with 13.5, 13.5 and 27.9% and in females with 17.3, 14.0, and 23.3% lower mean body weight at 1, 2.5 or 5 mg/kg/day, respectively. The mean body weight in 5 mg/kg/day females further decreased to 28.5% lower compared to control on Day 29.

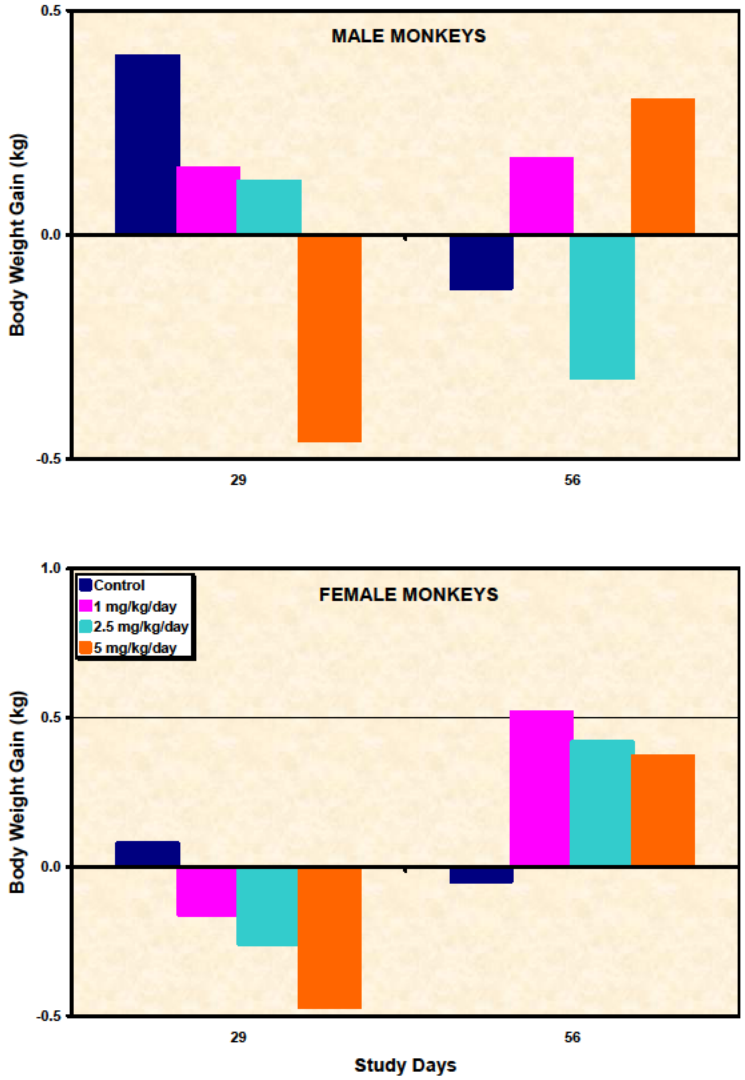
Figure 8 Mean Body Weight of Male and Female Cynomolgus Monkeys During 28 Day Study





The net mean body weight gain on Day 29 and 56 for male and female monkeys is shown graphically below (Figure 9). The 5 mg/kg/day males lost weight compared to their starting mean body weight on Day -1 during the dosing period and rebounded during the recovery period although there was only 1 male that survived to recovery. The body weight loss corresponded with observations of low and scant food consumption.

Figure 9 Mean Body Weight Gain of Male and Female Cynomolgus Monkeys at the End of the Dosing and Recovery Periods



Food Consumption

Evaluation of food consumption in NHP studies is usually qualitative because monkeys rarely eat the entire biscuit or they drop biscuits that fall to the cage pan or outside the cage making it difficult to quantitatively measure food consumption. The testing facility reported incidence of food consumption measurements expressed as number of

observations of normal, low, or scant food consumption divided by the total number of observations (Table 27).

During the dosing period, males at 2.5 and 5 and females at 1, 2.5 and 5 mg/kg/day, had higher incidence of low and scant food consumption compared to control with the greatest incidence in 5 mg/kg/day male and female monkeys that corresponded with lower mean body weight. During the recovery period Days 30 to 56, the incidence of normal food consumption was similar across the groups.

Table 27 Incidence of Food Consumption

(Excerpted from Applicant's submission)

Group	AP24534 Dose (mg/kg)	Feed Consumption	Prestudy (Days -6 to -1)[a]	Days 1–29[a]	Days 30–56[a]
1M	0	Normal	28/30 (93.3)	113/135 (83.7)	46/52 (88.5)
		Low	2/30 (6.7)	21/135 (15.6)	5/52 (9.6)
		Scant	0/30 (0.0)	1/135 (0.7)	1/52 (1.9)
2M	1	Normal	26/30 (86.7)	113/135 (83.7)	51/52 (98.1)
		Low	2/30 (6.7)	16/135 (11.9)	1/52 (1.9)
		Scant	2/30 (6.7)	6/135 (4.4)	0/52 (0.0)
3M	2.5	Normal	29/30 (96.7)	94/135 (69.6)	51/52 (98.1)
		Low	1/30 (3.3)	39/135 (28.9)	1/52 (1.9)
		Scant	0/30 (0.0)	2/135 (1.5)	0/52 (0.0)
4M	5	Normal	27/30 (90.0)	37/119 (31.1)	25/26 (96.2)
		Low	2/30 (6.7)	46/119 (38.7)	0/26 (0.0)
		Scant	1/30 (3.3)	36/119 (30.3)	1/26 (3.9)
1F	0	Normal	29/30 (96.7)	132/135 (97.8)	45/52 (86.5)
		Low	1/30 (3.3)	3/135 (2.2)	7/52 (13.5)
		Scant	0/30 (0.0)	0/135 (0.0)	0/52 (0.0)
2F	1	Normal	27/30 (90.0)	111/135 (82.2)	44/52 (84.6)
		Low	3/30 (10.0)	23/135 (17.0)	5/52 (9.6)
		Scant	0/30 (0.0)	1/135 (0.7)	3/52 (5.8)
3F	2.5	Normal	29/30 (96.7)	107/135 (79.3)	48/52 (92.3)
		Low	1/30 (3.3)	20/135 (14.8)	3/52 (5.8)
		Scant	0/30 (0.0)	8/135 (5.9)	1/52 (1.9)
4F	5	Normal	28/30 (93.3)	47/125 (37.6)	25/26 (96.2)
		Low	2/30 (6.7)	52/125 (41.6)	1/26 (3.8)
		Scant	0/30 (0.0)	26/125 (20.8)	0/26 (0.0)

M = males; F = females.
Numerator: # of observations of normal, low or scant food consumption;
Denominator: Total # observations. Frequency is shown in parenthesis.

Ophthalmic and Cardiology Evaluations

Ophthalmic exams were performed before treatment, within 6 days of the Day 30 necropsy and within 3 days of the Day 57 necropsy. Electrocardiograms were measured before Day 1 and on Day 29 (all animals) or Day 56 for recovery animals. No AP24534-related ophthalmic or cardiology abnormalities at the end of the dosing or recovery periods.

Hematology and Coagulation

Blood collection time points for hematology and clinical chemistry were Week -1 and on Day 8, 15 and 30 and on Day 57 for recovery monkeys.

Reversible reductions in hemoglobin, hematocrit, mean corpuscular volume (males only) and mean corpuscular hemoglobin in 5 mg/kg/day male and female monkeys occurred after 28 days of dosing (Table 28). Values for these parameters were comparable to control by the end of the recovery period.

Table 28 AP24534-related Fold Changes in Hematological Parameters

Parameter	Dose (mg/kg/day)	Male		Female	
		Day 30	Day 57	Day 30	Day 57
Hemoglobin (g/dL)	5	**0.83x***	0.99x	**0.86x***	0.98x
Hematocrit (%)	5	**0.83x***	0.79x	**0.89x***	1.04x
Mean Corpuscular Volume (fL)	5	**0.90x***	1.01x	0.93x	0.98x
Mean Corpuscular Hemoglobin (pg)	2.5	1.00x	1.03x	**0.92x***	0.92x
	5	**0.89x***	1.02x	**0.90x***	0.93x

**Bold values indicate the mean value was significantly different from group 1 controls (p≤0.05).*

On Day 57, the single 5 mg/kg/day male assigned for recovery had abnormally high white blood cell counts with associated abnormally high absolute neutrophil counts and elevated absolute mean monocyte counts. These values were unremarkable in the single female assigned to this group for recovery. Therefore, the toxicological significance of a potential delayed AP24534-effect on the white blood cell parameters could not be established.

Statistically significant AP24534-related effects on coagulation parameters occurred at the end of the dosing period at 5 mg/kg/day:

- Males had increased fibrinogen (1.4x control) on Day 30 and the 5 mg/kg/day
- Females had transient increases in mean activated partial thromboplastin time (1.14x control) and fibrinogen concentration (1.5x control) on Day 15.
- The prothrombin time was not affected at any interval for both male and female monkeys.

Clinical Chemistry

Increased serum globulin in the 2.5 and 5 mg/kg/day females on Days 8 and/or 15 and decreased serum albumin in the 5 mg/kg/day male and female monkeys could be directly AP24534-related or secondary to the cachexia and low food intake caused by AP24534 treatment.

Serum amylase and lipase levels were tested at Day 57 on recovery monkeys based on microscopic findings in the pancreas of some 2.5 and 5 mg/kg/day monkeys euthanized

on Day 30. No clear AP24534 effect was observed but an exception was one 5 mg/kg/day male monkey which had approximately 8 fold increased lipase value compared to control on Day 30 that corresponded with acute changes in the pancreas characterized by diffuse, moderate acinar cell necrosis accompanied by diffuse interstitial edema, multifocal acute fibrinous inflammation, and diffuse interstitial fibroplasia.

Thyroid Hormones

Samples for thyroid hormone analysis were collected 1 week prior to treatment and on Days 30 and 57 in all remaining animals.

Statistically significant decreases occurred in mean T_3 levels in 5 mg/kg/day males and significant increases in mean T_4 levels occurred in all AP24534-treated males (Table 29). Similar increases in mean T_4 levels occurred in 5 mg/kg/day females but not reach statistical significance. Thyroid stimulating hormone levels increased compared to control in the 5 mg/kg/day females on Day 30. On Day 57, mean T4 levels remained lower than controls in the high dose group and in the mid dose females.

Table 29 AP24534-related Fold Changes in Thyroid Hormones

Parameter	Dose (mg/kg/day)	Male		Female	
		Day 30	Day 57	Day 30	Day 57
Thyroid Stimulating Hormone (ng/mL)	5	1.18x	0.80x	**1.48x***	1.24x
Triiodothyronine – T_3 (ng/dL))	5	**0.37x***	0.93x	0.79x	0.92x
Thyroxine – T_4 (ng/dL)	1	**1.16x***	0.97x	1.05x	0.88x
	2.5	**1.61x***	1.23x	1.09x	0.80x
	5	**1.63x***	0.72x	1.54x	0.75x

**Bold values indicate the mean value was significantly different from group 1 controls (p≤0.05). Significant differences cannot be calculated for recovery groups (n=2).*

AP24534-related changes in thyroid hormones corresponded with microscopic findings of follicular atrophy of the thyroid at 2.5 and 5 mg/kg/day on Day 30. Mild follicular atrophy of the thyroid in the recovery male at 5 mg/kg/day corresponded with decreased level of T_4. No microscopic correlates occurred for one 2.5 mg/kg/day and one 5 mg/kg/day female that had decreased levels of T_4 on Day 57.

Urinalysis

Urine samples were collected 1 week prior to treatment and on Days 30 and 57 in all remaining animals. AP24534-treated male and female monkeys showed a tendency for increased urinary excretion of protein; however, there were not microscopic correlates in the kidneys and whether a relationship to AP24534 administration exists is uncertain.

Gross Pathology

AP24534-related macroscopic findings at terminal sacrifice on Day 30 included:

- Pancreas, diffusely thickened due to either diffuse fibrosis or interstitial edema and inflammation, and, in one of the two 5 mg/kg/day males the pancreas was adhered to the spleen;
- Skin crusts in limbs, face, neck, shoulders, and inguinal regions microscopically associated with serocellular crusts and/or hyperkeratosis;
- Thymus small in size microscopically associated with lymphoid depletion.

Fluid in the abdominal cavity in 1 of 5 females at 2.5 mg/kg/day and dark discoloration of adrenal glands in 2 of 2 males at 5 mg/ kg/day are of questionable relationship to AP24534 administration. Additional findings at Day 30 and/or 57 were sporadic and unrelated to AP24534 administration.

Table 30 Macroscopic Pathology in Cynomolgus Monkeys at Terminal Sacrifice Day 30

Tissue / Lesion	Number of Monkeys Affected					
	Males			Females		
Dose (mg/kg/day)	**1**	**2.5**	**5**	**1**	**2.5**	**5**
Number of monkeys examined	**3**	**3**	**2**	**3**	**3**	**3**
AP24534-related						
PANCREAS Diffusely thickened	--	--	2	--	--	--
Adhesion to spleen	--	--	1	--	--	--
SKIN Crusts	--	--	2	--	--	3
THYMUS Small	--	--	2	--	1	2
Potential AP24534-related						
ABDOMINAL CAVIDITY Fluid tan colored	--	--	--	--	--	1
ADRENAL GLANDS Dark discoloration	--	--	2	--	--	--

Organ Weights

AP24534-related organ weight changes at terminal sacrifice (Day 30) included thymus, ovary, uterus and adrenal glands. Dose-dependent decreases in mean thymus weights occurred in all AP24534-treated females, but decreases were seen only in the 5 mg/kg/day males (Table 31). Lymphoid depletion in thymus of the high dose males and females was evident microscopically, but the decreased thymus weight noted at 1 and 2.5 mg/kg/day was considered the result of physiologic involution rather than lymphoid depletion.

Table 31 Absolute and Relative Thymus Weight on Day 30

(Excerpted from Applicant's submission)

Group Number	Males			Females		
	Absolute Thymus Weight (g)	Thymus to Body Weight (g)	Thymus to Brain Weight (g)	Absolute Thymus Weight (g)	Thymus to Body Weight (g)	Thymus to Brain Weight (g)
1	1.893	0.047	0.023	4.413	0.157	0.070
2	2.763	0.083	0.040	2.363	0.097	0.033
3	1.830	0.050	0.027	2.257	0.083	0.033
4	0.490	0.015	0.010	0.887	0.033	0.017

A dose-dependent trend in decreased absolute and relative ovary and uterus weight in AP24534-treated females was evident (Table 32). Decreased ovary weight was a possible AP24534-related effect associated with increased follicular atresia while decreased uterine weight was secondary to endometrial atrophy related to the ovarian changes of follicular atresia.

Table 32 Absolute and Relative Ovary and Uterus Weight on Day 30

(*Excerpted from Applicant's submission*)

Group Number	Ovaries			Uterus		
	Absolute Ovary Weight (g)	Ovary to Body Weight (g)	Ovary to Brain Weight (g)	Absolute Uterus Weight (g)	Uterus to Body Weight (g)	Uterus to Brain Weight (g)
1	0.3697	0.0117	0.0060	3.903	0.127	0.063
2	0.2177	0.0090	0.0033	1.873	0.077	0.027
3	0.2460	0.0087	0.0040	2.037	0.070	0.030
4	0.1410	0.0060	0.0023	0.750	0.033	0.013

A few statistically significant differences in mean organ weight occurred for males at 1 and 2.5 mg/kg/day that had no dose-response patter or microscopic correlates, and included: increased adrenal weight in 2.5 mg/kg/day males; decreased brain and seminal vesicle weights in 1 and 2.5 mg/kg/day males. Variations in organ weight of male reproductive system, seminal vesicles, testes, epidydimides, and prostate gland were attributed to differences in the onset of sexual maturity in individual male monkeys. There were no notable AP24534-related organ weight changes at the end of the recovery period.

Histopathology

Microscopic findings for early decedents, 2 males and 1 female at 5 mg/kg/day, were reviewed in the mortality section. Microscopic findings were graded by the pathologist on a scale 1 to 4, minimal<mild<moderate<marked, according to the intensity and extent of change.

Adequate Battery: ***Yes***
Peer Review: ***No***

Histological Findings at Terminal Euthanasia
AP24534-related microscopic findings occurred in lymphoid tissues, thyroid glands, pancreas, skin and reproductive organs. Decreased lymphocyte numbers in the thymus, spleen, mandibular and mesenteric lymph nodes, and gut-associated lymphoid tissues (when present in section) ranged from minimal to moderate. Lymphoid depletion in the thymus was the most prominent and affected both cortical and medullary regions; lymphoid depletion in the spleen involved the periarteriolar lymphoid sheets with absence of follicles; and lymphoid depletion in the lymph nodes included a reduction of cortical and paracortical zones with absence of follicles in the cortex.

Both surviving high dose males had AP24534-related changes in the pancreas. One male had 8 fold increased serum lipase, corresponding with acute changes that included diffuse, moderate acinar cell necrosis accompanied by diffuse interstitial edema, multifocal acute fibrinous inflammation and diffuse interstitial fibroplasia. The second male presented changes characterized by diffuse interstitial fibrosis and diffuse acinar cell atrophy. One 2.5 mg/kg/day male and both 5 mg/kg/day male and female monkeys had single discrete foci of acinar cell regeneration accompanied by minimal focal fibrosis and/or chronic inflammation.

AP24534-related changes in the thyroid glands included minimal to moderate follicular atrophy noted with greater intensity in males than females at 2.5 and 5 mg/kg/day. Follicle atrophy was the most notable change and was multifocal in distribution with effects in the lining epithelia and mixed cell infiltrates (neutrophils, eosinophils, macrophages and/or lymphocytes) and included stromal condensation rather than fibrosis. Less frequent changes were minimal to mild individual follicular cell necrosis, presence of pigments in cytoplasm of interstitial macrophages, hemorrhage, edema, follicular cell regeneration and interstitial fibroplasia.

A single 5 mg/kg/day male had minimal germ cell degeneration characterized by a slight decrease in the number of spermatids in some seminiferous tubules as well as infrequent spermatid giant cells. This male also had evidence of cellular debris in the epididymal tubules. Although ovaries from all females had evidence of follicular development, all three 5 mg/kg/day females had evidence of increased atresia (degeneration and resorption of an ovarian follicle before it reaches maturity and ruptures) along with a paucity of secondary follicles and lack of tertiary follicles compared to control females. The lack of significant follicle development resulted in atrophy of uterine endometrium in these 5 mg/kg/day females.

Histological Findings at Recovery Euthanasia
Multifocal, mild follicular atrophy and presence of pigments in cytoplasm of interstitial macrophages of the thyroid gland as well as decreased number of spermatids in some seminiferous tubules accompanied by cellular debris within lumens of epididymal tubules were present in the single remaining high dose male at recovery euthanasia.

Table 33 Summary of AP24534-Related Histopathology Findings

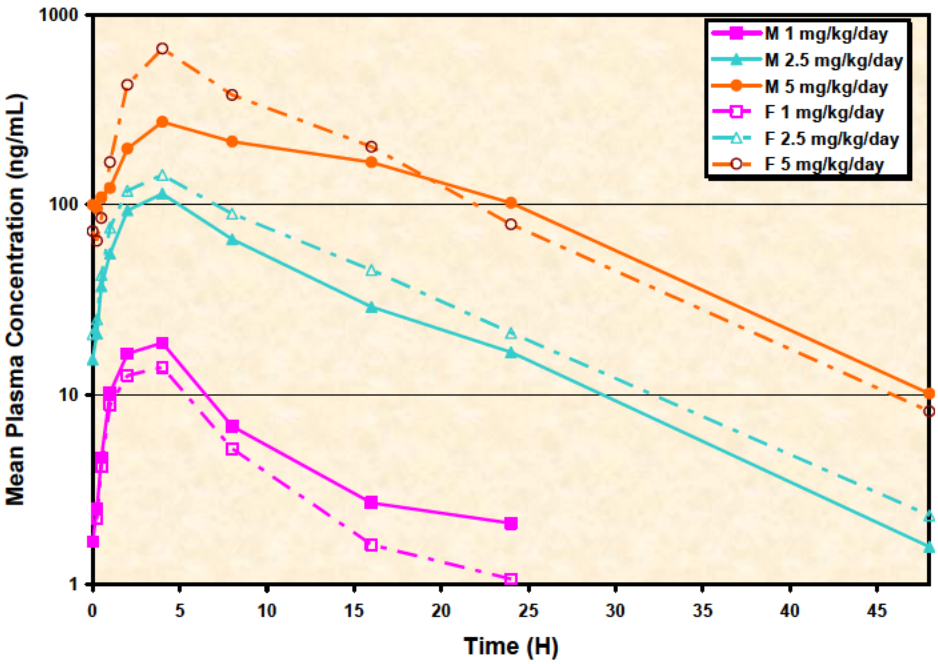
Microscopic Findings			Number of Monkeys Affected							
			Males				Females			
Dose (mg/kg/day)			0	1	2.5	5	0	1	2.5	5
Number of animals examined			3	3	3	2	3	3	3	3
Organ	Finding	Grade	Day 30 Terminal Euthanasia							
Lymph node, mesenteric	Lymphoid depletion	minimal	-	-	-	1	-	-	-	1
	Lymphoid depletion	mild	-	-	-	1	-	-	1	-
Lymph node, mandibular	Lymphoid depletion	minimal	-	-	-	2	-	-	-	2
	Lymphoid depletion	mild	-	-	-	-	-	-	1	1
Pancreas	Atrophy, acinar cell, diffuse	marked	-	-	-	1	-	-	-	-
	Fibrosis, diffuse	marked	-	-	-	1	-	-	-	-
	Fibrosis, focal	minimal	-	-	1	1	-	-	-	1
	Necrosis, acinar cell	minimal	-	-	-	1	-	-	-	-
		moderate	-	-	-	1	-	-	-	-
	Inflammation, chronic, focal	minimal	-	-	-	-	-	-	1	1
	Inflammation, subacute, focal	minimal	-	-	1	-	-	-	-	-
	Inflammation, acute, fibrinous multifocal	mild	-	-	-	1	-	-	-	-
	Edema, diffuse	moderate	-	-	-	1	-	-	-	-
	Fibroplasia, interstitum, diffuse	mild	-	-	-	1	-	-	-	-
	Fibroplasia, interstitum, focal	minimal	-	-	-	-	-	-	-	1
	Regeneration, acinar cell focal	minimal	-	-	1	1	-	-	-	2
	Adhesion	moderate	-	-	-	1	-	-	-	-
Skin	Crust	mild	-	-	1	-	1	1	-	-
		moderate	-	-	1	1	-	-	-	-
		marked	-	-	-	1	1	-	-	-
Spleen	Lymphoid depletion	minimal	-	-	-	2	-	-	1	2
Thymus	Lymphoid depletion	mild	-	-	-	-	-	-	1	1
		moderate	-	-	-	2	-	-	-	1
Thyroid	Atrophy, follicle, multifocal	minimal	-	-	-	1	-	-	1	1
		mild	-	-	-	1	-	-	-	-
		moderate	-	-	1	-	-	-	-	-
	Necrosis, single cell, epithelium, follicle	minimal	-	-	1	-	-	-	-	-
	Infiltration, mononuclear cell	minimal	-	2	1	1	-	1	1	2
	Infiltration, mixed cell	minimal	-	-	-	2	-	-	1	-
		mild	-	-	-	-	-	-	-	1
		moderate	-	-	1	-	-	-	-	-
	Fibroplasia, interstitium	minimal	-	-	1	-	-	-	-	-
	Regeneration, follicular cell	minimal	-	-	1	-	-	-	-	-
	Pigment	mild	-	-	1	-	-	-	-	-
	Edema, interstitium	mild	-	-	-	-	-	-	-	1
	Inflammation, granulomatous, interstitium, fat, focal	minimal	-	-	-	-	-	1	-	-
Lungs	Inflammation, granulomatous	mild	-	-	-	1	-	-	1	1
		moderate	-	-	-	1	-	-	-	-
Ovary	Atresia, follicular, increased		-	-	-	-	-	-	-	3
Testis	Degeneration, germinal epithelium	minimal	-	-	-	1	-	-	-	-
			Day 57 Recovery Euthanasia							
Testis	Degeneration, germinal epithelium	minimal	-	-	-	1	-	-	-	-
Thyroid	Atrophy, follicle, multifocal	mild	-	-	-	1	-	-	-	-
- lesion or tissue not present in monkeys examined										

Toxicokinetics

Blood collection time points for toxicokinetic analysis were before dosing on Days 15 and 28, at 15 and 30 min and 1, 2, 4, 8, 16 and 24 hours after dosing on Days 1, 15 and 28, and at 48 hours after dosing on Day 28. Both AP24534 and the metabolite AP24567 were measured in plasma samples using a validated turbo ion spray LC/MS/MS method calibrated over a range of 1-1000 ng/ml. All analytical runs were deemed acceptable by the Study Director. One sample from a control female (1h; Day 15) had measurable AP24534 (3.93 ng/mL); the reason for this is unknown.

After oral administration, mean AP24534 plasma concentration profiles over time were similar in male and female monkeys on Days 1, 15 and 28 at all dose levels. Figure 10 shows the mean plasma concentration over time for Day 28.

Figure 10 Mean Plasma Concentration Time Profiles of AP24534 in Male and Female Monkeys after 28 Days Dosing



In general, systemic exposures to AP24534, as measured by C_{max} and AUC_{last}, were greater than the relative increase in AP24534 dose (Table 36). Values for C_{max} and AUC_{last} were in general higher on Day 15 than on Day 1 and comparable on Days 15 and 28, indicating accumulation of AP24534 in monkeys after 15 or 28 days repeated exposure. Mean terminal half-lives for AP24534 ranged from 4.6 to 11.4 hours and were in general comparable for males and females at each dose level. T_{max} occurred most often in the range from 2 to 4 hours at this dose range.

The metabolite, AP24567, was not detected in plasma samples from monkeys dosed at 1 mg/kg/day except for few plasma samples from Day 15 and 28. Calculations of TK

parameters of the metabolite AP24567 was based on plasma sample values from 2.5 and 5 mg/kg/day dose levels.

Table 34 Summary of Toxicokinetic Parameters Mean Values for AP24534 in Cynomolgus Monkeys

(Excerpted from Applicant's submission)

Group	Dosage (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 1								
2	1	M	13.8	4	24	97.2	146[b]	8.7[b]
		F	6.33	2	8	45.0	96.6[b]	6.1[b]
3	2.5	M	92.3	4	24	847	922	6.3
		F	46.4	4	24	483	758[c]	9.3[c]
4	5	M	92.6	4	24	1060	885[b]	5.1[b]
		F	150	4	24	1970	947[d]	4.6[d]
Day 15								
2	1	M	28.0	4	24	205	250[e]	7.2[e]
		F	16.5	2	24	128	199[b]	6.8[b]
3	2.5	M	151	4	24	1510	1710	6.0
		F	113	4	24	1060	1130	5.7
4	5	M	442	4	24	7010	12200[d]	11.4[d]
		F	771	4	24	10500	9740[e]	8.6[e]
Day 28								
2	1	M	20.0	2	24	162	181[e]	7.9[e]
		F	15.8	4	24	115	147[c]	8.4[c]
3	2.5	M	114	4	48	1430 (1240)[f]	1640[e]	7.0[e]
		F	145	4	48	1920 (1670)[f]	3060[d]	8.2[d]
4	5	M	272[d]	4[d]	48[d]	5660 (4310)[d,f]	5770[d]	7.8[d]
		F	662[e]	4[e]	48[e]	8040 (7000)[e,f]	8130[e]	8.3[e]

C_{max} = maximum concentration; t_{max} = time of maximum concentration; t_{last} = time of last observable concentration; AUC_{last} = area under the plasma mean concentration-time curve through t_{last}; AUC = area under the plasma mean concentration-time curve; $t_{½}$ = terminal elimination half-life; M = male; F = female.

[a] M edian for t_{max} and t_{last}; n=5.
[b] n=2.
[c] n=1 .
[d] n=3 .
[e] n=4 .
[f] For comparison purposes, the AUC_{0-24} (value in parentheses) was also calculated.

The metabolite, AP24567, was not detected in plasma samples from monkeys dosed at 1 mg/kg/day except for few plasma samples from Day 15 and 28. Calculations of TK parameters of the metabolite AP24567 was based on plasma sample values from 2.5 and 5 mg/kg/day dose levels. AP24567 mean plasma concentration over time was similar in male and female monkeys on Days 1, 15 and 28. In general, increases in AUC_{last} were higher on Day 15 or 28 compared to Day 1, indicating accumulation of the metabolite AP24567 in monkeys after 15 or 28 days repeated exposure (Table 38). Mean terminal half-lives for AP24567 ranged from 5.2 to 18.6 hours. T_{max} occurred

most often in the range from 2 to 8 hours. C_{max} and AUC_{last} values for AP24534 were ~20-fold higher than corresponding values for the metabolite AP24567.

Table 35 Summary of Toxicokinetic Parameters Mean Values for Metabolite AP24567 in Cynomolgus Monkeys

(Excerpted from Applicant's submission)

Group	Dosage[b] (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 1								
2	1	M	0[c]	NA	NA	0[c]	NE	NE
		F	0[c]	NA	NA	0[c]	NE	NE
3	2.5	M	5.97	4	8	48.4	171[e]	5.6[e]
		F	6.22	4	8	35.1	NE	NE
4	5	M	6.57	4	16	56.5	NE	NE
		F	12.6	4	16	150	171[e]	5.2[e]
Day 15								
2	1	M	BQL[d]	4[e]	4[e]	0.684	NE	NE
		F	BQL[d]	3[f]	3[f]	1.03	NE	NE
3	2.5	M	6.62	4	8	57.1	174[e]	10.5[e]
		F	6.28	4	16	57.1	103[f]	6.6[f]
4	5	M	24.6	8	24	452	508[e]	16.1[e]
		F	35.9	8	24	677	1250[e]	18.6[e]
Day 28								
2	1	M	BQL[d]	4[f]	4[f]	0.900	NE	NE
		F	0[c]	NA	NA	0[c]	NE	NE
3	2.5	M	5.75	2	4	67.2	191[f]	6.7[f]
		F	6.98	4	16	93.8	287[f]	9.8[f]
4	5	M	10.8[g]	4[g]	24[g]	197[g]	NE	NE
		F	19.7[h]	8[g]	24[g]	359[h]	754[e]	8.5[e]

C_{max} = maximum concentration; t_{max} = time of maximum concentration; t_{last} = time of last observable concentration; AUC_{last} = area under the plasma mean concentration-time curve through t_{last}; AUC = area under the plasma mean concentration-time curve; $t_{½}$ = terminal elimination half-life; M = male; NA = not available; NE = not estimated for any animals, due to insufficient characterization of the terminal phase of the concentration-time curves; F = female; BQL = below quantifiable limits.

[a] Median for t_{max} and t_{last}; n=5.
[b] Dosage of AP24534.
[c] All plasma concentrations for all animals were BQL.
[d] The mean C_{max} was BQL.
[e] n=1.
[f] n=2.
[g] n=3.
[h] n=4.

Dose Formulation Analysis

Samples were analyzed using a validated HPLC-UV method. All dose formulations from Days 1 and 28 were within the acceptable limits of ±10% error and were stable at room temperature for at least 28 days.

Study title: AP24534: A 6-month Oral Toxicity Study in Cynomolgus Monkeys with a 2-month Recovery

Study no.:	QAA00194
Study report location:	NDA Section 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 10, 2008
GLP compliance:	FDA, OECD, MHLW
QA statement:	Statement included but was not signed by the QA representative
Drug, lot #, and % purity:	AP24534 (ponatinib), lot ABL411071, 99.6% by HPLC (88% as free base). A salt correction factor was used for dose formulation preparations.

Key Study Findings

- No unscheduled deaths occurred during the study.
- Elevated levels of ALT and AST occurred at all dose levels on Day 184 that ranged from 2- to 4.5-fold in ALT and 2- to 9.2-fold in AST, relative to Day -6 values. There were no microscopic correlates observed in the affected monkeys.
- Elevations in transaminases were not present during the recovery period.
- Mild thymus lymphoid depletion occurred at all dose levels
- Myocardial necrosis in 2/8, 4/8 and 1/8 at 0.25, 0.75 and 2 mg/kg/day, respectively.

Methods

Doses:	0, 0.25, 0.75, and 2.0 mg/kg/day (free base) 0, 3, 9, and 24 mg/m^2/day
Frequency of dosing:	Once daily
Route of administration:	Orogastric gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	25 mM Citrate Buffer, pH 2.75
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*)
Number/Sex/Group:	6/sex/group
Age:	2 to 3.8 years
Weight:	2.4 to 4.0 kg
Unique study design:	4 monkeys/sex/group assigned for terminal sacrifice; 2 monkeys/sex/group assigned for a 28-days recovery period.
Deviation from study protocol:	Report was not signed by the QA representative.

Results

Mortality

No unscheduled deaths occurred.

Clinical Signs and Physical Examinations

Diarrhea and alopecia requiring veterinary treatment occurred in 3 high dose group animals during the recovery period. No other AP24534-related clinical signs or physical examination observations occurred.

Body Weights and Food Consumption

No AP24534-related effects on body weight or food consumption were observed during the dosing or recovery periods.

Ophthalmic and Cardiology Evaluations

Ophthalmic exams were performed pre-dose and on Days 29, 87 and during final week of the dosing or recovery period. Electrocardiograms were obtained pre-dose, on Days 29, 91 and during the final week of the dosing or recovery period.

No AP24534-related ophthalmic or cardiology abnormalities at the end of the dosing or recovery period.

Hematology and Coagulation

Samples were collected for hematology and clinical chemistry on Days -35 and -6, predose on Day 28 and 91, and on Days 184 and 239 for recovery monkeys

No AP24534-related hematology or coagulation abnormalities were observed.

Clinical Chemistry

Increased ALT and AST values occurred in treated monkeys at the end of the dosing period (Day 184; see table below). The incidence of these changes was higher at 0.75 and 2 mg/kg/day but there were no corresponding microscopic findings at terminal euthanasia. Transaminase values were normal at recovery euthanasia for Male 3006 and Female 3106 at 0.75 mg/kg/day and Male 4005 at 2 mg/kg/day. There was an inconsistent occurrence of the changes among treatment groups and lack of corresponding microscopic changes.

Thyroid Hormones and Urinalysis

No AP24534-related changes in thyroid hormones levels or urinalysis observations at the end of the dosing or recovery period.

Table 36 Changes in ALT and AST on Day 184 Relative to Day -6 (Six Month Monkey Study)

(Excerpted from Applicant's submission)

AP24534 Dose (mg/mg/day)	Sex	Animal No.	Change in ALT	Change in AST
0.25	F	2102	1.92×	3.98×
0.75	M	3004	3.34×	4.31×
		3006	3.76×	5.87×
0.75	F	3103	2.39×	4.36×
		3106	2.40×	9.15×
2	M	4001	4.52×	6.22×
		4003	2.26×	3.38×
		4005	1.94×	2.16×

Gross Pathology and Organ Weights

No AP24534-related macroscopic or organ weight changes at Day 184 or 239.

Histopathology

Adequate Battery: ***Yes***
Peer Review: ***No***

AP24534-associated microscopic findings were of minimal or mild severity (Table 37). Myocardial necrosis was observed, and occurred at a higher incidence in AP24534-treated males and females, and was also present in recovery animals. Lymphoid depletion in the thymus was prevalent, and also persisted through the recovery period. Hepatocellular necrosis was observed in the livers of recovery animals 3006 (male, 0.75 mg/kg) and 2105 (female 0.25 mg/kg). Transaminase values in animal 2105 were normal throughout the study, but were elevated (see table above) for animal 3006 on Day 184 but had returned to baseline values on Day 239.

Table 37 Microscopic Findings from 6-Month Monkey Study

	Males				Females			
Dose (mg/kg)	**0**	**0.25**	**0.75**	**2.0**	**0**	**0.25**	**0.75**	**2.0**
Number of Animals in Group	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Terminal Groups								
Cervix -lumen, hemorrhage	--	--	--	--	0	1b	0	1a
Heart -endocardium hemorrhage	0	1b	0	0	0	0	0	0
Heart -myocardial necrosis	0	1a	1a	1a	1a	1a	3a	0
Heart -infiltration	1a	1a	0	1a	0	0	0	0
Heart -fibrosis	0	1a	0	0	0	0	1a	0
Heart *-within normal limits*	*3*	*3*	*3*	*2*	*3*	*3*	*1*	*4*

	Males				Females			
Dose (mg/kg)	**0**	**0.25**	**0.75**	**2.0**	**0**	**0.25**	**0.75**	**2.0**
Number of Animals in Group	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Lymph node, mandibular -hematopoiesis	0	1b	1b	1b	1a	3a 1b	2a 1b	3b
Kidney -infiltration	0	0	1a	1a	0	1a	0	0
Thymus -lymphoid depletion	1b	0	0	2b	2b	1b	1b	2b
Recovery Groups								
Number of Animals in Group	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Cervix, -lumen, hemorrhage	--	--	--	--	0	1b	1a	0
Heart -myocardial necrosis	0	0	0	1a	0	1a	0	0
Heart -infiltration	0	0	0	0	0	1a	0	0
Kidney -infiltration	2a	0	0	0	0	1b	0	0
Liver -hepatocellular necrosis	0	0	1a	0	0	1a	0	0
Lymph node, mandibular -hematopoiesis	0	0	0	1a 1b	1a	1b	1b	1a
Thymus -lymphoid depletion	0	0	0	1a	1a	0	0	1b

--: not evaluated
a, minimal; b, mild; c, moderate; d, marked

Toxicokinetics

Samples were collected for TK analysis before dosing on Days 28 and 91, at 15 min and 1, 2, 4, 8, and 24 hours after dosing on Days 1, 28, 91 and 182, and at 48 hours after dosing on Day 182.

Systemic exposure to AP24534, as measured by C_{max} and AUC_{0-24}, increased with dose from 0.25 to 2 mg/kg. The increases in C_{max} and AUC_{0-24} were higher than dose proportional with minor to moderate accumulation on Day 184 compared to Day 1 at 0.75 and 2 mg/kg/day (see tables and figures below).

Plasma concentration of AP24534 metabolite, AP24567, was quantifiable in some 0.75 mg/kg/day monkeys and in the majority of 2 mg/kg/day monkeys. The metabolites exposure was between 3.75 and 7.25% of the parent compound. Inadequate data for the metabolite AP24567 prevented estimation of TK parameters and/or conclusions regarding dose proportionality and accumulation.

Table 38 Summary of TK Analysis for AP24534 Following Oral Administration in Cynomolgus Monkey for 6 Months

(*Excerpted from Applicant's submission*)

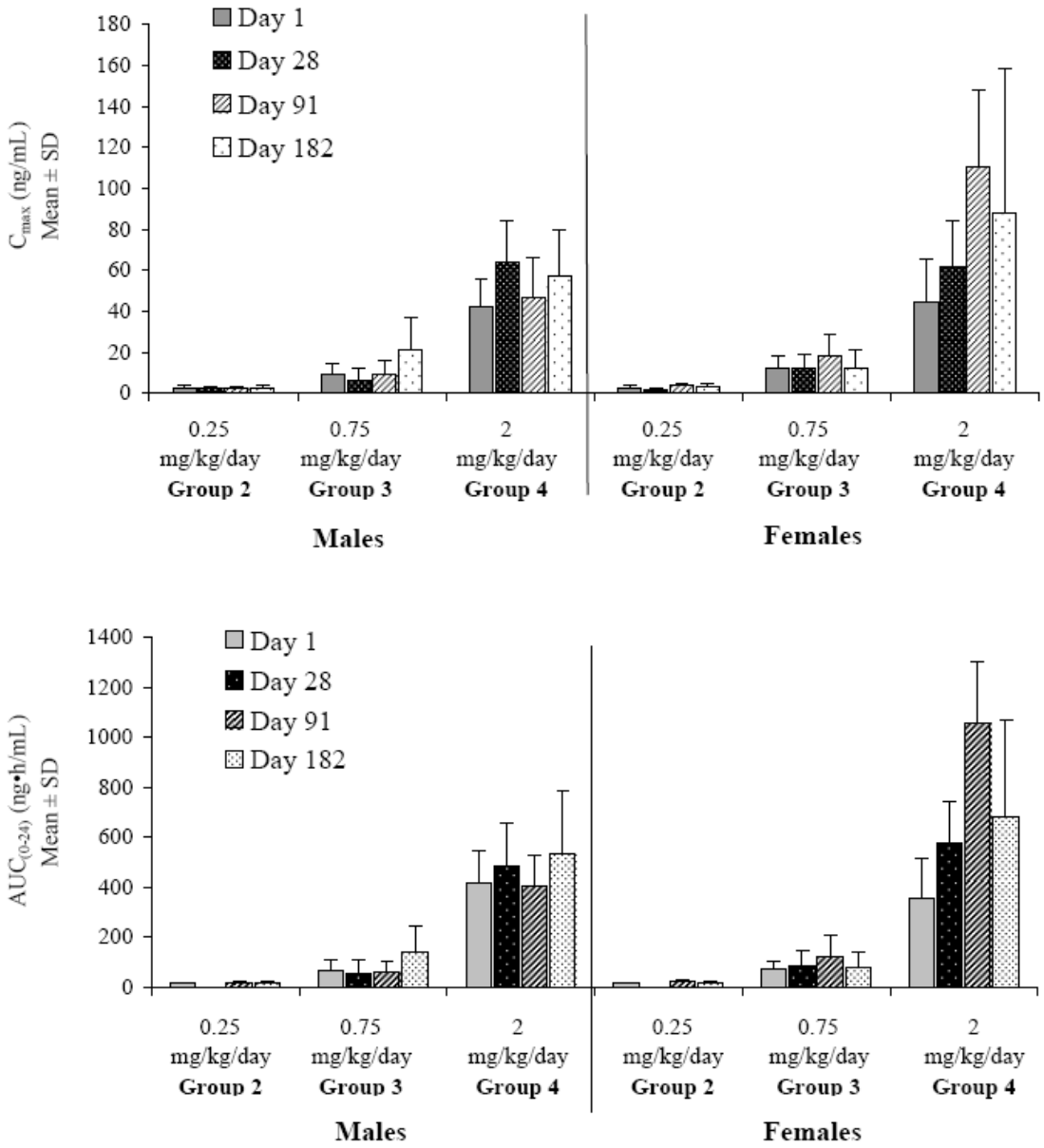
Gr	Dosage (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (h)	T_{last} (h)	AUC $_{(0\text{-tlast})}$ (ng·h/mL)	AUC $_{(0\text{-}24)}$ (ng·h/mL)	AUC $_{(0\text{-}\infty)}$ (ng·h/mL)	$T_{1/2}$ (h)
Day 1									
2	0.25	M	2.74	2.00	4.0	7.87	20.4	a	a
		F	2.76	2.00	4.0	7.97	20.1	a	a
3	0.75	M	9.39	2.0	8.0	62.7	68.2	75.3	3.35
		F	12.5	2.0	8.0	63.1	72.7	72.7	2.61
4	2	M	42.5	4.0	24.0	416	416	438	5.35
		F	44.8	2.0	24.0	356	356	420	4.97
Day 28									
2	0.25	M	2.67	2.0	4.0	7.92	a	a	a
		F	1.51	2.0	2.0	4.91	a	a	a
3	0.75	M	6.11	2.0	8.0	46.9	58.6	a	a
		F	12.5	2.0	16.0	84.1	87.6	106	5.09
4	2	M	63.8	3.0	24.0	483	483	504	4.97
		F	62.0	2.0	24.0	578	578	614	5.93
Day 91									
2	0.25	M	2.34	1.0	4.0	9.62	18.1	a	a
		F	3.56	2.0	4.0	13.7	27.4	a	a
3	0.75	M	9.46	2.0	8.0	54.6	61.8	85.2	3.57
		F	18.5	2.0	8.0	117	125	129	3.63
4	2	M	46.8	2.0	24.0	404	404	474	5.51
		F	110	3.0	24.0	1057	1057	1103	5.10
Day 182									
2	0.25	M	2.71	1.0	4.0	9.06	19.4	a	a
		F	3.32	1.0	4.0	8.89	17.4	a	a
3	0.75	M	20.9	2.0	24.0	138	141	167	3.92
		F	12.0	2.0	16.0	75.6	78.3	93.3	4.10
4	2	M	56.9	3.0	24.0	564	533	581	6.19
		F	87.9	2.0	48.0	750	681	771	8.98

Gr = group; C_{max} = maximum plasma concentration; T_{max} = time of maximum plasma concentration; T_{last} = time of last quantifiable plasma test article concentration; AUC $_{(0\text{-tlast})}$ = area under the plasma concentration-time curve from time zero to the time of the last quantifiable plasma test article concentration; AUC $_{(0\text{-}24)}$ = area under the plasma concentration-time curve from time zero to 24 hours after dose; AUC $_{(0\text{-}\infty)}$ = area under the plasma concentration-time curve from time zero to infinity; $T_{1/2}$ = terminal elimination half-life; M = male; F = female.

a = No reportable results.

Figure 11 Comparison of AP24534 Exposure in Cynomolgus Monkey Plasma Following Oral Administration of AP24534

(Excerpted from Applicant's submission)



7 Genetic Toxicology

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

Study title: *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay

Study no.:	6843-152
Study report location:	NDA Section 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 5, 2007
GLP compliance:	Yes; an exception was noted that "the stability and concentration of the dosing preparations were not analyzed." The Study Director notes that "the impact of not having verification of the stability, homogeneity, and/or concentration of the formulations cannot be fully evaluated at this time without additional information. This information may influence the evaluation of the results of this study."
QA statement:	Yes
Drug, lot #, and % purity:	AP24534 HCl salt, PAK-009-173, 99.8%

Key Study Findings

- Using a plate incorporation method, AP24534 (ponatinib) did not induce genotoxic responses in bacteria, with or without S9 metabolic activation. This study used the highest level recommended by ICH S2(R1) (5.0 mg/plate). Other than the exception to GLP compliance noted by the Study Director, the results are considered valid and adequate.

Methods (plate incorporation method)

Strains: *Salmonella typhimurium* (TA98, TA100, TAI535 and TAI537), and *Escherichia coli* tester strain *WP2uvr* A

Ponatinib used (mcg) in definitive study:

TA98, TA100, TA1535, TA1537		WP2*uvr*A	
With S9	Without S9	With S9	Without S9
10.0	10.0	-	-
33.3	33.3	-	-
50.0	50.0	-	-
75.0	75.0	-	-
100	100	100	100
150	150	333	333
200	200	500	500
333	333	1000	1000
500	500	3330	3330
1000	-	5000	5000
3330	-	-	-
5000	-	-	-

Basis of concentration selection: A pilot study was conducted on the test article using tester strains TA100 and *WP2uvrA* (one plate per concentration). Ten amounts of test article, from 6.67 to 5000 mcg/plate, were evaluated with and without S9. Inhibited growth (characterized by a reduced background lawn and/or a decrease in revertant frequencies) or complete toxicity was observed in tester strain TA100 at ≥333 mcg/plate with S9 and ≥66.7 mcg/plate without S9. Decreases in revertant frequencies also were observed in tester strain *WP2uvrA* at ≥3330 mcg/plate with and without S9. In addition, the test article precipitated from solution in the aqueous top agar at the highest two or three concentrations evaluated with and without S9.

Negative control: 25 mM citrate buffer (pH 2.75)

Positive control: Table provided in study report (p.11):

Table I. Positive Controls

Tester Strain	S9 Mix	Positive Control	Dose (µg/plate)
TA98	+	benzo[a]pyrene	2.5
TA98	–	2-nitrofluorene	1.0
TA100	+	2-aminoanthracene	2.5
TA100	–	sodium azide	2.0
TA1535	+	2-aminoanthracene	2.5
TA1535	–	sodium azide	2.0
TA1537	+	2-aminoanthracene	2.5
TA1537	–	ICR-191	2.0
WP2*uvr*A	+	2-aminoanthracene	25.0
WP2*uvr*A	–	4-nitroquinoline-N-oxide	1.0

Formulation/Vehicle: 25 mM citrate buffer (pH 2.75)

Incubation & sampling time: Plates were incubated for 52 ± 4 hours. The metabolic activation system consisted of commercially available S9 fraction prepared from livers obtained from Aroclor 1254 pretreated male Sprague-Dawley rats. Revertant colonies were counted by automated colony counter and/or by hand.

Study Validity

Bacteria strains used conform to ICH S2A recommendations. Positive and negative controls produced expected responses (see results tabulated below). Concentration selection for the plate incorporation method was adequate based upon use of the limit (i.e., 5000 mcg/plate).

Results

A pilot study was conducted using two strains (TA100 and WP2*uvr*A) and ten concentrations of test article, from 6.67 to 5000 mcg/plate. The test article precipitated from solution in the aqueous top agar of the 3330 mcg and 5000 mcg plates. Inhibited growth or complete toxicity was observed in strain TA100 at ≥333 mcg/plate with S9, and ≥66.7 mcg/plate without S9.

A definitive study (Trial No. B1) was conducted using 10.0, 33.3, 50.0, 100, 333 and 500 mcg/plate, with and without S9. Higher amounts of ponatinib were used in strain WP2*uvr*A: 100, 333, 500, 1000, 3330 and 5000 mcg/plate. All concentrations and controls were evaluated in triplicate.

- Growth was inhibited at the highest two concentrations in all 5 strains without S9 and in tester strains TA100, TA1535, TA1537 and WP2*uvr*A with S9. However, background growth appeared normal in tester strain TA98 at all concentrations with S9. Revertant frequencies for all amounts of test article in all 5 strains with S9 and 4/5 strains without S9 (not TA98) were similar to or less than those observed in concurrent negative cultures. Concentration-dependent increases in revertant frequencies, to approximately 2-fold control values were observed in strain TA98 without S9. All revertant frequencies were within acceptable ranges for vehicle controls.

Table 39 Average Colony Counts for the Bacterial Mutagenesis Assay (Definitive Study)

Average colony count per plate with S9:

mcg/plate	TA98	TA100	TA1535	TA1537
10.0	22	129	25	8
33.3	20	136	24	6

mcg/plate	WP2-uvrA
100	24
333	17

mcg/plate	TA98	TA100	TA1535	TA1537	mcg/plate	WP2-uvrA
50.0	25	123	4	6	*500*	16
100	31	140	6	100	*1000*	14
333	23	38	9	4	*3330*	8
500	21	5	5	1	*5000*	8
-control	23	135	22	5	*-control*	21
+control	432	777	174	105	*+control*	261

Average colony count per plate without S9:

mcg/plate	TA98	TA100	TA1535	TA1537	mcg/plate	WP2-uvrA
10.0	14	114	26	7	*100*	11
33.3	17	114	29	9	*333*	14
50.0	21	112	22	4	*500*	18
100	18	102	21	7	*1000*	14
333	9	17	5	3	*3330*	13
500	7	2	1	2	*5000*	0
-control	10	119	21	6	*-control*	19
+control	358	1086	797	112	*+control*	167

A second assay (Trial No. C1) was conducted that used the same amounts as the initial study, but added concentrations of 75.0, 150 and 200 mcg/plate with and without S9 (to expand the number of concentrations were the elevated revertant frequencies were observed). Additional concentrations were used with strain TA98 (1000, 3330 and 5000 mcg/plate with S9) to ensure a cytotoxic concentration was reached.

- Growth was inhibited in all 5 strains at the highest one to three concentrations evaluated with and without S9, and the test article was incompletely soluble at the high amount used for TA98. Revertant frequencies for all amounts in all 5 strains with and without S9 approximated or were less than control values.

Table 40 Average Colony Counts for the Bacterial Mutagenesis Assay (Confirmatory Study)

Average colony count per plate with S9:

mcg/plate	TA98	TA100	TA1535	TA1537	mcg/plate	WP2-uvrA
10.0	16	102	11	10	*100*	14
33.3	24	115	12	8	*333*	12
50.0	21	82	11	10	*500*	13
75.0	24	-	-	-	*1000*	15
100	25	113	8	10	*3330*	11
150	22	-	-	-	*5000*	7

mcg/plate	TA98	TA100	TA1535	TA1537
200	23	-	-	-
333	25	7	6	5
500	27	3	2	3
1000	11	-	-	-
3330	0	-	-	-
5000	0	-	-	-
-control	23	103	11	7
+control	312	821	107	88

mcg/plate	WP2-uvrA
-control	16
+control	450

"-" = concentration not tested

Average colony count per plate without S9:

mcg/plate	TA98	TA100	TA1535	TA1537
10.0	16	104	11	7
33.3	15	104	13	7
50.0	15	90	10	5
75.0	15	-	-	-
100	11	78	10	4
150	12	-	-	-
200	15	-	-	-
333	9	0	1	2
500	4	0	0	0
-control	19	87	8	5
+control	388	1094	765	390

mcg/plate	WP2-uvrA
100	16
333	15
500	11
1000	13
3330	8
5000	7
-control	17
+control	248

"-" = concentration not tested

A partial re-test (Trial No. D1) was performed using strain TA98 with S9 to confirm results observed at the higher concentrations.

- Growth was inhibited at ≥500 mcg/plate, and the test article was insoluble at ≥1000 mcg/plate. Revertant frequencies for all concentrations were similar to or less than vehicle control values.

Table 41 Average Colony Counts for the Bacterial Mutagenesis Assay (Partial Retest)

mcg/plate	TA98
10.0	41
33.3	30
50.0	30
75.0	32
100	30
150	39
200	35

mcg/plate	TA98
333	30
500	22
1000	2
3330	0
5000	0
-control	30
+control	307

Conclusion

The test article (AP24534) produced negative results in the bacterial mutagenicity assay that were confirmed by a second study. The increase in revertant frequencies using strain TA98 without S9 activation observed in the initial mutagenicity study (Trial No. B1) was not reproducible in a subsequent assay (Trial No. C1).

7.2 In Vitro Assays in Mammalian Cells

Study title: Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

Study no.:	6843-153
Study report location:	NDA Section 4.2.3.3.1 (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 5, 2007
GLP compliance:	Yes; an exception was noted by the Study Director that "the stability, homogeneity and/or concentration of the dosing preparations were not provided. Since the concentrations analyzed were based on toxicity, this deviation had no impact on the study."
QA statement:	Yes
Drug, lot #, and % purity:	AP24534 HCl salt, PAK-009-173, 91.3% A correction factor of 0.913 was used to calculate the concentration of the stock formulations.

Key Study Findings

- Using cultured human lymphocytes, AP24534 (ponatinib) did not induce chromosomal aberrations with or without S9 metabolic activation. The results are considered valid and adequate.

Methods

Cell line:	Primary cultures of human whole blood lymphocytes from healthy, adult donors (nonsmokers without a history of radiotherapy, chemotherapy, or drug usage and lacking current viral infections).
Concentrations in definitive study:	0.375, 0.750, 1.50, 3.00 mcg/ml
Basis of concentration selection:	An initial assay was conducted using concentrations
Negative control:	25 mM citrate buffer, pH 2.75
Positive control:	Without S9: 1.0 mcg/ml Mitomycin C With S9: 40.0 mcg/ml Cyclophosphamide
Formulation/Vehicle:	25 mM citrate buffer, pH 2.75
Incubation & sampling time:	The definitive study treated cells for 3 hours, and harvested them ~22h later. For the last two hours of incubation, 0.1 mcg/ml Colcemid® was added. Cells were treated for 22 hours for the confirmatory study. The metabolic activation system consisted of commercially available S9 fraction prepared from livers obtained from Aroclor 1254 pretreated male Sprague-Dawley rats.

Study Validity

The study appears valid based on:

- The use of appropriate negative and positive controls.
- An adequate number of cells were evaluated (100/plate)
- Two replicates of each treatment were evaluated
- Dose selection was based on toxicity as measured by a reduction in the average % mitotic index

Results

An initial assay was conducted using concentrations between 17 mcg/ml and 2500 mcg/ml with and without activation. Because precipitates formed and hemolysis occurred at the higher concentrations used, and excessive toxicity was observed at all concentrations, the assay was repeated using significantly lower concentrations.

A second experiment (Trial No. B2) used concentrations between 0.375 mcg/ml and 20.0 mcg/ml. Data from cultures without excessive toxicity is tabulated below.

Table 42 Results from In Vitro Chromosomal Aberration Assay (Definitive Assay)

Treatment	Mean Mitotic Index	% Mitotic Index Reduction	Aberrations/cell (average of duplicate treatments)
Without S9 (3h treatment)			
Negative control	11.3	--	0.5
Vehicle control	10.8	0	1.0
Positive control (MMC)	--	--	47
0.375 mcg/ml	11.5	0	0.5
0.750 mcg/ml	10.3	5	0.5
1.50 mcg/ml	7.9	27	0.5
3.00 mcg/ml	5.3	51	0.5
With S9 (3h treatment)			
Negative control	10.3	--	2.0
Vehicle control	11.3	0	0.5
Positive control (MMC)	--		35
0.375 mcg/ml	11.1	2	0.5
0.750 mcg/ml	8.7	23	1.0
1.50 mcg/ml	6.7	41	1.0
3.00 mcg/ml	5.3	53	0.5

In cultures treated with ≥3.00 mcg/ml with and without activation, ≥50% of cells mitotic index was reduced as compared to the vehicle control. Therefore, chromosomal aberrations were analyzed from cultures treated with 0.375, 0.750, 1.50 and 3.00 mcg/ml and not at higher concentrations. There were no increases in chromosomal aberrations, polyploidy, or endo-reduplication.

Based on the results from initial studies, a confirmatory study (without activation only) was conducted. Treatment duration was 22 hours. Again, there were no increases in chromosomal aberrations, polyploidy, or endo-reduplication.

Table 43 Results from In Vitro Chromosomal Aberration Assay (Confirmatory Assay)

Treatment	Mean Mitotic Index	% Mitotic Index Reduction	Aberrations/cell (average of duplicate treatments)
Without S9 (22h treatment)			
Negative control	11.0	--	0.5
Vehicle control	11.3	0	0.0
Positive control (MMC)	--	--	47.0
0.188 mcg/ml	11.6	0	0.5
0.375 mcg/ml	8.8	22	0.5
0.750 mcg/ml	7.5	34	1.0

1.50 mcg/ml	5.7	50	--
3.00 mcg/ml	5.1	55	0.0

Conclusions

The test article demonstrated excessive toxicity (average mitotic index reduction >50%) at concentrations greater than 3.0 mcg/ml. There were no increases in chromosomal aberrations, polyploidy or endo-reduplication at concentrations between 0.375 and 3.0 mcg/ml, with or without metabolic activation. A longer duration treatment (22 hours) without activation did not result in an increase in chromosomal abnormalities.

7.3 In vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: In vivo Mouse Bone Marrow Micronucleus Assay

Study no:	6843-154
Study report location:	NDA Section 4.2.3.3.2.1 (EDR)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 6, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AP24534 HCl salt, PAK-009-173, 91.3%

Key Study Findings

- AP24534 (ponatinib) did not induce statistically significant increases in bone marrow micronucleus formation.

Methods

Doses in definitive study:	125, 500, 2000 (reduced to 1000) mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	20 ml/kg
Formulation/Vehicle:	25 mM Citrate buffer, pH 2.75
Species/Strain:	CD-1® (ICR)BR mice
Number/Sex/Group:	5 males/group
	The decision to use only males in the micronucleus assay was based on results from the dose range finding study where no relevant differences in toxicity were noted between the sexes.
Basis of dose selection:	A dose range finding study identified 2000 mg/kg as the upper dose limit, but this dose in the definitive study resulted in excessive toxicity and mortality. An additional group of 5 males was dosed at 1000 mg/kg and used for a 48 hour time point.
Negative control:	vehicle
Positive control:	80 mg/kg cyclophosphamide

Tabulated study design from report:

Target Dose Level (mg/kg)	Stock Concentration (mg/mL)	Dosing Volume (mL/kg)	Route of Administration	Animals/Harvest Timepoint 24 Hour Male	48 Hour Male
Positive Control, 80	8	10	Oral Gavage	5	-
Vehicle Control, 0	0	20	Oral Gavage	5	5
125	6.25	20	Oral Gavage	5	-
500	25	20	Oral Gavage	5	-
2000	100	20	Oral Gavage	5	-
1000	50	20	Oral Gavage	-	5[a]

Vehicle Control = Sodium Citrate, Positive Control = Cyclophosphamide

[a] Due to toxicity seen at the 2000 mg/kg level, 48 hr harvest timepoint, 5 extra animals were dosed at 1000 mg/kg as replacements..

Study Validity

- Bone marrow was extracted and at least 2000 polychromatic erythrocytes (PCEs) were analyzed for micronuclei.
- Cytotoxicity was assessed by scoring the number of PCEs and normochromatic erythrocytes (NCEs) in at least the first 500 total erythrocytes for each animal.
- The positive control demonstrated a statistically significant increase in micronuclei formation.

- The proportion of immature erythrocytes among total erythrocytes was not less than 20% of the control value.

Results

Analysis of the diluted dosing solutions demonstrated all solutions were within approximately 6% of the expected drug concentrations.

The dose-range finding study used a single dose of 2000 mg/kg administered once by oral gavage to 3 males and 3 females. Animals were observed 1 hour, 1 day and 2 days following dosing. One female was found dead 1 Day post-dosing. Clinical observations included hypoactivity in males and females on Day 1 and hunched posture and squinted eyes in 2/3 males on Days 1 and 2. Results tabulated in the report indicate that there may be a gender difference in the toxicities observed but there are too few observations for making a clear determination.

For the micronucleus assay, mice treated with 2000 mg/kg had clinical signs of toxicity that included squinted eyes, hypoactivity, slight hypoactivity, hunched posture and/or irregular respiration. All the animals in the high dose group were found dead after 1 day of dosing. As a result, an additional group of 5 animals were dosed at 1000 mg/kg and used for the 48 hour harvest timepoint. Animals in this group had clinical signs of toxicity that included squinted eyes, hunched posture and/or slight hypoactivity.

AP24534 did not induce statistically significant increases in micronucleated PCEs at any test article dose examined (125, 500, 2000 and 1000 mg/kg). Results from both the positive and negative controls trended as expected.

Regarding toxicity evidenced by the PCE:NCE ratio, doses of 2000 and 1000 mg/kg resulted in decreased PCE:NCE ratio when compared to the 125 and 500 mg/kg dose. However, when compared to the vehicle control, the PCE:NCE ratio was higher for all but the 2000 mg/kg dose. The significance of this finding is unknown.

Table 44 Micronucleus Assay Summary Table

(Excerpted from Applicant's Submission)

Assay No.: 29042-0-455OECD
Test Article: AP24534
Initiation of Dosing: 21 February 2007

Treatment	Dose	Harvest Time	% Micronucleated PCEs Mean of 2000 per Animal ± S.E. Males	Ratio PCE:NCE Mean ± S.E. Males
Controls				
Vehicle	Citrate Buffer 20 mL/kg	24 hr	0.09 ± 0.03	0.48 ± 0.04
		48 hr	0.05 ± 0.02	0.60 ± 0.09
Positive	CP 80 mg/kg	24 hr	0.94 ± 0.18*	0.48 ± 0.06
Test Article	125 mg/kg	24 hr	0.04 ± 0.03	0.78 ± 0.06*
	500 mg/kg	24 hr	0.03 ± 0.02	0.74 ± 0.08*
	2000 mg/kg	24 hr	0.14 ± 0.04	0.48 ± 0.04
	1000 mg/kg	48 hr	0.07 ± 0.03	0.68 ± 0.06

* Significantly greater than the corresponding vehicle control, $p \leq 0.05$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte
Citrate Buffer = 25mM Citrate Buffer pH 2.75

7.4 Other Genetic Toxicity Studies

No other genotoxicity assays were conducted.

8 Carcinogenicity

Carcinogenicity studies were not conducted.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

No conducted

9.2 Embryonic Fetal Development

Study title: Oral (Gavage) Embryo-Fetal Development Study of AP24534 in Rats

Study no.:	20009232
Study report location:	NDA Section 4.2.3.5.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 18, 2011
GLP compliance:	FDA, OECD. MHLW
QA statement:	Statement included and signed
Drug, lot #, and % purity:	AP24534, lot F10-02702, 92.9 % A salt correction factor of 1.08 % was used for dose formulation preparations.
Report Issues:	None

Key Study Findings

- AP24534 produced maternal toxicity at 3 mg/kg/day (18 mg/m^2/day) including one dam euthanized early on GD 19 and adverse clinical signs, and decreased body weight gain and food consumption.
- All surviving 3 mg/kg/day dams had resorptions, the number of live fetuses was significantly reduced and mean percent post-implantation loss was significantly increased compared to control.
- Mean fetal bodyweight, males and females, in the 3 mg/kg/day were all significantly reduced compared to control.
- Fetal gross external alterations of edema, abdominal distension, and short tail occurred at 3 mg/kg/day.
- Skeletal and soft tissue alterations and differences in ossification site averages occurred at 1 and 3 mg/kg/day.
- Maternal NOAEL defined at 1 mg/kg/day (6 mg/m^2) corresponded to a mean AP24534 C_{max} of 23.6 ng/mL and AUC_{0-t} of 314 ng*h/mL.
- No developmental effects were observed at 0.3 mg/kg/day.

Methods

Doses:	0, 0.3, 1.0, 3.0 mg/kg/day 0, 1.8, 6, 18 mg/m^2/day
Frequency of dosing:	Once daily

Dose volume:	5 mL/kg
Route of administration:	Gavage
Formulation/Vehicle:	25 mM Citrate Buffer, pH 2.75
Species/Strain:	female Crl:CD(SD) Sprague Dawley rats
Number/Sex/Group:	25 female rats/group
Satellite groups:	3 additional female rats in Group 1 6 additional female rats in Groups 2 through 4 for toxicokinetic analysis divided in 2 subgroups of 3 females each
Study design:	Female rats mated with male breeder rats, one male rat per female rat. GD 0 defined as the day that spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug observed in situ. Twenty five mated females per group were dosed GD 7-17 and euthanized on GD 21. Toxicokinetic rats were dosed GD 7-17 and euthanized on GD18 following the collection of blood samples
Deviation (s) from study protocol:	Documented three deviations none considered to have impacted the overall integrity of the study or interpretation of study results.

Results

Mortality

One damn at the highest dose level (3 mg/kg/day) was euthanized on GD 19 due to AP24534-related adverse clinical sings that included decreased motor activity, ataxia, hunched posture, bradypnea, ptosis, pale ears, thin body condition, fecal-, urine- and blood-stained fur, chromorhinorrhea, and mild to moderate dehydration.

Clinical Signs

Clinical observations for general appearance and postdose observations were conducted daily during the dosing and postdose period. AP24534-related adverse clinical sings in the highest dose level (3 mg/kg/day) included mild to moderate dehydration, soft or liquid feces, urine-stained abdominal fur, red perivaginal substance and pale ears. Other adverse clinical signs were previously described for the unscheduled euthanasia in the 3 mg/kg/day females. The onset of these clinical signs occurred on GD 14 through 16.

Body Weight and Body Weight Changes

Body weight measurements were conducted daily during the dosing period. Mean body weight of 3 mg/kg/day females was significantly lower than control females starting on GD 14 through GD 21, Figure 12. Mean body weight gain was significantly lower compared to control during the dosing period GD 7 to18, the post-dose period GD 18 to

21, and during the entire gestation period following dose initiation GD 7 to 21, Table 45. The reviewer considered the reductions in mean body weight and mean body weight gain at 3 mg/kg/day AP24534-related and adverse based on the magnitude and duration of the change and it corresponded with significantly lower mean absolute food consumption.

Mean body weight gain of 1 mg/kg/day females was slightly lower but not significantly different compared to control during the dosing period GD 7 to18 and the post-dose period GD 18 to 21 and corresponded with significantly lower mean absolute food consumption during the postdose period GD 18 to 21. The mean body weight gain was significantly lower during the entire gestation period following dose initiation GD 7 to 21, Table 45. The reduction in mean body weight gain at 1 mg/kg/day was AP24534-related but not adverse based on the low magnitude and short duration of the change. Gravid uterine weight measurements were not recorded nor specified in the protocol.

Figure 12 Mean Body Weight of Pregnant Rats Dosed with AP24534 during Gestation Days 7 to 18

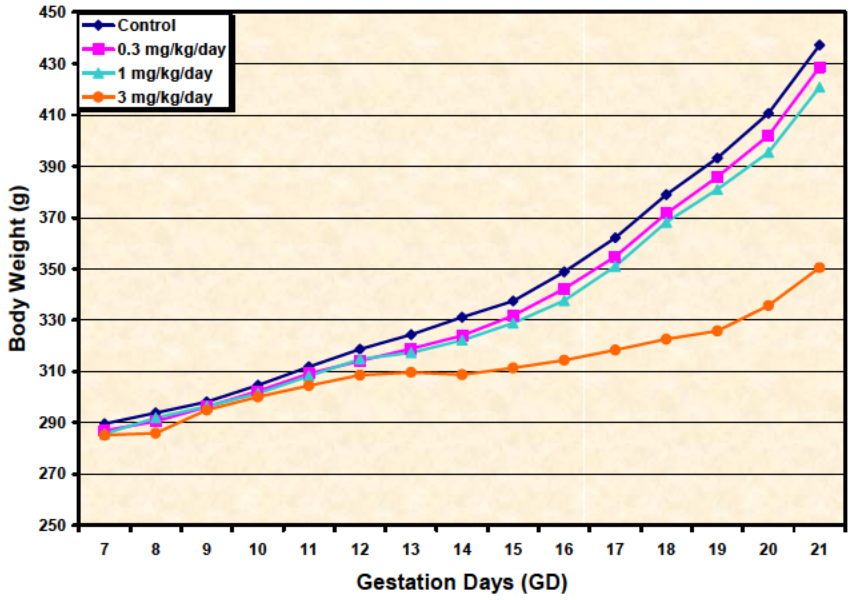


Table 45 Summary of Maternal Body Weight Changes of Pregnant Rats Dosed with AP24534 Compared to Control

Percent Changes in Maternal Body Weight Change Compared to Control Values				
Dose Level (mg/kg/day)	Control[a] (g)	0.3	1	3
GD 7 - 18	+89.2	94.8	92.5	41.9**
GD 18 - 21	+58.9	96.6	89.3	40.1**
GD 7 - 21	+147.3	95.9	91.8*	44.3**

a Mean body weight change for the Control Group
* Significantly different from the vehicle control group value ($p \leq 0.05$)
** Significantly different from the vehicle control group value ($p \leq 0.01$)

Food Consumption

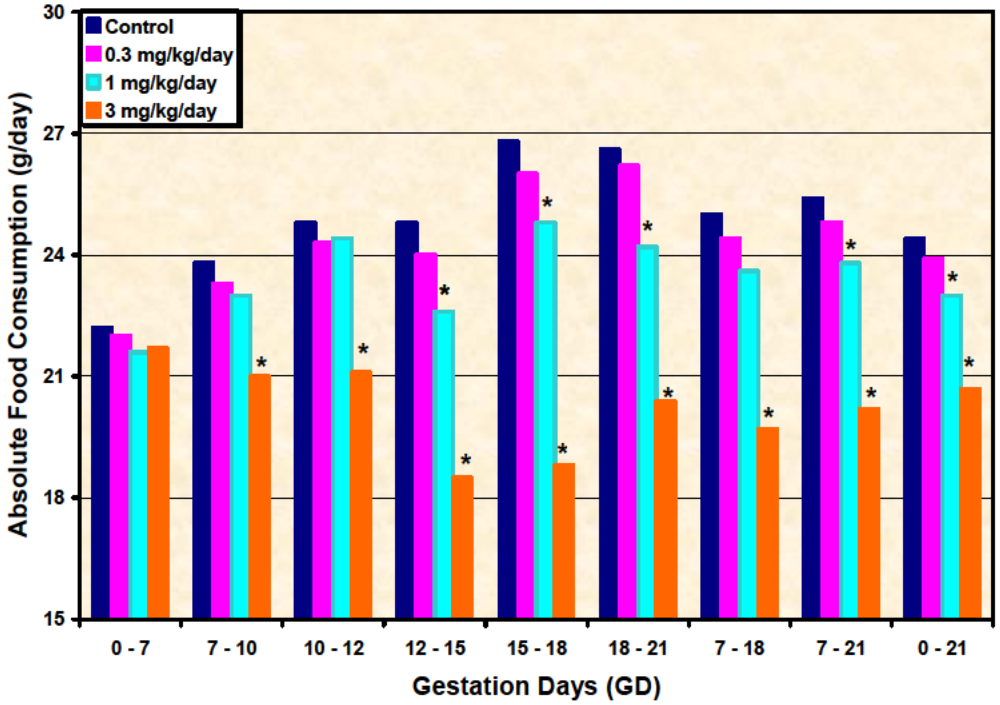
Food consumption measurements were conducted daily during the dosing period. Absolute food consumption (g/day) in the 3 mg/kg/day females was significantly lower during all measured periods, Table 46, and corresponded with significantly reductions in mean body weight and mean body weight gain. Absolute food consumption (g/day) in the 1 mg/kg/day females was significantly lower during the postdose period GD 18 to 21, Table 46. The lower absolute food consumption in the highest dose level group was AP24534-related and adverse based on the magnitude and duration of the change.

Table 46 Summary of Food Consumption of Pregnant Rats Dosed with AP24534 Compared to Control

Percent Changes in Absolute Food Consumption (g/day) Compared to Control Values			
Dose Level (mg/kg/day)	0.3	1	3
GD 7 - 18	97.6	94.4	78.8**
GD 18 - 21	98.5	91.0*	76.7**
GD 7 - 21	97.6	93.7*	79.5**

* Significantly different from the vehicle control group value ($p \leq 0.05$)
** Significantly different from the vehicle control group value ($p \leq 0.01$)

Figure 13 Absolute Food Consumption of Pregnant Rats Dosed with AP24534



Toxicokinetics

Blood collection time points for toxicokinetics were at 0 and 8 h from 3 females in the control group and at 0, 2, 4, 8, 16 and 24 hours from 3 females alternating subgroups in groups 2 through 4. All satellite female rats were pregnant except one female in the 0.3 mg/kg/day group.

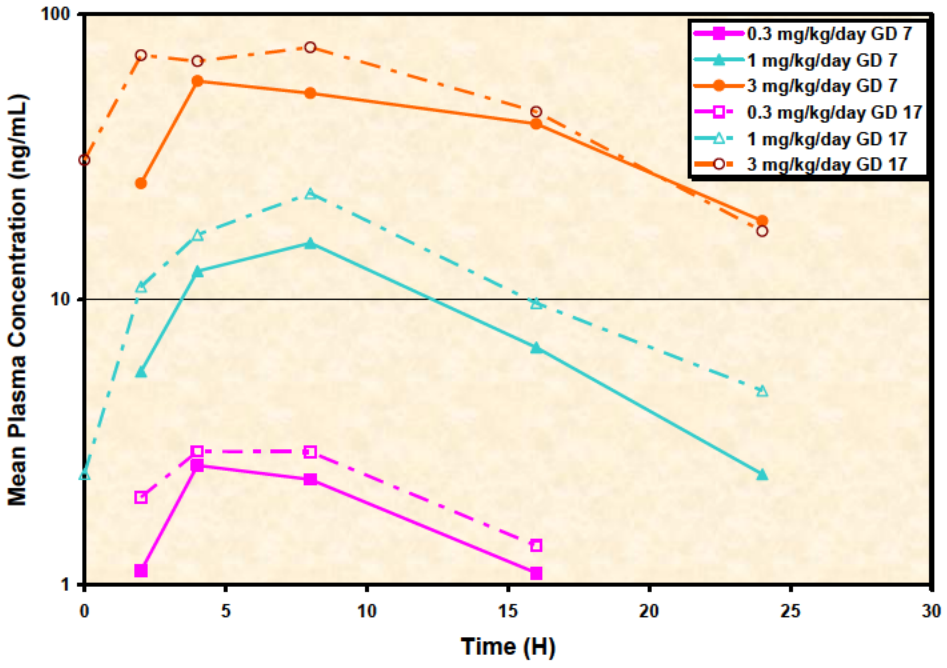
Table 47 Summary of Toxicokinetic Parameters of AP24534 in Plasma of Pregnant Rats

(*Excerpted from Applicant's submission*)

Occasion	Group No.	Dose Level (mg/kg/day)	Tmax (h)	Cmax (ng/mL)	AUC(0-t) (ng•h/mL)	T1/2 (h)
GD 7	2	0.3	4	2.62	28.5	RNR
	3	1	8	15.8	208	5.94
	4	3	4	58.5	952	RNR
GD 17	2	0.3	4	2.94	35.9	RNR
	3	1	8	23.6	314	6.97
	4	3	8	76.7	1275	7.48

RNR Result not reported because the AUC(0-inf) was extrapolated by more than 20% or Rsq was < 0.800.

Figure 14 Mean Plasma Concentration of AP24534 in Pregnant Rats following Oral Administration on GD 7 through GD 17



Exposure to AP24534, as measured by C_{max} and AUC_{0-t}, generally increased more than proportional to the dose given in the dose range of 0.3 to 3 mg/kg/day, Table 47. The C_{max} occurred between 4 and 8 hours, Figure 14, and the estimated half-life ranged between 5.94 and 7.48 hours. Half-life could not be estimated for the metabolite due to high variability of the data. Accumulation of AP24534 or its metabolite was minimal in pregnant females after daily administration of AP24534 from GD 7 through 17 at 0.3, 1, and 3 mg/kg/day, Figure 14.

Quantifiable conversion of AP24534 to its metabolite, AP24567, occurred only at the 3 mg/kg/day dose level, where plasma concentrations of AP24567 peaked at 8 and 2 hours postdose on GD 7 and 17, respectively. Exposure to metabolite AP24567 at 3 mg/kg/day was less than 3% on both GD 7 and 17, Table 48.

Table 48 Summary of Toxicokinetic Parameters of Metabolite AP24567 in Plasma of Pregnant Rats Following Oral Administration of AP24534

(*Excerpted from Applicant's submission*)

Occasion	Group No.	Dose Level (mg/kg/day)	Tmax (h)	Cmax (ng/mL)	AUC(0-t) (ng•h/mL)	T1/2 (h)
GD 7	4	3	8	1.60	18.6	NE
GD 17	4	3	2	2.22	24.9	RNR

NE Parameter not estimable from data set.
RNR Result not reported because the AUC(0-inf) was extrapolated by more than 20% or Rsq was < 0.800.

Dose Formulation Analysis

Mean AP24534 concentrations for all dose formulations prepared in 25 mM citrate buffer, pH 2.75 and measured using a validated HPLC-UV methods were within acceptable limits (± 10% of the nominal concentration), Table 49.

Table 49 Dose Formulation Analysis of AP24534

(*Excerpted from Applicant's submission*)

Sample (Preparation Date)	Group	Nominal Concentration (mg/mL)	Mean Measured Concentration (mg/mL)	Mean Bias (%)
Start of Study	1	0	< LOD*	NA
(01 Apr 2011)	2	0.06	0.05815	-3.1
	3	0.2	0.2072	3.6
	4	0.6	0.6216	3.6
End of Study	1	0	ND	NA
(14 Apr 2011)	2	0.06	0.06052	0.9
	3	0.2	0.2043	2.2
	4	0.6	0.6162	2.7

ND None detected.
NA Not applicable.
LOD Limit of Detection.
*Sample replicate 2 of 2 is ND

Necropsy

Unremarkable

Laparohysterectomy Evaluations

The reproductive tract was dissected from the abdominal cavity, opened, and contents examined. The number and distribution of corpora lutea, implantation sites, placenta (size, color or shape), live and dead fetuses and early and late resorptions were evaluated, and fetal weight was measured. AP24534 at 3 mg/kg/day had effects on the number of live fetuses, early, late and total resorptions. All surviving dams at 3 mg/kg/day presented resorptions. The calculated mean of these parameters and the percentage of post-implantation loss were adverse and significantly different compared to control with one dead fetus in the 3 mg/kg/day group, Table 50. As a result, the litter size and the mean number of live fetuses were adverse and significantly reduced in the 3 mg/kg/day compared to control, Table 51.

Table 50 Summary of Laparohysterectomy Evaluations

Dose (mg/kg/day)	0	0.3	1	3
Number of females tested	25	25	25	25
Number of females pregnant	23	25	24	24
Unscheduled euthanasia	0	0	0	1
Early delivery	1	0	0	0
Number of surviving pregnant females	22	25	24	23
Dams with any resorptions N (%)	10 (45.4)	12 (48.0)	13 (54.2)	23 (100.0)**
Corpora lutea Mean number per liter	325 14.8	376 15.0	364 15.2	349 15.2
Implantations Mean number per liter	316 14.4	365 14.6	346 14.4	330 14.3
Pre-implantation loss Mean Percent pre-implantation loss	9 2.6	11 2.9	18 4.9	19 5.4
Live fetuses Dead fetuses	304 0	346 0	326 0	169** 1
Post-implantation loss Number Early resorptions Mean Late resorptions Mean Mean Percent post-implantation loss	 11 0.5 1 0.0 3.8	 18 0.7 0 0.0 5.2	 20 0.8 0 0.0 5.8	 146 6.4** 14 0.6** 48.8**

Percent Pre-implantation loss = [(# corpora lutea - # implantations) / # corpora lutea] x 100
Percent Post-implantation loss = [(# implantations - # live fetuses) / # implantations] x 100
* Significantly different $p \leq 0.05$
** Significantly different $p \leq 0.01$

Table 51 Summary of Litter Observations

Dose (mg/kg/day)	0	0.3	1	3
Number of females tested	25	25	25	25
Litters with one or more live fetuses	22	25	24	23
Implantations	316	365	346	330
Mean number per litter	14.4	14.6	14.4	14.3
Live fetuses	304	346	326	169**
Mean number per litter	13.8	13.8	13.6	7.3**
Sex distribution				
Males	147	176	171	79
Females	157	170	155	90
Mean Percent male fetuses per litter	48.4	50.9	52.5	46.8
Mean fetal body weight (g) per litter	5.6	5.6	5.6	4.7**
Male fetuses	5.7	5.8	5.8	4.9**
Female fetuses	5.5	5.5	5.4	4.5**

Mean fetal bodyweight, and mean male and female fetuses body weight in the highest dose level (3 mg/kg/day) were significantly reduced compared to control, Table 51. The effects on fetal body weight were adverse because other signals of developmental toxicity at the 3 mg/kg/day.

Fetal Evaluations for Alterations

Fetuses were examined for external, visceral, and skeletal abnormalities. Fetal alterations were defined in the report as:

1. Malformations, irreversible changes that occur at low incidences in this species and strain
2. Variations, common findings in this species and strain and reversible delays or accelerations in development.

Litter averages were calculated for specific fetal ossification sites as part of the evaluation of the degree of fetal ossification.

All other fetal alterations were considered unrelated to treatment with AP24534 because:

- the litter incidence, the more relevant parameter, and/or the fetal incidence for the alteration were generally comparable with the respective vehicle control group values and historical control data for the testing facility
- the litter incidence, the more relevant parameter, did not demonstrate a dose-dependent pattern of effect, and/or,
- the fetal and/or litter incidences were within the range of historical control data for the testing facility

AP24534-related fetal findings at 1 and 3 mg/kg/day reflected increases or significant increases in fetal gross external malformations, with associated skeletal findings and alterations in soft tissue morphology.

Fetal Gross External Alterations

Table 52 AP24534-Related Fetal Gross External Alterations

Dose (mg/kg)		0	0.3	1	3
Total number of litters examined		22	25	24	23
Total number of fetuses examined		304	346	326	169
Body: Edema	Litter incidence (%)	-	-	-	12 (52.2)**
	Fetal incidence (%)	-	-	-	21 (12.4)**
Body: Abdominal Distention	Litter incidence (%)	-	-	-	1 (4.3)
	Fetal incidence (%)	-	-	-	1 (0.6)
Tail: Short	Litter incidence (%)	-	-	-	6 (26.1)**
	Fetal incidence (%)	-	-	-	9 (5.3)**

** Significantly different $p \leq 0.01$

Fetal Soft Tissue Alterations

Table 53 summarizes AP24534-related soft tissue malformations and variations as described in the report. An interventricular septal defect malformation was observed in one fetus at 1 mg/kg/day and five fetuses from four different litters at 3 mg/kg/day. The fetus at 1 mg/kg/day also had head exencephaly, fore and/or limbs flexed and/or rotated. Vessels and the urogenital system appeared as the main targets for AP24534-related soft tissue alterations.

Table 53 AP24534-Related Soft Tissue Alterations (M, malformations, V, variations)

Dose (mg/kg)		0	0.3	1	3
Total number of litters examined		22	25	24	23
Total number of fetuses examined		148	164	157	76
M- Heart: Interventricular septal defect	Litter incidence (%)	-	-	1 (4.2)	4 (22.2)**
	Fetal incidence (%)	-	-	1 (0.6)	5 (6.6)**
M- Vessels: Interrupted aortic arch	Litter incidence (%)	-	-	-	6 (33.3)**
	Fetal incidence (%)	-	-	-	6 (7.9)**
V- Vessels: Urinary artery descends left of urinary bladder	Litter incidence (%)	-	-	-	2 (11.1)**
	Fetal incidence (%)	-	-	-	3 (3.9)**
V- Vessels: Left subclavian artery arises from pulmonary artery	Litter incidence (%)	-	-	-	4 (22.2)**
	Fetal incidence (%)	-	-	-	4 (5.3)**
V- Vessels: Right subclavian passes dorsal to the trachea and esophagus	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	2 (2.6)**
V- Vessels: Right subclavian passes left of the left subclavian	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	2 (2.6)**

Dose (mg/kg)		0	0.3	1	3
Total number of litters examined		22	25	24	23
Total number of fetuses examined		148	164	157	76
M- Vessels: arise in incorrect order	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	1 (1.3)
M- Vessels: Persistent truncus arteriosus	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	1 (1.3)
M- Vessels: Aorta descends to the right	Litter incidence (%)	-	-	-	2 (11.1)**
	Fetal incidence (%)	-	-	-	2 (2.6)**
M- Vessels: Transposed	Litter incidence (%)	-	-	-	2 (11.1)**
	Fetal incidence (%)	-	-	-	2 (2.6)**
M- Vessels: Pulmonary artery descends to the right	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	1 (1.3)
M- Vessels: Ductus arteriosus patent	Litter incidence (%)	-	-	-	2 (11.1)**
	Fetal incidence (%)	-	-	-	2 (2.6)**
V- Vessels: Pulmonary artery passes dorsal to aorta	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	1 (1.3)
M- Vessels: Semi lunar valves absent	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	1 (1.3)
M- Lungs: Right lobe apical absent	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	1 (1.3)
V- Liver: White areas	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	1 (1.3)
M- Kidneys: absent	Litter incidence (%)	-	-	-	16 (88.9)**
	Fetal incidence (%)	-	-	-	34 (44.7)**
M- Kidneys: Small	Litter incidence (%)	-	-	-	11 (61.1)**
	Fetal incidence (%)	-	-	-	24 (31.6)**
V- Kidneys: High set	Litter incidence (%)	-	-	-	2 (11.1)**
	Fetal incidence (%)	-	-	-	3 (3.9)**
M- Genitalia: Absent	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	1 (1.3)
M- Genitalia: Undescended testes	Litter incidence (%)	-	-	-	3 (16.7)**
	Fetal incidence (%)	-	-	-	5 (6.63)**
M- Uterus: Absent	Litter incidence (%)	-	-	-	4 (22.2)**
	Fetal incidence (%)	-	-	-	5 (6.6)**
M- Uterus: Reduced to a ligament	Litter incidence (%)	-	-	-	6 (33.3)**
	Fetal incidence (%)	-	-	-	8 (10.5)**
M- Ovary: Absent	Litter incidence (%)	-	-	-	4 (22.2)**
	Fetal incidence (%)	-	-	-	5 (6.6)**
M- Ureter (s) Absent	Litter incidence (%)	-	-	-	15 (83.3)**
	Fetal incidence (%)	-	-	-	33 (43.4)**
M- Ureters: Constricted	Litter incidence (%)	-	-	-	1 (5.6)
	Fetal incidence (%)	-	-	-	1 (1.3)
M- Ureter: Elongated	Litter incidence (%)	-	-	-	2 (11.1)**
	Fetal incidence (%)	-	-	-	3 (3.9)**
M- Ureter: Marked dilation	Litter incidence (%)	-	-	-	6 (33.3)**
	Fetal incidence (%)	-	-	-	9 (11.8)**
M- Ureter: Extreme dilation	Litter incidence (%)	-	-	-	2 (11.1)**
	Fetal incidence (%)	-	-	-	2 (2.6)**

** Significantly different $p \leq 0.01$

Fetal Skeletal Alterations

Table 54 summarizes AP24534-related skeletal malformations and variations as described in the report. Most of the alterations in the control and 0.3 were variations at a low incidence. The 1 and 3 mg/kg/day had significant increases in the litter and/or fetal incidence of skeletal alterations and fetal ossification sites compared to control, Table 55.

Table 54 AP24534-Related Skeletal Alterations (M, malformations, V, variations)

Dose (mg/kg)		0	0.3	1	3
Total number of litters examined		22	25	24	23
Total number of fetuses examined		156	182	170	93
V- Cervical vertebrae: Arch, 6th cervical vertebra has the appearance of the 7th	Litter incidence (%)	-	-	6 (25.0)	15 (65.2)**
	Fetal incidence (%)	-	-	7 (4.1)	21 (22.6)**
V- Cervical vertebrae: Arch incompletely ossified	Litter incidence (%)	1 (4.5)	2 (8.0)	4 (16.7)	14 (60.9)**
	Fetal incidence (%)	2 (1.3)	3 (1.6)	4 (2.4)	25 (26.9)**
V- Cervical vertebrae: Arch irregular shaped	Litter incidence (%)	-	-	-	2 (8.7)
	Fetal incidence (%)	-	-	-	2 (2.2)**
V- Thoracic vertebrae: Centrum bifid	Litter incidence (%)	-	1 (4.0)	2 (8.3)	12 (52.2)**
	Fetal incidence (%)	-	1 (0.5)	2 (1.2)	17 (18.3)**
V- Thoracic vertebrae: Centrum unilateral ossification	Litter incidence (%)	-	-	-	3 (13.0)**
	Fetal incidence (%)	-	-	-	3 (3.2)**
M- Thoracic vertebrae: Arches fused	Litter incidence (%)	-	-	-	2 (8.7)
	Fetal incidence (%)	-	-	-	2 (2.2)**
M- Thoracic vertebrae: Hemivertebrae	Litter incidence (%)	-	-	-	2 (8.7)
	Fetal incidence (%)	-	-	-	2 (2.2)**
M- Thoracic vertebrae: 15 vertebrae present	Litter incidence (%)	-	-	-	1 (4.3)
	Fetal incidence (%)	-	-	-	1 (1.1)
V- Lumbar vertebrae : Centrum bifid	Litter incidence (%)	-	-	-	4 (17.4)
	Fetal incidence (%)	-	-	-	4 (4.3)
V- Lumbar vertebrae: Arch incompletely ossified	Litter incidence (%)	-	1 (4.0)	1 (4.2)	-
	Fetal incidence (%)	-	1 (0.5)	1 (0.6)	-
M- Lumbar vertebrae: Hemivertebrae	Litter incidence (%)	-	-	-	1 (4.3)
	Fetal incidence (%)	-	-	-	1 (1.1)
M- Lumbar vertebrae 6 present	Litter incidence (%)	-	-	-	1 (4.3)
	Fetal incidence (%)	-	-	-	1 (1.1)
M- Sacral vertebrae: Arch small	Litter incidence (%)	-	-	-	1 (4.3)
	Fetal incidence (%)	-	-	-	1 (1.1)
M- Caudal vertebrae: 4 present	Litter incidence (%)	-	-	-	1 (4.3)
	Fetal incidence (%)	-	-	-	2 (8.7)

Dose (mg/kg)		0	0.3	1	3
Total number of litters examined		22	25	24	23
Total number of fetuses examined		156	182	170	93
M- Caudal vertebrae: 3 present	Litter incidence (%)	-	-	-	2 (2.2)**
	Fetal incidence (%)	-	-	-	1 (1.3)
M- Ribs: Fused	Litter incidence (%)	-	-	-	11 (47.8)**
	Fetal incidence (%)	-	-	-	13 (14.0)**
M- Ribs: Short	Litter incidence (%)	-	1 (4.0)	-	8 (34.8)**
	Fetal incidence (%)	-	1 (0.5)	-	9 (9.7)**
V- Ribs: Proximate	Litter incidence (%)	-	-	-	12 (52.2)**
	Fetal incidence (%)	-	-	-	14 (15.0)**
V- Ribs: Wavy	Litter incidence (%)	1 (4.5)	-	-	6 (26.1)**
	Fetal incidence (%)	1 (0.6)	-	-	7 (7.5)**
V- Ribs: Irregular shape	Litter incidence (%)	-	-	-	3 (13.0)**
	Fetal incidence (%)	-	-	-	3 (3.2)**
V- Ribs: Bowed	Litter incidence (%)	-	-	-	2 (8.7)
	Fetal incidence (%)	-	-	-	2 (2.2)**
M- Manubrium: fused	Litter incidence (%)	-	-	-	19 (82.6)**
	Fetal incidence (%)	-	-	-	45 (48.4)**
M- Manubrium: Irregular shaped	Litter incidence (%)	-	-	-	6 (26.1)**
	Fetal incidence (%)	-	-	-	7 (7.5)**
M- Manubrium: Small	Litter incidence (%)	-	-	-	3 (13.0)**
	Fetal incidence (%)	-	-	-	4 (4.3)**
V- Sternal Centra: Incompletely ossified	Litter incidence (%)	1 (4.5)	-	1 (4.2)	4 (17.4)
	Fetal incidence (%)	1 (0.6)	-	1 (0.6)	4 (4.3)**
V- Sternal Centra: Asymmetric	Litter incidence (%)	-	-	3 (12.5)	6 (26.1)**
	Fetal incidence (%)	-	-	4 (2.4)	11 (11.8)**
V- Sternal Centra: Fused	Litter incidence (%)	-	-	-	13 (56.5)**
	Fetal incidence (%)	-	-	-	29 (31.2)**
M- Sternal Centra: Duplicated	Litter incidence (%)	-	-	-	1 (4.3)
	Fetal incidence (%)	-	-	-	1 (1.1)
M- Sternal Centra: Irregular Shaped	Litter incidence (%)	-	-	-	3 (13.0)**
	Fetal incidence (%)	-	-	-	4 (4.3)**
M- Xiphoid: Duplicated	Litter incidence (%)	-	-	-	1 (4.3)
	Fetal incidence (%)	-	-	-	1 (1.1)
V- Pelvis: Pubis incompletely ossified	Litter incidence (%)	-	1 (4.0)	3 (12.5)	5 (21.7)
	Fetal incidence (%)	-	1 (0.5)	5 (2.9)*	6 (6.4)**

* Significantly different $p \leq 0.05$
** Significantly different $p \leq 0.01$

Table 55 AP24534-Related Fetal Ossification Sites Observations

Dose (mg/kg)		0	0.3	1	3
Total number of litters examined		22	25	24	23
Total number of fetuses examined		156	182	170	93
Vertebrae Thoracic Lumbar Caudal	Mean Ossification sites per fetuses per litter	 13.04 5.94 7.4	 13.07 5.92 7.32	 13.10 5.89 6.62**	 13.38** 5.62** 5.30**
Ribs (Pairs)	Mean Ossification sites per fetuses per litter	13.04	13.06	13.08	13.28**

Dose (mg/kg)		0	0.3	1	3
Total number of litters examined		22	25	24	23
Total number of fetuses examined		156	182	170	93
Sternum Sternal Centers	Mean Ossification sites per fetuses per litter	3.98	4.00	4.00	3.82**
Forelimb Phalanges	Mean Ossification sites per fetuses per litter	7.92	7.96	7.62	6.79**
Hindlimb Metatarsals Phalanges	Mean Ossification sites per fetuses per litter	 4.95 6.33	 4.93 6.38	 4.9 6.04	 4.41** 5.00**

9.3 Prenatal and Postnatal Development

Not conducted.

10 Special Toxicology Studies

Phototoxicity study results are provided in Report 20011779 titled "Single-Dosage Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of AP24534 on Eyes and Skin in Pigmented Rats." This study was not reviewed in detail but the following summary information is included here for completeness:

- The study concluded there was no evidence of cutaneous phototoxicity after a single oral administration of 0, 2.5, 5 or 10 mg/kg AP24534 followed by a single exposure of solar-simulated UV radiation to female CRL:LE (Long-Evans) rats. There was evidence of a low-level ocular phototoxicity at doses of 5 and 10 mg/kg. Changes observed were diffuse superficial corneal edema observed in 2/5 rats (3 eyes) and a corneal scar in one eye in a third rat in the 10 mg/kg group. There was also lenticular epithelial hyperplasia in the 5 and 10 mg/kg group above background. There was also a dose-dependent reduction in body weight that was not considered adverse.

11 Integrated Summary and Safety Evaluation

The proposed indication for ponatinib is CML or Ph+ ALL that is resistant or intolerant to prior tyrosine kinase inhibitor therapy. Results from studies using recombinant proteins, cell-based assays or mouse tumor models support the conclusion that ponatinib has activity towards the BCR-ABL T315I mutation and may provide a novel treatment option for this patient population.

However, ponatinib can inhibit many other intracellular and receptor tyrosine kinases in vitro. Investigation of a panel of >200 kinases using a radiometric phosphor-transfer method demonstrated ponatinib inhibited VEGFR, FGFR, PDGFR, EPH and SRC family members with IC50 concentrations below 20 nM, which was similar to the IC50s for

native and T315I BCR-ABL. It is possible that the pleiotropic kinase inhibitory action of ponatinib has a causative role in the serious toxicities that were observed during clinical investigation of ponatinib, including myocardial infarctions and strokes.

Results from animal studies that may be reflective of the clinical toxicities observed include:

- Physical examination findings in the 28-Day monkey study showed systolic heart murmurs in one 1 mg/kg/day male (low Grade I/VI), one 2.5 mg/kg/day female (Grade III/VI) and two 5 mg/kg/day monkeys (male: Grade II/VI; female: Grade I/VI). No murmurs were noted pre-study in monkeys with these findings or in monkeys during the 6-month toxicology study. These findings, plus the systolic murmurs seen in the single dose monkey study and evidence of a possible dose-dependent effect on necrosis of myocardial cells in the 6-month monkey study may be relevant to the clinically observed myocardial toxicities. Arterial thromboembolic events and hypertension are recognized clinical side-effects of VEGF inhibitors (e.g., bevacizumab, ziv-aflibercept).
- Pancreatic alterations were observed in the 28-Day monkey study in groups administered doses of 2.5 and 5 mg/kg/day (acinar cell necrosis/regeneration, chronic inflammation, edema, fibrosis and/or acinar atropy) and lipase was elevated in two males from the 5 mg/kg/day group. These were the only pancreatic changes observed in the toxicology studies and their relevance to clinically observed cases of pancreatitis is unknown.
- Elevated ALT and AST values occurred in treated monkeys at the end of the 6-month dosing period at all doses that ranged from 2- to 4.5-fold in ALT and 2- to 9.2-fold in AST, relative to Day -6 values. There were no microscopic correlates observed in the affected monkeys and elevations in transaminases were not present during the recovery period.

Ponatinib exhibited a similar nonclinical toxicological profile as other TKIs and included findings affecting the hematopoietic and lymphoid systems, liver, thyroid gland, skin, and the testis and ovaries.

Table 56 Summary of Major Findings and AUC Values for the Repeat Dose Toxicology Studies using AP24534

Species	Dose mg/kg/day (mg/m^2/day)	AUC ng*h/mL	Major Findings
Human	45 mg/day	1296	Arterial Occlusive or thromboembolic events (AOTE) Any Grade 23%; SAE 17%
Rats 28 Days	1.5 (9)[1]	M - 1260 F - 704	Mortality 2/23. ↓ BW, ALP, T3. ↑ AST, LYM, EOS.
	3 (18)	M - 1920 F - 1580	Mortality 6/23. ↓ BW, LYM, BSO, ALP, T3, ALP, liver/spleen wt. ↑ NEU, MNO, EOS, RTC, ALT, AST.

Species	Dose mg/kg/day (mg/m^2/day)	AUC ng*h/mL	Major Findings
	6 (36)	M - 2970 F - 2510	Mortality 12/23. ↓ BW, AST, BUN, TRIG, liver/spleen/prostate/ lungs wt. ↓↑ LYM, WBC, NEU. ↑ MNO, EOS. Bone marrow myeloid hyperplasia, thymus necrosis, variable changes in the stomach.
Rats 6 Months	0.25 (1.5)	M - 91.9 F - 23.9	No AP24534-related findings.
	0.75 (4.5)	M - 559 F - 374	Mortality 6/68. ↓ BW/FC, thymus wt. ↑ NEU, MNO, EOS, FIB, ovarian wt. Kidney/femoral bone nonreversible microscopic findings.
	2 (12)	M - 1924 F - 1375	Mortality 20/68. ↓ BW/FC, thymus wt. ↑ NEU, MNO, EOS, FIB, CRE, BUN, ovarian wt. Kidney/femoral bone nonreversible microscopic findings, lymphoid depletion thymus.
Monkeys 28 Days	1 (12)	M - 162 F - 115	Mild dry flaky skin/ erythema. Systolic heart murmurs (Grade I/VI) and harsh lung sounds. ↓ BW/FC. ↑ T4. Thyroid mononuclear infiltration.
	2.5 (30)	M - 1430 F: 1920	Moderate dry flaky skin/ erythema. Systolic heart murmurs (Grade III/VI) and harsh lung sounds. ↓ BW/FC, MCH. ↑ T4. Lymphoid depletion thymus/ spleen, pancreas chronic inflammation, thyroid necrosis and atrophy, granulomatous inflammation lungs.
	5 (60)	M: 5660 F - 8040	Mortality 3/10. Moderate-severe dry flaky skin/ erythema. Systolic heart murmurs (Grade I/VI and II/VI) and harsh lung sounds. ↓ BW/FC, HGB, HCT, MCV, MCH, T3, thymus/ovaries/uterus wt. ↑ Lipase, TSH, T4. Lymphoid depletion thymus/ spleen, pancreas chronic inflammation, thyroid necrosis and atrophy, granulomatous inflammation lungs, ovary atresia, testis germinal epithelium degeneration.
Monkeys 6 Months	0.25 (3)	M - 9.1 F - 8.9	AP24534-related ↑ALT, AST in few animals. Myocardial necrosis 2/8. Mild thymus lymphoid depletion.
	0.75 (9)	M - 138 F - 75.6	AP24534-related ↑ALT, AST in few animals. Myocardial necrosis 4/8. Mild thymus lymphoid depletion.
	2 (24)	M - 533 F - 681	AP24534-related ↑ALT, AST in few animals. Myocardial necrosis 1/8. Mild thymus lymphoid depletion.
Rats EFD	0.3 (1.8)	35.9	Few skeletal alterations.
	1 (6)	314	↓ BW/FC, ↑ litter and fetal incidence of heart with interventricular septal defect. Numerous skeletal alterations.
	3 (18)	1275	Mortality 1/25. ↓ BW/FC. 100% Dams with resorptions, ↓ fetal BW, # live fetuses. ↑ Litter and fetal incidence of edema, short tail. Numerous soft tissue and skeletal alterations.

There were no dose-dependent ponatinib-related effects noted on pulmonary function in conscious rats, neurologic effects in mice, or cardiac function in telemeterized dogs.

Effects observed on reproductive organs during the repeat-dose toxicology studies included degeneration of the epithelium of seminiferous tubules in the testes of both rats

and monkeys, and follicular atresia in monkey ovaries and related uterine epithelium atrophy (Table 57).

Table 57 Pathology Results for Reproductive Organs from Repeat-Dose Toxicology Studies

Species and Duration	Rat 28 day	Rat 6 month	Monkey 28 day	Monkey 6 month
Doses used (mg/kg/day)	1.5, 3, 6	0.25, 0.75, 2	1, 2.5, 5	0.25, 0.75, 2
Organ Weights				
– Ovary	↑ all doses[BW, brain]	↑ (0.75, 2 mg/kg)[BW,brain]	↓ all doses[BW,brain]	↓ 2 mg/kg[BW]
– Uterus		↑ (0.75, 2 mg/kg)[BW]	↓ all doses[BW,brain]	↓ 2 mg/kg
– Testis		↓ (2 mg/kg)[brain]	↓ all doses[BW,brain]	↑ all doses[BW]
– Prostate		↓ (2 mg/kg)	↓ all doses[BW]	
– Epididymis		↓ (2 mg/kg)[brain]	↓ all doses[BW,brain]	↑ all doses
– Seminal Vesicles		↓ (2 mg/kg)[brain]	↓ all doses[BW,brain]	
Histopathology Results	minimal degeneration of seminferous tubule epithelium in the testes of one 3 mg/kg male	minimal degeneration of seminiferous tubule epithelium in testes of two 0.75 mg/kg recovery males	follicular atresia in the ovary and atrophy of uterine endometrium in all 5 mg/kg females; Minimal/mild degeneration of germinal epithelium of the testis in three 5 mg/kg terminal group males and one recovery male	

[BW]: relative to body weight; [brain]: relative to brain weight

The embryo-fetal development study administered ponatinib orally to pregnant rats from at doses of 0, 0.3, 1, and 3 mg/kg. Soft tissue and skeletal alterations and differences

in the number of ossification site averages were observed at the mid and high doses used and maternal toxicity, including mortality, was observed at the high dose. Additional fetal toxicities observed at the high dose included increased post-implantation loss (early, late and total resorptions); reduced body weight; gross external alterations; multiple soft tissue and skeletal alterations, as well as differences in the number of ossification site averages.

Ponatinib was not genotoxic when evaluated in three separate assays (Ames assay for mutagenicity, *in vitro* chromosomal aberration assay or *in vivo* mouse micronucleus assay). Carcinogenicity studies were not completed based on the serious and life-threatening nature of the proposed clinical indication. Results from a phototoxicity study indicate that ponatinib does not cause dermal toxicity but ocular effects were observed at the mid and high doses used (5 and 10 mg/kg)

The recommended clinical dose of ponatinib is 45 mg/day (26.5 mg/m^2/day based on an average 60 kg person), which corresponds to estimated steady state C_{min} and C_{max} of 34 and 77 ng/mL and $AUC_{(0-\tau)}$ of 1296 ng*h/mL.

Oral doses of ponatinib in rats were tolerated with no adverse effects up to 1.5 mg/m^2/day when administered for 6 months and corresponded to a mean AP24534 AUC_{last} of 91.9 and 23.9 ng*h/mL and C_{max} of 5.7 and 4.3 ng/mL for males and females, respectively. Thus, exposure levels with no toxic effects in male and female rats, when as measured AUC, were approximately 13 times lower than the human exposure. Exposure levels with no toxic effects as measured by C_{max} was approximately 6 and 8 times lower than the C_{min} of human exposure. Ponatinib was toxic in rats at doses ≥ 9 mg/m^2/day.

Oral doses of ponatinib in monkeys were tolerated with no adverse effects up to 24 mg/m^2/day when administered for 6 months that corresponded to a mean AP24534 AUC_{0-24} of 533 and 681 ng*h/mL and C_{max} of 56.9 and 87.9 ng/mL for males and females, respectively. Thus, exposure in male monkeys as measured by AUC was approximately half of the human exposure in both male and female monkeys. Exposure as measured by C_{max} was within the range of C_{min} and C_{max} of human exposure while exposure in female monkeys was slightly higher than the C_{max} of human exposure. Ponatinib was toxic at oral doses ≥ 30 mg/m^2/day in monkeys when administered daily for 28 days.

Ponatinib was tolerated in pregnant rats up to 6 mg/m^2/day and corresponded to a AUC_{0-t} of 314 ng*h/mL and C_{max} of 23.6 ng/mL. Thus, exposure levels with no toxic effects in pregnant rats as measured by AUC were approximately 4 times lower than the human exposure. Ponatinib produced maternal toxicity at ≥ 18 mg/m^2/day (AUC_{0-t} 1275 ng*h/mL) and it was fetotoxic at doses ≥ 6 mg/m^2/day (AUC_{0-t} 314 ng*h/mL). Exposure levels with no toxic effects as measured by C_{max} were approximately 1.4 times lower than the C_{min} of human exposure.

Detailed findings from the repeat dose toxicology studies and the embryonic-fetal development study are tabulated in the Appendix/Attachments section.

12 Appendix/Attachments

Table 58 Doses Used in AP24534 Toxicology Studies

Study	Dose*, mg/kg	Dose*, mg/m^2
Single Dose		
– Mouse	0, 50, 150, 450	
– Rat	0, 10, 30, **100**	0, 60, 180, **600**
– Monkey	0, 5, 15, 45	0, 60, 180, 540
Repeat Dose		
– 28-Day Rat	0, **1.5, 3, 6**	0, **9, 18, 36**
– 6-Month Rat	0, 0.25, **0.75, 2**	0, 1.5, **4.5, 12**
– 28-Day Monkey	0, 1, 2.5, **5**	0, 12, 30, **60**
– 6-Month Monkey	0, 0.25, 0.75, 2	0, 3, 9, 24

*Ponatinib-related mortalities occurred in groups receiving doses shown in bold.

Study ARP280: Analysis of the In Vitro Kinase Selectivity Profile of AP24534 and its Principal Metabolite AP24600.

Tabulated Results were copied from the study report (Table 1) and from the response to the Information Request sent to ARIAD (Table 2) on November 5, 2012 (SDN 37/eCTD #35):

Table 1 Single concentration broad panel kinase screen with 1 μM AP24534, Lot 1*

Kinase()†	% Kinase activity remaining	Kinase	% Kinase activity remaining	Kinase	% Kinase activity remaining
Abl(h)	1	Fer(h)	64	PAK5(h)	101
Abl(m)	1	Fes(h)	12	PAK6(h)	94
Abl(T315I)(h)	6	FGFR1(h)	0	PAR-1Bα(h)	85
ALK(h)	105	FGFR1(V561M)(h)	0	PASK(h)	85
ALK4(h)	91	FGFR2(h)	0	PDGFRα(h)	25
Arg(h)	3	FGFR3(h)	2	PDGFRα(D842V)(h)	3
AMPK(r)	94	FGFR4(h)	4	PDGFRß(h)	21
Arg(m)	2	Fgr(h)	0	PDK1(h)	106
ARK5(h)	97	Flt1(h) [VEGFR1]	-3	PhKγ2(h)	96
ASK1(h)	96	Flt3(D835Y)(h)	40	Pim-1(h)	59
Aurora-A(h)	83	Flt3(h)	7	Pim-2(h)	107
Axl(h)	46	Flt4(h) [VEGFR3]	2	PKA(b)	117
Blk(m)	1	Fms(h)	-1	PKA(h)	97
Bmx(h)	1	Fyn(h)	5	PKBα(h)	87
BRK(h)	15	GRK5(h)	107	PKBß(h)	92
BrSK1(h)	96	GRK6(h)	101	PKBγ(h)	89
BrSK2(h)	108	GSK3α(h)	103	PKCα(h)	103
BTK(h)	14	GSK3ß(h)	127	PKCßI(h)	99
CaMKI(h)	71	Hck(h)	1	PKCßII(h)	97
CaMKII(r)	96	HIPK1(h)	98	PKCγ(h)	99
CaMKIIß(h)	108	HIPK2(h)	100	PKCδ(h)	87
CaMKIIγ(h)	98	HIPK3(h)	99	PKCε(h)	86
CaMKIδ(h)	91	IGF-1R(h)	77	PKCη(h)	94
CaMKIIδ(h)	75	IKKα(h)	30	PKCι(h)	96
CaMKIV(h)	96	IKKß(h)	42	PKCμ(h)	97
CDK1/cyclinB(h)	105	IR(h)	81	PKCθ(h)	106
CDK2/cyclinA(h)	87	IRR(h)	12	PKCζ(h)	102
CDK2/cyclinE(h)	86	IRAK1(h)	30	PKD2(h)	75
CDK3/cyclinE(h)	84	IRAK4(h)	85	PKG1α(h)	94
CDK5/p25(h)	112	Itk(h)	29	PKG1ß(h)	101
CDK5/p35(h)	116	JAK2(h)	56	Plk3(h)	106
CDK6/cyclinD3(h)	76	JAK3(h)	3	PRAK(h)	90
CDK7/cyclinH/MAT1(h)	104	JNK1α1(h)	98	PRK2(h)	92
CDK9/cyclin T1(h)	101	JNK2α2(h)	38	PrKX(h)	79
CHK1(h)	99	JNK3(h)	80	PTK5(h)	-3
CHK2(h)	7	KDR(h) [VEGFR2]	2	Pyk2(h)	16
CK1γ1(h)	108	Lck(h)	2	Ret(h)	1
CK1γ2(h)	108	LIMK1(h)	98	RIPK2(h)	5
CK1γ3(h)	98	LKB1(h)	111	ROCK-I(h)	90
CK1δ(h)	103	LOK(h)	7	ROCK-II(h)	72
CK1(y)	94	Lyn(h)	2	ROCK-II(r)	38
CK2(h)	96	Lyn(m)	1	Ron(h)	70
CK2α2(h)	102	MAPK1(h)	105	Ros(h)	64

Table 1 Single concentration broad panel kinase screen with 1 μM AP24534, Lot 1* continued

Kinase()†	% Kinase activity remaining	Kinase	% Kinase activity remaining	Kinase	% Kinase activity remaining
CLK3(h)	87	MAPK2(h)	102	Rse(h)	78
cKit(h)	17	MAPK2(m)	99	Rsk1(h)	87
cKit(D816V)(h)	77	MAPKAP-K2(h)	102	Rsk1(r)	93
cKit(D816H)(h)	7	MAPKAP-K3(h)	108	Rsk2(h)	84
cKit(V560G)(h)	-1	MARK1(h)	102	Rsk3(h)	75
cKit(V654A)(h)	3	MEK1(h)	98	Rsk4(h)	92
c-RAF(h)	1	MELK(h)	30	SAPK2a(h)	-1
CSK(h)	21	Mer(h)	22	SAPK2a(T106M)(h)	18
cSRC(h)	1	Met(h)	84	SAPK2b(h)	2
DAPK1(h)	106	MINK(h)	16	SAPK3(h)	26
DAPK2(h)	108	MKK4(m)	88	SAPK4(h)	64
DCAMKL2(h)	102	MKK6(h)	4	SGK(h)	91
DDR2(h)	1	MKK7ß(h)	87	SGK2(h)	95
DMPK(h)	94	MLCK(h)	97	SGK3(h)	88
DRAK1(h)	80	MLK1(h)	50	SIK(h)	12
DYRK2(h)	92	Mnk2(h)	31	Snk(h)	110
eEF-2K(h)	89	MRCKα(h)	101	SRPK1(h)	106
EGFR(h)	82	MRCKß(h)	107	SRPK2(h)	115
EGFR(L858R)(h)	28	MSK1(h)	61	STK33(h)	84
EGFR(L861Q)(h)	14	MSK2(h)	45	Syk(h)	94
EGFR(T790M)(h)	106	MSSK1(h)	98	TAK1(h)	1
EGFR(T790M,L858R)(h)	59	MST1(h)	94	TBK1(h)	100
EphA1(h)	1	MST2(h)	56	Tie2(h)	-1
EphA2(h)	-1	MST3(h)	305	TrkA(h)	-1
EphA3(h)	0	MuSK(h)	2	TrkB(h)	0
EphA4(h)	-2	NEK2(h)	115	TSSK1(h)	95
EphA5(h)	0	NEK3(h)	96	TSSK2(h)	113
EphA7(h)	1	NEK6(h)	105	WNK2(h)	75
EphA8(h)	-1	NEK7(h)	105	WNK3(h)	87
EphB1(h)	0	NEK11(h)	24	VRK2(h)	88
EphB2(h)	-1	NLK (h)	84	Yes(h)	2
EphB3(h)	3	p70S6K(h)	13	ZAP-70(h)	109
EphB4(h)	5	PAK2(h)	94	ZIPK(h)	102
ErbB4(h)	37	PAK3(h)	82	PI 3-Kinase□(h)	96
FAK(h)	73	PAK4(h)	99	PI 3-Kinase□(h)	89
				PI 3-Kinase□(h)	86

*Data are % kinase activity remaining and are presented as the mean of two runs.

†(h,r, or m) refers to protein source as human, rat, or mouse respectively

Table 2 Mean IC50 (nM) data for 108 kinases, AP24534 lot 7

IC50 ≤2 nM		IC50 ≤20 nM		IC50 ≤200 nM		IC50 >200 nM	
Kinase	IC50 (nM)	Kinase	IC50 (nM)	Kinase	IC50 (nM)	Kinase	IC50 (nM)
ABL	0.4	BLK	6.1	BMX/ETK	47	AKT2/PKBb	>1000
ABL (H396P)	0.3	CSK	12.7	BRK	51	ALK	>1000
ABL (M351T)	0.3	DDR2	16.1	EPHA1	143	Aurora A	>1000
ABL (Q252H)	0.4	EPHA2	2.1	ERBB4/HER4	176	Aurora B	543
ABL (T315I)	2.0	EPHA3	6.7	JAK1	32	Aurora C	>1000
ABL (Y253F)	0.3	EPHA7	8.5	JAK2	169	AXL	>1000
ABL2/ARG	0.8	EPHA8	2.5	JAK3	91	BTK	849
EPHA4	1.1	EPHB4	10.2	KIT (D816V)	152	BTK(E41K)	>1000
EPHA5	0.7	FGFR1	2.2	KIT (V654A)	78	CDK2/cyclin E	>1000
EPHB1	1.2	FGFR1 (V561M)	7.3	P38b	173	CTK/MATK/HYL	>1000
EPHB2/HEK5	0.6	FGFR3	18.2	P70S6K	94	EGFR	>1000
EPHB3	1.1	FGFR4	7.7	PYK2/FAK2	35	EGFR (L858R/T790M)	>1000
FGFR2	1.6	FLT1/VEGFR1	3.7	TYK2	177		
FGFR2 (N549H)	0.4	FLT3	12.6			EGFR (L858R)	211
FGR	0.5	FLT4	2.3			EGFR (L861Q)	536
FRK/PTK5	1.3	FMS	8.6			EGFR (T790M)	>1000
FYN	0.4	KDR/VEGFR2	2.9			ERBB2/HER2	>1000
HCK	0.1	KIT	12.5			FAK/PTK2	>1000
KIT (V560G)	0.4	KIT (D816H)	16.0			FER	560
LCK	0.3	P38a	9.8			FES/ FPS	768
LYN	0.2	PDGFRα (D842V)	15.6			FLT3 (D835Y)	948
LYNB	0.2	PDGFRα (T674I)	3.0			IGF1R	>1000
PDGFRα	1.1	PDGFRβ	7.7			IR	>1000
PDGFRα (V561D)	0.8	RAF/RAF1	13.7			IRR/INSRR	>1000
RET	0.2	RET (V804L)	3.7			ITK	>1000
RET (V804M)	1.4	SRC	5.4			MER	406
Yes	0.9	TIE2	14.3			MET	>1000
		TRKA/NTRK1	11.4			mTOR	>1000
		TRKB/NTRK2	15.1			MUSK	694
		TRKC/NTRK3	13.2			PKA	613
						PKCtheta	>1000
						RON/MST1R	>1000
						ROS	>1000
						SRC (T341M)	>1000
						SYK	>1000
						TEC	>1000
						TYK1/LTK	>1000
						TYRO3/SKY	>1000
						ZAP70	>1000

The above table was submitted on November 8 and replaces the original version submitted in Study Report ARP280.

Table 59 General Toxicology Tabulated Summary

Repeat Dose Toxicity Studies				
Species	**Route Duration**	**Number/sex/ dose**	**mg/kg (mg/m^2)**	**AP24534-related Findings**
Rats	Oral 28 days Recovery 28 days	15/sex (MS) 4 or 8/sex (TK)	1.5 (9) 3 (18) 6 (36)	STD10 was 1.5 mg/kg (9 mg/m^2/day) 9 mg/m^2/day: mortality 2/23; Cold to touch and swelling in early decedents; thinning fur. Males, ↓ ALP, T3 levels, liver and spleen weight. Females, ↑ lymphocytes, eosinophils, ↑ AST. Normal values at recovery. Day 28 AUClast ♂1260 ♀704 ng*h/mL 18 mg/m^2/day: mortality 6/23; Porphyrin stain, scant feces, rough hair coat, urine stain and thin in early decedents; dry flaky skin, thinning fur, rough hair coat. ↓ BW/FC ♂♀; Males, ↑ neutrophils, monocytes, eosinophils, ALT, ↓ lymphocytes, ALP, T3 levels. ↓ liver, prostate, and pituitary weight. Females, ↑ lymphocytes, neutrophils, monocytes, eosinophils, RTC, AST, ALT. ↓ basophils, ALP, ovary weight. Normal values at recovery. Day 28 AUClast ♂1920 ♀1580 ng*h/mL 36 mg/m^2/day: mortality 12/23; Hunched posture, labored breathing, cold to touch, porphyrin stain, squint eyes, scant feces, and rough hair coat in early decedents; dry flaky skin, thinning fur, rough hair coat, discolored urine. ↓ BW/FC ♂♀; Males ↑ neutrophils, monocytes, eosinophils, ↓ lymphocytes, WBC, T3 and T4 levels, thymus, testes and seminal vesicles weight; Females, ↑ neutrophils, monocytes, eosinophils, ↓ lymphocytes, ovary weight. Early decedents, minimal to moderate myeloid hyperplasia of the bone marrow, minimal to mild cartilaginous hyperplasia of the epiphyseal plate of the femur, variable changes in the stomach, including minimal to mild hyperkeratosis of the squamous epithelium (non-glandular stomach), minimal to mild submucosal edema and moderate thymus necrosis. Day 8 AUClast ♂ 2970 ♀2510 ng*h/mL.
				NOAEL 1 mg/kg/day (12 mg/m^2/day) corresponded to mean AP24534 Cmax of 20 and 15.8 ng/mL and AUClast of 162 and 115 ng*h/mL in male and female monkeys, respectively. 12 mg/m^2/day: less severe dry flaky skin, skin erythema, scratching, soft feces. ↓BW/FC ♂. Systolic heart murmurs (Grade I/VI) and harsh lung sounds. ↑T4 /thyroid infiltration mononuclear ♂♀.

Monkeys	Oral 28 days Recovery 28 days	5/sex 2/sex recovery	1 (12) 2.5 (30) 5 (60)	30 mg/m^2/day: moderate dry flaky skin, skin erythema, scratching, soft feces, ↓ BW/FC. Systolic heart murmurs (Grade III/VI) and harsh lung sounds. ↓ MCH ♀; ↑ T4 ♂♀; small thymus ♀; lymphoid depletion mesenteric/mandibular ♀; pancreas chronic inflammation ♀, pancreas regeneration acinar cell ♂, lymphoid depletion thymus, spleen ♀, thyroid atrophy ♂♀, thyroid necrosis ♂, thyroid infiltration mononuclear ♂♀, thyroid infiltration mixed cells ♀, lungs granulomatous inflammation ♀. Day 28 AUClast ♂ 1430 ♀1920 ng*h/mL 60 mg/m^2/day: mortality 2/5 males, 1/5 females. Dehydration, ocular discharge, marked soft feces, cold to touch, lethargy, scratching, dry, flaky skin, moderate to marked skin erythema, pale mucous membrane, thinness and weight loss in early decedents; dry flaky skin, skin erythema, scratching, soft feces, ↓ BW/FC. Systolic heart murmurs (Grade I/VI and II/VI) and harsh lung sounds. ↓ hemoglobin, hematocrit, MCV and MCH, ↑ lipase ♂; ↑ TSH ♀, ↓ T3 ♂, ↑ T4 ♂♀; thickened pancreas ♂, skin crusts ♂♀, small thymus ♂♀; ↓ thymus weight ♂♀, ↓ ovaries and uterus weight; abnormal microscopic findings in the pancreas, thymus and thyroid glands; lymphoid depletion mesenteric/mandibular ♂♀, necrosis pancreas acinar cells ♂, pancreas regeneration acinar cell ♂♀, pancreas chronic inflammation ♀, lymphoid depletion thymus, spleen ♂♀, thyroid atrophy ♂♀, thyroid infiltration mononuclear ♂♀, thyroid infiltration mixed cells ♂♀, lungs granulomatous inflammation ♂♀, ovary atresia ♀, testis degeneration germinal epithelium ♂. Day 28 AUClast ♂ 5660 ♀8040 ng*h/mL.
				NOAEL 0.25 mg/kg/day (1.5 mg/m^2/day) corresponded to mean AP24534 Cmax of 5.7 and 4.3 ng/mL and AUClast of 91.9 and 23.9 ng*h/mL in male and female rats, respectively. 1.5 mg/m^2/day: No AP24534-related findings. 4.5 mg/m^2/day: Mortality 6/68. Dose-related clinical signs of labored breathing and/or in irregular pattern, cold to touch, lethargy, hunched posture, dehydration, rough hair coat, thin, and scant or soft feces; ↓ BW/FC ♂♀; ↑ ANEU, AMNO and/or AEOS, reversible at recovery ♂♀; skin crusts ♂♀ and accumulation of firm nodules inguinal skin ↑ incidence ♀; ↓ thymus weight ♂ non-reversible microscopic findings femoral bone included ↓ number of chondrocytes along the

Rats	Oral 6 months Recovery 2 months	25/sex (MS) 3 or 9/sex (TK)	0.25 (1.5) 0.75 (4.5) 2 (12)	growth plate, ↓ numbers of chondrocytes in the proliferation and hypertrophy zones, islands of residual cartilage were present, but also at ↓ numbers and ↓ trabecular bone in several males. Non-reversible microscopic findings kidney ♂♀ included ↑ basophilia of tubules in the cortex, thickening of tubule basement membranes, variable infiltrates of mononuclear cells in the interstitium, occasional interstitial fibrosis, and variable accumulations of hyaline droplets in renal tubule epithelium. Day 182 AUClast ♂ 559 ♀374 ng*h/mL. 12 mg/m^2/day: mortality 20/68. Similar dose-related clinical signs than in 4.5 mg/m^2/day; ↓ BW/FC ♂♀; abnormal ↑ ANEU, AMNO and/or AEOS, reversible at recovery ♂♀; abnormal ↑ in FIB ♂♀ correlated with ↑ ANEU and AMNO; skin crusts ♂♀ and accumulation of firm nodules inguinal skin ↑ incidence ♀; ↓ thymus weight ♂, ↑ ovarian weight ♀; non-reversible microscopic findings femoral bone similar to finding at 4.5 mg/m^2/day; non-reversible microscopic findings kidney ♂♀ included ↑ basophilia of tubules in the cortex, thickening of tubule basement membranes, variable infiltrates of mononuclear cells in the interstitium, occasional interstitial fibrosis, and variable accumulations of hyaline droplets in renal tubule epithelium. Microscopic findings correlated with significantly ↑ CRE and BUN values in ♂ at 2.0 mg/kg/day; reversible microscopic findings thymus included ↑ incidence of lymphoid depletion with decreases in cortex and medulla. Day 182 AUClast ♂ 1924 ♀1375 ng*h/mL.
Monkeys	Oral 6 months Recovery 2 months	6/sex 2/sex recovery	0.25 (3) 0.75 (9) 2 (24)	NOAEL 2 mg/kg/day (24 mg/m^2/day) corresponded to mean AP24534 Cmax of 56.9 and 87.9 ng/mL and AUC0-24 of 533 and 681 ng*h/mL in male and female monkeys, respectively. Plasma levels of the metabolite AP24567 was between 3.75 and 7.25% of the parent compound at 0.75 and 2 mg/kg/day, respectively. ↑ALT, AST in few animals. Myocardial necrosis 1/8. Mild thymus lymphoid depletion. 0.75 mg/kg/day (9 mg/m^2/day). ↑ALT, AST in few animals. Myocardial necrosis 4/8. Mild thymus lymphoid depletion. Day 182 AUClast ♂ 138 ♀75.6 ng*h/mL. 0.25 mg/kg/day (3 mg/m^2/day). ↑ALT, AST in few animals. Myocardial necrosis 2/8. Mild thymus lymphoid depletion. Day 182 AUClast ♂ 9.1 ♀8.9 ng*h/mL.

Table 60 Reproductive and Developmental Toxicology Tabulated Summary

Embryonic Fetal Development	
NOAEL	Maternal 1 mg/kg/day, 6 mg/m^2/day corresponded to mean AP24534 Cmax of 23.6 ng/mL and AUC0-t of 314 ng*h/mL. Metabolism of AP24534 to AP24567 was quantifiable only at the 3 mg/kg/day dose level. Developmental 0.3 mg/kg/day (1.8 mg/m^2/day)
Toxicokinetics	0.3 mg/kg/day, 1.8 mg/m^2/day AUC0-t 35.9 ng*h/mL. 1 mg/kg/day, 6 mg/m^2/day AUC0-t 314 ng*h/mL. 3 mg/kg/day, 18 mg/m^2/day corresponded AUC0-t 1275 ng*h/mL.
Species	Sprague-Dawley rat
Methods	Virgin female rats mated with male breeder rats 25 mated females/group dosed GD 7-17 and euthanized on GD 21 Toxicokinetic rats dosed GD 7-17 and euthanized on GD18
Doses	0, 0.3, 1.0, 3.0 mg/kg/day 0, 1.8, 6, 18 mg/m^2/day
Mortality and Clinical Signs	1 ♀/25 at 18 mg/m^2/day GD 19 with decreased motor activity, ataxia, hunched posture, bradypnea, ptosis, pale ears, thin body condition, fecal-, urine- and blood-stained fur, chromorhinorrhea, and mild to moderate dehydration 1 ♀/25 control early deliver GD 21 with bent tail on GD 14 through 21, unremarkable body weight gain and food consumption 18 mg/m^2/day: additional clinical signs onset GD 14 – 16, mild to moderate dehydration, soft or liquid feces, urine-stained abdominal fur, red perivaginal substance and pale ears
Body Weight and Food Consumption	1.8 mg/m^2/day: unremarkable 6 mg/m^2/day: AP24534-related but not adverse ↓ bw GD 7-18 and 18-21; ↓ bw gain GD 7-21; ↓ FC GD 18-21 and 7-21. 18 mg/m^2/day: AP24534-related and adverse ↓ bw GD 14-21; ↓ bw gain GD 7-18, 18-21 and 7-21; ↓ FC GD 7-18, 18-21 and 7-21.
Necropsy	Unremarkable
Laparohysteroctomy Evaluations	18 mg/m^2/day: 100% dams with resorptions ↓ live fetuses, ↑ early and late resorptions and post-implantation loss ↓ fetal ♂♀ bw
Fetal Gross External Alterations	18 mg/m^2/day: ↑ litter and fetal incidence of edema and short tail
Fetal Soft Tissue Alterations	6 mg/m^2/day: ↑ litter and fetal incidence of interventricular septal defect – M Heart 18 mg/m^2/day: ↑ litter and fetal incidence of: interventricular septal defect, interrupted aortic arch, urinary artery descending left of urinary bladder, left subclavian artery arising from pulmonary artery, right subclavian passing dorsal to the trachea and esophagus, right subclavian passing left of the left subclavian, vessels arising in incorrect order or

	transposed, persistent truncus arteriosus, aorta descending to the right, pulmonary aorta descending to the right or passing dorsal to aorta, ductus arteriosus patent, semilunar valves absent, lung right lobe apical absent, liver with white areas, kidneys absent, small or high set, genitalia absent or undescended testes, uterus absent or reduced to a ligament, ovary absent, ureters absent, constricted, elongated, or with marked or extreme dilation.
Fetal Skeletal Alterations	Control: cervical vertebrae arch incompletely ossified litter (4.5%) fetal (1.3%); wavy ribs litter (4.5) fetal (0.6%); sternal centra incompletely ossified litter (4.5%) fetal (0.6%); 1.8 mg/m^2/day: cervical vertebrae arch incompletely ossified litter (8%) fetal (1.6%); thoracic vertebrae centrum bifid litter (4%) fetal (0.5%); lumbar vertebrae hemivertebrae litter (4%) fetal (0.5%); short ribs litter (4%) fetal (0.5%); pelvis pubis incompletely ossified litter (4%) fetal (0.5%). 6 mg/m^2/day: cervical vertebrae arch 6th with appearance of the 7th litter (25%) fetal (4.1%); cervical vertebrae arch incompletely ossified litter (16.7%) fetal (2.4%); thoracic vertebrae centrum bifid litter (8.3%) fetal (1.2%); lumbar vertebrae hemivertebrae litter (4.2%) fetal (0.6%); sternal centra incompletely ossified litter (4.2%) fetal (0.6%); sternal centra asymetric litter (12.5%) fetal (2.4%); pelvis pubis incompletely ossified litter (12.5%) fetal (2.9%**). 18 mg/m^2/day: cervical vertebrae arch 6th with appearance of the 7th litter (65.2%**) fetal (22.6%**); cervical vertebrae arch incompletely ossified litter (60.9%**) fetal (26.9%**); cervical vertebrae arch irregular shaped litter (8.7%) fetal (2.2%**); thoracic vertebrae centrum bifid litter (52.2%**) fetal (18.3%**); thoracic vertebrae centrum unilateral ossification litter (13.0%**) fetal (3.2%**); thoracic vertebrae arches fused litter (8.7%) fetal (2.2%**); thoracic vertebrae hemivertebrae litter (8.7%) fetal (2.2%**); thoracic vertebrae 15 vertebrae present litter (4.3%) fetal (1.1%); lumbar vertebrae centrum bifid litter (17.4%) fetal (4.3%); lumbar vertebrae hemivertebrae litter (4.3%) fetal (1.1%); lumbar vertebrae 6 present litter (4.3%) fetal (1.1%); sacral vertebrae arch small litter (4.3%) fetal (1.1%); caudal vertebrae 4 present litter (4.3%) fetal (8.7%); caudal vertebrae 3 present litter (2.2%**) fetal (1.3%); ribs fused litter (47.8%**) fetal (14%**); ribs short litter (34.8%**) fetal (9.7%**); ribs proximate litter (52.2%**) fetal (15%**); ribs wavy (26.1%**) fetal (7.5%**); ribs irregular shape litter (13.0%**) fetal (3.2%); ribs bowed (8.7%) fetal (2.2%**); manubrium fused litter (82.6%**) fetal (48.4%**); manubrium irregular shaped litter (26.1%**) fetal (7.5%**); manubrium small litter (13.0%**) fetal (4.3%**); sternal centra incompletely ossified litter (17.4%) fetal (4.3%**); sternal centra asymetric litter (26.1%**) fetal (11.8%**); sternal centra fused litter (56.5%**) fetal (31.2%**); sternal centra duplicated litter (4.3%) fetal (1.1%); sternal centra irregular shaped litter (13.0%**) fetal (4.3%**); xiphoid duplicated litter (4.3%) fetal (1.1%); pelvis pubis incompletely ossified litter (21.7%) fetal (6.4%**).
Fetal Ossification Sites	6 mg/m^2/day: mean caudal vertebrae 6.62** 18 mg/m^2/day: mean thoracic (13.38**), lumbar (5.62**), and caudal (5.30**) vertebrae; pair ribs 13.28**; sternum sternal centers 3.82**; forelimb phalanges 6.79**; hindlimb metatarsals (4.41**) and phalanges (5.0**).

** Statistically significant

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/s/

M S RICCI
11/19/2012

PEDRO L DEL VALLE
11/19/2012

HALEH SABER
11/19/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 203469 **Applicant: ARIAD Pharmaceuticals** **Stamp Date: Sept. 28, 2012**

Drug Name: Ponatinib **NDA Type: NME**

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		
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		All toxicology studies used oral administration.
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		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)		X	Impurity issues will be addressed, as needed, during the review.
11	Has the applicant addressed any abuse potential issues in the submission?		X	Not applicable.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X	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.

M. Stacey Ricci, M.Eng., ScD and Pedro L. DelValle, Ph.D.

Reviewing Toxicologist/Pharmacologist Date

Haleh Saber, Ph.D.

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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

/s/

M S RICCI
09/28/2012

PEDRO L DEL VALLE
09/28/2012

HALEH SABER
09/28/2012