CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202324Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

INLYTA (axitinib)

Date: January 23, 2012
To: File for NDA 202324
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. Goheer, Putman and Aziz and labeling and secondary memorandum provided by Dr. Palmby. I concur with Dr Palmby's conclusion that INLYTA may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
01/23/2012

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 202324
Supporting document/s:	1
Applicant's letter date:	April 13, 2011
CDER stamp date:	April 14, 2011
Product:	INLYTA (axitinib)
Indication:	Advanced renal cell carcinoma (RCC) after failure of one prior systemic therapy
Applicant:	Pfizer Inc 10646 Science Center Drive San Diego CA 92121
Review Division:	Division of Hematology Oncology Toxicology (Division of Oncology Products 1)
Reviewer:	M. Anwar Goheer, Ph.D. Alexander H. Putman, Ph.D. Robeena Aziz, MPH, Ph.D.
Acting Supervisor/Team Leader:	Todd Palmby, Ph.D.
Division Director:	John Leighton, Ph.D. (Robert Justice, M.D., M.S.)
Project Manager:	Lisa Skarupa

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1.1.3 Labeling Review

All pertinent information from nonclinical studies conducted with axitinib that is included in the package insert can be found in the original Pharmacology/Toxicology review for NDA 202324. This labeling review includes calculations of exposure (AUC) ratios in animals as compared to humans at the recommended starting dose of 5 mg orally twice daily. The following sections of the package insert for axitinib were modified from the version proposed by the Applicant with input from the Pharmacology/Toxicology team and represent the FDA recommendations:

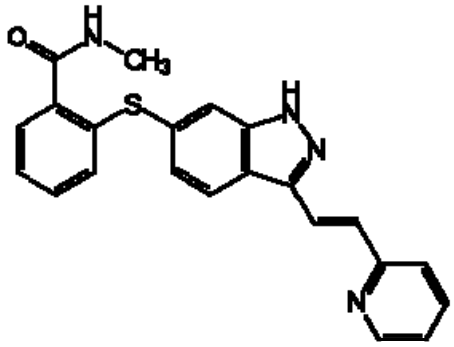
Under **HIGHLIGHTS** OF PRESCRIBING INFORMATION:

----------INDICATIONS AND USAGE----------
INLYTA is a kinase inhibitor indicated for the treatment of advanced renal cell carcinoma after failure of one prior systemic therapy. (1)
----------WARNINGS AND PRECAUTIONS----------
• INLYTA can cause fetal harm when administered to a pregnant woman based on its mechanism of action. Women of childbearing potential should be advised of the potential hazard to the fetus and to avoid becoming pregnant while receiving INLYTA. (5.12, 8.1)

Under **FULL PRESCRIBING INFORMATION**:

Recommended Labeling	Comments/Justification
5 WARNINGS AND PRECAUTIONS **5.12 Pregnancy** INLYTA can cause fetal harm when administered to a pregnant woman based on its mechanism of action. There are no adequate and well-controlled studies in pregnant women using INLYTA. In developmental toxicity studies in mice, axitinib was teratogenic, embryotoxic and fetotoxic at maternal exposures that were lower than human exposures at the recommended clinical dose. Women of childbearing potential should be advised to avoid becoming pregnant while receiving INLYTA. If this drug is used during pregnancy, or if a patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus *[see Use in Specific Populations (8.1)].*	
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** Pregnancy Category D *[see Warnings and Precautions (5.12)]*. There are no adequate and well-controlled studies with INLYTA in pregnant women. INLYTA can cause fetal harm when administered to a pregnant woman based on its mechanism of action. Axitinib was teratogenic, embryotoxic and fetotoxic in mice at exposures lower than human exposures at the recommended starting dose. If this drug is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Oral axitinib administered twice daily to female mice prior to mating and through the first week of pregnancy caused an increase in post-implantation loss at all doses tested	In the fertility and early embryonic development study (#06GR118), the AUC_{0-24} in female mice at 15 mg/kg/dose oral axitinib twice daily was 2850 ng*hr/mL, which is approximately 10 times the AUC in humans at the recommended starting dose of 265 ng*hr/mL. In the embryo-fetal developmental toxicity study (#06GR117), female mice receiving 1.5 mg/kg/dose twice daily had an AUC_{0-24} of 142 ng*hr/mL, which is approximately 0.5 times the human AUC of 265 ng*hr/mL. Mice in this study had an AUC_{0-24} of 39.5 ng*hr/mL at a dose of 0.5 mg/kg/dose twice daily, which is approximately 0.15 times the human AUC of 265 ng*hr/mL.

(≥ 15 mg/kg/dose, approximately 10 times the systemic exposure (AUC) in patients at the recommended starting dose). In an embryo-fetal developmental toxicity study, pregnant mice received oral doses of 0.15, 0.5 and 1.5 mg/kg/dose axitinib twice daily during the period of organogenesis. Embryo-fetal toxicities observed in the absence of maternal toxicity included malformation (cleft palate) at 1.5 mg/kg/dose (approximately 0.5 times the AUC in patients at the recommended starting dose) and variation in skeletal ossification at ≥ 0.5 mg/kg/dose (approximately 0.15 times the AUC in patients at the recommended starting dose).	
8.3 Nursing Mothers It is not known whether axitinib is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from INLYTA, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.	
8.4 Pediatric Use The safety and efficacy of INLYTA in pediatric patients have not been studied. Toxicities in bone and teeth were observed in immature mice and dogs administered oral axitinib twice daily for 1 month or longer. Effects in bone consisted of thickened growth plates in mice and dogs at ≥15 mg/kg/dose (approximately 6 and 15 times, respectively, the systemic exposure (AUC) in patients at the recommended starting dose). Abnormalities in growing incisor teeth (including dental caries, malocclusions and broken and/or missing teeth) were observed in mice administered oral axitinib twice daily at ≥ 5 mg/kg/dose (approximately 1.5 times the AUC in patients at the	In the 28-day repeat-dose toxicology study in mice (#6750-145) the mean AUC_{0-24} at the dose of 15 mg/kg/dose oral axitinib twice daily was 1600 ng*hr/mL, which is approximately 6 times the AUC in humans at the recommended starting dose of 265 ng*hr/mL. In the 28-day repeat-dose toxicology study in dogs (#6750-143) the mean AUC_{0-24} at the dose of 15 mg/kg/dose twice daily was 4150 ng*hr/mL, which is approximately 15 times the AUC in humans at the recommended starting dose of 265 ng*hr/mL. In the 28-day repeat-dose toxicology study in mice (#6750-145) the mean AUC_{0-24} at the dose of 5 mg/kg/dose twice daily was 370 ng*hr/mL, which is approximately 1.5

recommended starting dose). Other toxicities of potential concern to pediatric patients have not been evaluated in juvenile animals.	times the AUC in humans at the recommended starting dose of 265 ng*hr/mL.
11 DESCRIPTION INLYTA (axitinib) is a kinase inhibitor. Axitinib has the chemical name *N*-methyl-2-[3-((*E*)-2-pyridin-2-yl-vinyl)-1*H*-indazol-6-ylsulfanyl]-benzamide. The molecular formula is $C_{22}H_{18}N_4OS$ and the molecular weight is 386.47 Daltons. The chemical structure is:  Axitinib is a white to light-yellow powder with a pKa of 4.8. The solubility of axitinib in aqueous media over the range pH 1.1 to pH 7.8 is in excess of 0.2 μg/mL. The partition coefficient (n-octanol/water) is 3.5. INLYTA is supplied as red, film-coated tablets containing either 1 mg or 5 mg of axitinib together with microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, magnesium stearate, and Opadry II red 32K15441 as inactive ingredients. The Opadry II red 32K15441 film coating contains lactose monohydrate, HPMC 2910/Hypromellose 15cP, titanium dioxide, triacetin (glycerol triacetate), and red iron oxide.	
12 CLINICAL PHARMACOLOGY	

12.1 Mechanism of Action Axitinib has been shown to inhibit receptor tyrosine kinases including vascular endothelial growth factor receptors (VEGFR)-1, VEGFR-2, and VEGFR-3 at therapeutic plasma concentrations. These receptors are implicated in pathologic angiogenesis, tumor growth, and cancer progression. VEGF-mediated endothelial cell proliferation and survival were inhibited by axitinib *in vitro* and in mouse models. Axitinib was shown to inhibit tumor growth and phosphorylation of VEGFR-2 in tumor xenograft mouse models.	

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies have not been conducted with axitinib.

Axitinib was not mutagenic in an *in vitro* bacterial reverse mutation (Ames) assay and was not clastogenic in the *in vitro* human lymphocyte chromosome aberration assay. Axitinib was genotoxic in the *in vivo* mouse bone marrow micronucleus assay.

INLYTA has the potential to impair reproductive function and fertility in humans. In repeat-dose toxicology studies, findings in the male reproductive tract were observed in the testes/epididymis (decreased organ weight, atrophy or degeneration, decreased numbers of germinal cells, hypospermia or abnormal sperm forms, reduced sperm density and count) at ≥15 mg/kg/dose administered orally twice daily in mice (approximately 7 times the systemic exposure (AUC) in patients at the recommended starting dose) and ≥ 1.5 mg/kg/dose administered orally twice daily in dogs (approximately 0.1 times the AUC in patients at the recommended starting dose). Findings in the female reproductive tract in mice and dogs included signs of delayed sexual maturity, reduced or absent corpora lutea, decreased uterine weights and uterine atrophy at ≥ 5 mg/kg/dose (approximately 1.5 or 0.3 times the AUC in patients at the recommended starting dose compared to mice and dogs, respectively).

In a fertility study in mice, axitinib did not affect mating or fertility rate when administered orally twice daily to males at any dose tested up to 50 mg/kg/dose following at least 70 days of administration

Axitinib was reported as genotoxic in the *in vivo* mouse bone marrow micronucleus assay rather than clastogenic, because results indicated an aneugenic mechanism lead to the increase in micronucleated polychromatic erythrocytes.

In the 26-week repeat-dose toxicology study in mice (#6750-148) and dogs (#6750-150), effects on the testes and epididymis were observed in males at ≥15 mg/kg/dose and ≥1.5 mg/kg/dose, respectively, oral axitinib twice daily. The AUC_{0-24} in males at 15 mg/kg/dose twice daily was 1885 ng*hr/mL in mice and at 1.5 mg/kg/dose twice daily was 12.3 ng*hr/mL in dogs, which is approximately 7 and 0.1 times, respectively, the AUC in humans at the recommended starting dose of 265 ng*hr/mL.

Findings in the female reproductive tract were observed at ≥5 mg/kg/dose twice daily in mice and dogs. In the 26-week repeat-dose toxicology study in mice (#6750-148), the AUC_{0-24} was 457.04 ng*hr/mL in females at 5 mg/kg/dose twice daily, which is approximately 1.5 times the AUC in humans (265 ng*hr/mL) at the recommended starting dose. In the 28-day repeat dose toxicology study in dogs (#6750-143), the AUC_{0-24} was 70 ng*hr/mL in females at 5 mg/kg/dose twice daily, which is approximately 0.3 times the AUC in humans (265 ng*hr/mL) at the recommended starting dose.

In the fertility and early embryonic development study in mice (#06GR118), the AUC_{0-24} in males at 50 mg/kg/dose twice daily, the highest dose tested, was 15200 ng*hr/mL, which is approximately 57 times the AUC (265 ng*hr/mL) in humans at the recommended starting

(approximately 57 times the AUC in patients at the recommended starting dose). In female mice, reduced fertility and embryonic viability were observed at all doses tested (≥ 15 mg/kg/dose administered orally twice daily) following at least 15 days of treatment with axitinib (approximately 10 times the AUC in patients at the recommended starting dose).	dose. The AUC_{0-24} in females at 15 mg/kg/dose twice daily in this study was 2850 ng*hr/mL, which is approximately 10 times the AUC (265 ng*hr/mL) in humans at the recommended starting dose.

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/s/

M A GOHEER
01/23/2012

TODD R PALMBY
01/23/2012

MEMORANDUM

Date: January 6, 2012
From: Todd R. Palmby, Ph.D.
Acting Supervisory Pharmacologist
Division of Hematology, Oncology Toxicology
To: File for NDA 202324 axitinib
Re: Approvability for Pharmacology and Toxicology
Indication: Treatment of patients with advanced renal cell carcinoma after failure of one prior systemic therapy

Non-clinical pharmacology and toxicology studies to support axitinib NDA 202324 for the treatment of renal cell carcinoma after failure of one prior systemic therapy were reviewed by Anwar Goheer, Ph.D, Alexander H. Putman, Ph.D., and Robeena Aziz, MPH, Ph.D. Information included studies conducted with orally administered axitinib investigating the drug's pharmacology, toxicokinetics and ADME, safety pharmacology, general toxicology (mouse and dog), and genetic toxicity (*in vivo* and *in vitro*). Reproductive and developmental toxicology studies were conducted in mice to assess the effects of axitinib on fertility and embryo-fetal development. The studies cited in the review consist primarily of original research studies conducted by the applicant.

Pharmacology studies submitted to the NDA support that axitinib is a kinase inhibitor which binds to and inhibits the activity of multiple receptor tyrosine kinases including vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2 and VEGFR-3.

The most common adverse reactions observed with axitinib in patients (≥ 20% according to Highlights section of the label) were diarrhea, hypertension, fatigue, decreased appetite, nausea, dysphonia, palmar-plantar erythrodysesthesia (hand-foot) syndrome, weight decrease, vomiting, asthenia, and constipation. Safety pharmacology studies conducted with axitinib in mice, rats and dogs identified the potential for increased systolic blood pressure and decreased heart rate. In repeat-dose studies, toxicities in bone and teeth, spleen and thymus (in mice), and elevated cholesterol and triglycerides (in dogs) were not observed clinically, but may be relevant to patient risk under certain circumstances. Toxicities were observed throughout the gastrointestinal tract in mice and dogs.

Axitinib was not mutagenic in an *in vitro* bacterial reverse mutation (Ames) assay and was not clastogenic in the *in vitro* human lymphocyte chromosome aberration assay. However, axitinib was genotoxic in the *in vivo* mouse bone marrow micronucleus assay. Kinetochore staining results from the *in vivo* micronucleus assay indicated that the increases in micronucleated polychromatic erythrocytes were due to an aneugenic mechanism.

Axitinib may impair reproductive function and fertility in males and females. In repeat-dose toxicology studies in mice and dogs, findings in the male reproductive tract were observed in the testes/epididymis at exposures approximately equivalent to and lower than patient exposure, respectively. Findings in the female reproductive tract in mice and dogs included signs of delayed sexual maturity, reduced or absent corpora lutea, decreased uterine weights and uterine atrophy at exposures approximately equivalent to exposure in patients.

In a fertility study in mice, axitinib did not affect mating or fertility rate when administered to males at any dose tested. Reduced fertility and embryonic viability were observed in female mice at all doses tested. Doses in this study resulted in systemic exposures greater than exposures in patients.

Axitinib is embryotoxic, fetotoxic, and teratogenic to mice, at exposures lower than human exposures at the recommended human starting dose. During a fertility and early embryonic development study, axitinib administered to female mice prior to mating and through the first week of pregnancy caused an increase in post-implantation loss. In an embryo-fetal developmental toxicity study, pregnant mice received oral axitinib twice daily during the period of organogenesis. Embryo-fetal toxicities observed in the absence of maternal toxicity included malformations (cleft palate) and variations in skeletal ossification (interfrontal ossification sites, incomplete ossification of the supraoccipitals). A no effect level for adverse embryo-fetal effects was not identified in this study. The potential benefit of axitinib in pregnant women in this patient population may outweigh the potential risk to the developing fetus. Therefore, Pregnancy Category D is recommended for the use of axitinib in this patient population.

Recommendations: I concur with Drs. Goheer's, Putman's and Aziz's conclusion that pharmacology and toxicology data support the approval of NDA 202324 for axitinib. There are no outstanding nonclinical issues that would preclude the approval of axitinib for the proposed indication.

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/s/

TODD R PALMBY
01/06/2012

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PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 202324
Supporting document/s:	1
Applicant's letter date:	April 13, 2011
CDER stamp date:	April 14, 2011
Product:	INLYTA (axitinib)
Indication:	Advanced renal cell carcinoma (RCC) after failure of one prior systemic therapy
Applicant:	Pfizer Inc 10646 Science Center Drive San Diego CA 92121
Review Division:	Division of Hematology Oncology Toxicology (Division of Oncology Products 1)
Reviewer:	M. Anwar Goheer, Ph.D. Alexander H. Putman, Ph.D. Robeena Aziz, MPH, Ph.D.
Acting Supervisor/Team Leader:	Todd Palmby, Ph.D.
Division Director:	John Leighton, Ph.D. (Robert Justice, M.D., M.S.)
Project Manager:	Lisa Skarupa

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1 Executive Summary

1.1 Introduction

NDA 202324 was submitted to the U.S. Food and Drug Administration as a full New Drug Application (NDA) for axitinib for the indication of the treatment of patients with advanced renal cell carcinoma (RCC). Axitinib is a new molecular entity kinase inhibitor, which inhibits multiple kinases including vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, and VEGFR-3. The proposed clinical starting dose of 5 mg is administered orally as a tablet twice daily. Nonclinical pharmacology, pharmacokinetic and toxicology studies have been submitted to support the approval of axitinib for the proposed indication.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical primary pharmacology studies evaluated the ability of axitinib to inhibit the activity and function of Vascular Endothelial Growth Factor Receptors (VEGFRs), *in vitro* and *in vivo*, thereby inhibiting angiogenesis and tumor progression in experimental cancer models in mice. Utilizing biochemical and cell-based assays, axitinib was shown to inhibit receptor tyrosine kinases including VEGFR-1, VEGFR-2, and VEGFR-3 at therapeutic plasma concentrations. Additional *in vitro* studies demonstrated that axitinib was able to inhibit VEGF-stimulated endothelial cell survival, vascular tube formation, adhesion, migration, and induce endothelial cell apoptosis. Axitinib-induced alterations in endothelial cell function were demonstrated *in vivo*. Specifically, axitinib was shown to decrease vascular permeability and density in tumor xenografts in mice. Axitinib was shown to inhibit the phosphorylation of VEGFR-2 and PDGFR-β in tumor xenografts and produce tumor growth inhibition in experimental models of cancer in mice. Based on primary pharmacology data submitted with this NDA and on other FDA approved products which inhibit tyrosine kinases, the Established Pharmacologic Class of “kinase inhibitor” was determined to be both clinically meaningful and scientifically valid for axitinib.

In safety pharmacology studies, axitinib administered orally to mice and rats did not demonstrate signs of neurotoxicity or adverse effects on the respiratory system. Axitinib increased gastric emptying (stomach) in rats. There were no significant effects of axitinib on the *hERG* channel *in vitro* or on cardiac function (e.g., PR, QRS, QT, QTcB, QTcF) in dogs. Cardiovascular effects of axitinib may include increased systolic blood pressure and a concomitant decrease in heart rate, as demonstrated in mice, rats and dogs. Hypertension was observed in clinical trials conducted with axitinib.

Toxicities in bone and teeth were observed in immature mice and in dogs administered oral axitinib twice daily for 1 month or longer. Effects in bone consisted of thickened growth plates in mice and dogs at ≥15 mg/kg/dose. Abnormalities in growing incisor teeth (including dental caries and broken and/or missing teeth) were observed in mice

administered oral axitinib twice daily for 26-weeks at ≥5 mg/kg/dose. These toxicities are consistent with the activity of axitinib for inhibiting VEGFR, and should be considered when axitinib is administered to pediatric patients.

Other toxicities observed in mice and dogs administered axitinib included effects on the gastrointestinal tract with microscopic findings of inflammation, hyperplasia, necrosis, erosion and ulcers. These findings correlate to the gastrointestinal perforations observed clinically. In addition, elevated cholesterol and triglyceride levels in dogs suggest axitinib may have effects on lipid metabolism. In mice, lymphoid depletion was present in the spleen and thymus, but there were no significant correlating hematological findings.

The overall exposure (C_{max} and AUC) after repeated dosing in mice and dogs did not show significant and consistent accumulation. The increase in AUC value was slightly greater than dose proportional. This may suggest nonlinear toxicokinetics due to a combination of potential inhibition or saturation of elimination pathways.

In repeat-dose toxicology studies, findings in the male reproductive tract were observed in the testes/epididymis (decreased organ weight, atrophy or degeneration, decreased numbers of germinal cells, hypospermia or abnormal sperm forms, reduced sperm density and count) at ≥15 mg/kg/dose twice daily in mice and ≥1.5 mg/kg/dose twice daily in dogs. These doses correlate to systemic exposures (AUC) approximately 7 and 0.1 times, respectively, that in patients at the recommended starting dose in humans. Findings in the female reproductive tract in mice and dogs included signs of delayed sexual maturity, reduced or absent corpora lutea, decreased uterine weights and uterine atrophy at ≥5 mg/kg/day. This dose correlates to a systemic exposure (AUC) approximately 1.5 or 0.3 times, respectively, the exposure in patients at the recommended starting dose.

In a fertility study in mice, axitinib did not affect mating or fertility rate when administered to males at any dose tested up to 50 mg/kg/dose (approximately 57 times the AUC in patients at the recommended starting dose) following at least 70 days of administration. Reduced fertility and embryonic viability were observed in female mice at all doses tested (≥15 mg/kg/dose) following at least 15 days of treatment with axitinib. This dose correlates to a systemic exposure (AUC) approximately 10 times that in patients at the recommended starting dose. Data from repeat-dose toxicology studies and this fertility study indicate axitinib has the potential to impair reproductive function and fertility in humans.

In reproduction and developmental toxicity studies, axitinib was teratogenic, embryotoxic and fetotoxic at maternal exposures that were lower than human exposures at the recommended starting dose. During a fertility and early embryonic development study, axitinib administered to female mice prior to mating and through the first week of pregnancy caused an increase in post-implantation loss. In an embryo-fetal developmental toxicity study, pregnant mice received oral axitinib twice daily during the period of organogenesis. Embryo-fetal toxicities observed in the absence of maternal toxicity included malformations (cleft palate) and variations in skeletal ossification (interfrontal ossification sites, incomplete ossification of the supraoccipitals).

Standard genetic toxicology studies were conducted with axitinib. Axitinib was not mutagenic in an *in vitro* bacterial reverse mutation (Ames) assay and was not clastogenic in the *in vitro* human lymphocyte chromosome aberration assay. However, axitinib was genotoxic in the *in vivo* mouse bone marrow micronucleus assay.

1.3 Recommendations

1.3.1 Approvability

Approvable

There are no non-clinical findings that would preclude the approval of axitinib for the proposed indication.

1.1.2 Additional Non Clinical Recommendations None

1.1.3 Labeling See separate review

2 Drug Information

2.1 Drug Inlyta

2.1.1 CAS Registry Number (Optional) 319460-85-0

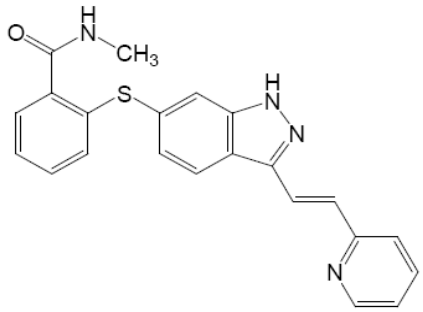
2.1.2 Generic Name axitinib

2.1.3 Code Name AG-013736

2.1.4 Chemical Name N-Methyl-2-[3-((E)-2-pyridin-2-yl-vinyl)-1H-indazol 6-ylsulfanyl]-benzamide

2.1.5 Molecular Formula/Molecular Weight $C_{22}H_{18}N_4OS$ / 386.47 Daltons

2.1.6 Structure



2.1.7 Pharmacologic class Kinase inhibitor

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

IND 63,662

2.3 Drug Formulation

Film coated immediate release tablet for oral administration

Composition of Axitinib 1 mg and 5 mg tablets

Component	Function	Reference to Standard	1 mg Theoretical Unit and/or Formula	5 mg Theoretical Unit and/or Formula
Axitinib	Active	Pfizer	1.000 mg[1]	5.000 mg[1]
Microcrystalline Cellulose[2]	(b) (4)	NF, Ph. Eur.,JP		(b) (4)
Lactose Monohydrate		NF, Ph. Eur.,JP		
Croscarmellose Sodium		NF, Ph. Eur.,JP		
Magnesium Stearate		NF, Ph. Eur.,JP		
(b) (4)				
Film Coat				
Opadry® II Red 32K15441		Pharmaceutical[3]		
Total Finished Tablet				

Note: NF=National Formulary; USP=the United States Pharmacopeia; Ph.Eur. =the European Pharmacopeia; JP=Japanese Pharmacopeia

(b) (4)

(excerpted from the Applicant's submission)

Composition of Opadry® 33 red (32K15441)

Name of Ingredients	Reference to Standard	Unit Formula (mg/tablet)	
		1 mg	5 mg
Hypromellose 2910 (b) (4)	USP, Ph. Eur, JP		(b) (4)
Titanium dioxide	USP, FCC, Ph. Eur, JP		
Lactose Monohydrate	NF, Ph. Eur, JP		
Triacetin (Glycerol Triacetate)	USP, FCC, JP, (Ph. Eur)		
Iron Oxide Red	NF, JPE		

(excerpted from the Applicant's submission)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

Axitinib drug substance contains the impurity (b) (4) which was demonstrated to be mutagenic in an *in vitro* Ames test. The proposed specification for controlling (b) (4) in the axitinib drug substance is (b) (4). At the level of (b) (4) would be administered orally to a patient receiving the maximum recommended daily dose of axitinib of 20 mg/day. This is equivalent to the toxicologic threshold of concern (TTC) for genotoxic impurities.[2] The proposed specification for (b) (4) in axitinib drug substance is acceptable.

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[1] Japan Chemical Industry Ecology-Toxicology & Information Center (JETOC), Japan; Mutagenicity Test Data of Existing Chemical Substances Based on the Toxicity Investigation System of the Industrial Safety and
Health Law, Supplement 3, 2005. Test summary result accessed via ToxNet: http://toxnet.nlm.nih.gov/

[2] United States Food and Drug Administration: Center for Drug Evaluation and Research, 2008.

The axitinib drug substance also contains (b) (4) as an impurity in the drug substance. The applicant proposed a specification for (b) (4) in the axitinib drug substance of (b) (4). Patients receiving the maximum recommended dose of axitinib, 20 mg/day, would be administered (b) (4) This is well under what would be considered a reasonable permitted daily exposure (PDE) (b) (4), so the proposed specification of (b) (4) in axitinib drug substance is acceptable.

2.6 Proposed Clinical Population and Dosing Regimen

The starting dose is 5 mg orally twice daily for the treatment of patients with advanced renal cell carcinoma.

2.7 Regulatory Background

Original IND (63,662) was submitted to the FDA on November 9, 2001.

3 Studies Submitted

3.1 Studies Reviewed

Studies were reviewed by Anwar Goheer, Ph.D. unless otherwise specified within each section.

PHARMACOLOGY

Primary Pharmacodynamics

Studies reviewed by Alexander H. Putman, Ph.D.

1. Biochemical Measurements of Receptor Tyrosine Kinase (Rtk) Inhibitory Potencies and Selectivity of AG-013736 (Axitinib). Study # AG-013736-NonclinPharm-001
2. Cellular Target Potency, Selectivity, and Functional Activity of AG-013736 (Axitinib). Study # AG-013736-NonclinPharm-002
3. AG-013736 *In Vivo* Target Modulation, Induction of Vascular Phenotypical Changes, Efficacious Plasma Concentrations, and Pharmacokinetics-Pharmacodynamics Correlation. Study # AG-013736-NonclinPharm-003
4. *In Vivo* Anti-Tumor Efficacy, Anti-Angiogenesis, and Biomarkers of AG-013736 (Axitinib) In Rodent Models of Cancer. Study # AG-013736-NonclinPharm-004

Secondary Pharmacodynamics

Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches.

Study reviewed by Robeena Aziz, MPH, Ph.D.

1. Profiling Screen Data Report: To evaluate, in Radioligand Binding, and Tissue assays, the activity of compound AGP-58 (PT# 1010519).

Safety Pharmacology

Studies reviewed by Robeena Aziz, MPH, Ph.D.

Neurological effects:

1. Irwin Dose-Range in Mice Including Body Temperature and Locomotor Assessment. Study # VFF/488.

Pulmonary effects:

1. Evaluation of Respiratory Parameters in the Conscious Rat Using Whole Body Bias Flow Plethysmography. Study # VFF/491.

Gastrointestinal effects:

1. Charcoal Propulsion Test in Mice Following Oral Administration. Study # VFF/489.
2. Assessment of Effects on Gastric Emptying in Rats Following Oral Administration (Phenol Red Method). Study # VFF/490.

Cardiovascular effects:

1. Assessment of the Potential Effect of AG-013736 on *hERG* Potassium Current Stably Expressed in HEK293 Cells. Study # AG013736HERG.
2. Ki Determination of AG-028458 (sulfoxide metabolite of AG-013736) in a Dofetilide Fluorescence Polarization Binding Assay. Study # DOF_GBLFP1_E-02102006.
3. Assessment of the Effect of PF-04621675 on hERG Potassium Current Stably Expressed in HEK293 Cells. Study # SP107.
4. Evaluation of Cardiovascular Effects of Oral AG-013736 in Mice. Study # SP4002.
5. Evaluation of Cardiovascular Effects of Oral AG-013736 in Mice. Study # SP4002-2.
6. Evaluation of Cardiovascular Effects of Oral AG-013736 in Mice. Study # SP0304.
7. Telemetric Evaluation of Cardiovascular Effects in the Conscious Dog. Study # VFF/492
8. Assessment of the Effects of AG-013736 on Blood Pressure and Heart Rate in Conscious, Telemetered Beagle Dogs. Study # SPT04-029.

GENERAL TOXICOLOGY

Single-dose toxicity

1. Single Dose Oral Gavage Toxicity and Toxicokinetic Study with AG-013736 in Dogs with a 14-Day Observation Period. Study # 6348-505.

Repeat-dose toxicity

1. 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with AG13736 in Mice with a 13-Week Interim Sacrifice and 4-Week Recovery Period. Study # 6750-148.
2. 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with AG13736 in Dogs with a 4-Week Recovery Period. Study # 6750-150.
3. 9-Month Oral Gavage Toxicity and Toxicokinetic Study of AG-013736 in Dogs with 8-Week Recovery. Study # 6348-470.

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Fertility and early embryonic development

1. Combined Oral (Gavage) Fertility and Developmental Toxicity Study in Mice Evaluating Twice Daily Administration of AG-013736. Study # LIA00238.

Embryofetal development

1. Oral (Gavage) Dosage-Range Finding Embryo-Fetal Development Toxicity Study in Mice Evaluating Twice Daily Administration of AG-013736. Study # LIA00236.
2. Oral (Gavage) Developmental Toxicity Study in Mice Evaluating Twice Daily Administration of AG-013736. Study # LIA00237.
3. Oral Dose Range-Finding Study of AG-013736 in Pregnant Rabbits. Study # 06GR178.

SPECIAL TOXICOLOGY

Local tolerance

1. Local Vascular Tissue Irritation Study ofAG-013736 in Female New Zealand White Rabbits. Study # 05VEG009.

Other toxicology studies

1. Determination of the Phototoxic Potential of AG-013736 in The 3T3 Neutral Red Uptake (NRU) Phototoxicity Assay. Study # 05228.

2. Single Dose Oral (Gavage) Phototoxicity Study of AG-013736 in Hairless Mice. Study # RSA00087.
3. AG-013736: Report for the In Vitro Compatibility of the Parenteral Formulation with Human Blood. Study # SA1000.

3.2 Studies Not Reviewed

PHARMACOKINETICS/TOXICOKINETICS

1. Method Validation for AG-013736 in Dog Plasma Using LC/MS/MS. Study # 6750-300.
2. Method Validation for AG-013736 (Lower LLOQ) in Dog Plasma Using LC/MS/MS. Study # 6750-302.
3. Analytical Validation Report for a Liquid Chromatography / Tandem Mass Spectrometry (LC/MS/MS) Method for the Quantification of AG013736 in Mouse Plasma. Study # AG013736-PDM-006.
4. LC/MS/MS Analysis of AG13736 in Dog Plasma To Support (b) (4) Study Number 6750-143. Study # 197-09-12141.
5. To Investigate the Freezer Stability of AG13736 in Human Plasma at -70 and -20° C. Study # AG13736-PDM-025.
6. To Investigate the Light Stability of AG13736 in Human Plasma. Study # AG13736-PDM-026
7. To Investigate the Light Stability of AG13736 in Human Blood. Study # AG13736-PDM-027.
8. Analytical Re-Validation Report for a Liquid Chromatography / Tandem Mass Spectrometry (LCIMS/MS) Method for the Quantification of AG013736 in Dog Plasma. Study # 197-06-12140.
9. Validation of a High Performance Liquid Chromatographic-Mass Spectrometric Method for the Analysis of AG013736 and PF-3482595 in K3 EDTA Mouse Plasma. Study # LIA00260LX-06-988.
10. Long-Term Matrix Stability Assessment of AG-013736 and PF-3482595 in K3 EDTA Rabbit Plasma. Study # LIA00243SX-07-503
11. Validation of a High Performance Liquid Chromatographic-Mass Spectrometric Method for the Analysis of AG013736 and PF-3482595 in K3 EDTA Rabbit Plasma. Study # LIA00242LX-06-1118.
12. Long-Term Stability Assessment of AG-013736 and PF-3482595 in K3 EDTA Rabbit Plasma. Study # LIA00326SX-07-1205.
13. Validation of a High Performance Liquid Chromatographic-Mass Spectrometric Method for the Analysis of AG013736 and PF-3482595 in K3 EDTA Rabbit Plasma. Study # LIA00307LX-08-077.
14. Validation of a High Performance Liquid Chromatographic-Mass Spectrometric Method for the Analysis of AG013736 and PF-3482595 in K3 EDTA Dog Plasma. Study # RSA00018LX-05-220.

Absorption

1. Pharmacokinetics and Bioavailability of Ag13736 in the Rat and Monkey. Study # AG013736-PDM-001.
2. Pharmacokinetics and Bioavailability of A613736 in Mice. Study # AG013736-PDM-012
3. Pharmacokinetics and Bioavailability of AG13736 in the Dog. Study # AG13736-PDM-013
4. Permeability of AG-013736 in the Caco-2 Cell Culture Model. Study # AG13736-PDM-021.
5. Pharmacokinetics from Oral Administration of AG13736 to Wistar Rats ([redacted] (b) (4) Study No. VFFI526). Study # AG13736-PDM-022.
6. Pharmacokinetics and Bioavailability of AGO13736 Clinical Tablets and Suspension in the Male Beagle Dog. Study # AG13736-PDM-033.
7. Pharmacokinetic Analysis of AG-013736 and AG-028458 in Plasma From A Study Conducted in Beagle Dogs Administered AG-013736 ([redacted] (b) (4) Study Number SPT04-029). Study # AG13736-PDM-047.
8. Pharmacokinetics of AG-013736 in Mice Following an Oral Bid Dose Administration. Study # AG13736_15Jul10_132224.
9. Toxicokinetics of AG-013736 in Mice Following Oral Administration. Study # AG-013736-SP-4009-2BA.

Distribution

1. In Vitro Binding of AG-013736 in Human Albumin and Human α1-Acid Glycoprotein. Study # 8216659.
2. (^{14}C)-AG13736: Tissue Distribution, Blood and Plasma Pharmacokinetics and Excretion of Radioactivity Following a Single Oral Administration (50 mg/kg) To the Mouse. Study # AG013736-PDM-004.
3. Determination of Protein Binding and Blood to Plasma Concentration Ratio for AG13736 in Mouse, Dog, and Human. Study # AG013736-PDM-017.
4. Protein Binding of AG-013736 in Rat Plasma. Study # AG013736/22Jan09/171550.

Metabolism

1. *In Vitro* Metabolism of AG13736 in Human and Animal Liver Microsomes and Hepatocytes. Study # AG013736-PDM-003.
2. The Identification of Human Cytochrome P450 Isoforms Involved in the Metabolism of AG13736. Study # AG013736-PDM-019.
3. Profiling and Structure Elucidation of Metabolites of AG-013736 in Beagle Dogs Following a Single Oral Dose of [^{14}C]AG-013736. Study # AG013736-PDM-037.
4. Profiling and Structure Elucidation of Metabolites of AG-013736 in Mice Following a Single Oral Dose of [^{14}C]AG-013736. Study # AG013736-PDM-038.
5. Profiling and Identification of Metabolites of AGO13736 in Healthy Male Volunteers Following a Single Oral Dose of [^{14} C] AG-013736. Study # AG013736-PDM-043.

6. Identification of UDP-Glucuronosyl Transferases (UGT) Responsible for the Glucuronidation of AG-013736 in Humans. Study # AG013736-PDM-048.
7. AG-013736 Incubations with Aroclor-Induced Rat S-9 Fraction. Study # AG013736-PDM-052.
8. Non-involvement of Human Liver FMOs and Human Liver Sulfoxide Reductase in Metabolism of AG-013736 *in Vitro.* Study # AG013736-PDM-060.
9. Definitive Phenotyping and Kinetic Characterization of AG-013736 Metabolism. Study # AG013736/16Jan09/162654.
10. Identification of AG-013736 Metabolites Generated *In Vitro* By Recombinant CYP3A4, CYP3A5, CYP1A2, and CYP2C19. Study # AG013736/20Mar09/181146.

Excretion

1. Excretion of AG13736 Following A Single Oral Dose of [^{14}C]-AG13736 to Male CD-1 Mice. Study # AG013736-PDM-029.
2. Excretion of AG13736 Following A Single Oral Dose of [^{14}C]-AG13736 to Beagle Dogs. Study # AG013736-PDM-036.

Pharmacokinetic drug Interactions

1. Effects of AG-013736 on CYP450 Enzyme Regulation in Primary Cultures of Human Hepatocytes. Study # RR764-05388.
2. Evaluation of Inhibition of The Catalytic Activity of cDNA-expressed Human Uridine 5'-Diphosphoglucuronosyl Transferase (UGT) 1A1 by The Test Substance, AG-013736. Study # 301040127.
3. Evaluation of AG13736 as an Inhibitor of Human Cytochrome P450. Study # AG013736-PDM-020.
4. Evaluation of the CYP Inhibition Characteristics of AG-013736 Towards CYP1A2 and of AG-013887 Towards CYP1A2, CYP2C8 and CYP3A4. Study # AG013736-PDM-039.
5. Drug-Drug Interaction Risk Assessment for Ag-013736 As A Perpetrator Using Simcyp. Study # AG-013736/05Jun09/161413.
6. Simulation of Intestinal Concentrations of Axitinib Using Gastroplus. Study # AG-013736/09Apr09/154709.
7. Permeability and Transport Evaluations of AG-013736. Study # AG-013736/10Dec08/111529.
8. Simulation of the Effects of A CYP3A Inhibitor and Inducer on the Pharmacokinetic of Axitinib (AG-013736) Using Simcyp®. Study # AG-013736/19Jun09/190355.
9. The *In Vitro* Study of P-Glycoprotein Inhibition by AG-013736 (Axitinib) In Caco-2 Cells. Study # AG-013736/21Jan09/055543.

Other Pharmacokinetic Studies

1. Determination of the Human Plasma Compatibility of the AG-013736 Clinical Intravenous Formulation. Study # AG-013736-PDM-049.
2. Pharmacokinetics of AG-01373 [redacted] (b) (4) in Dogs. Study # AG-013736-PDM-050.

3.3 Previous Reviews Referenced

See attachment 1 for reviews

PHARMACOKINETICS/TOXICOKINETICS

1. Determination of plasma levels in rats following oral administration ([redacted] (b) (4) study # VFF/526). [redacted] (b) (4)

GENERAL TOXICOLOGY

Single-dose toxicity

1. Acute oral toxicity study in mice with AG013736 ([redacted] (b) (4) study # 22337-0-800). [redacted] (b) (4) unaudited draft report. Vol. 01, page 238.

Repeat-dose toxicity

1. 14-Day gavage toxicity study with AG013736 in mice ([redacted] (b) (4) study # 6750-144). [redacted] (b) (4) audited draft report. Vol. 03, page 001.
2. 28-Day gavage toxicity study with AG013736 in mice ([redacted] (b) (4) study # 6750-145). [redacted] (b) (4) audited draft report. Vol. 04, page 115.
3. 14-Day gavage toxicity study with AG013736 in dogs ([redacted] (b) (4) study # 6750-142). [redacted] (b) (4) unaudited draft report. Vol. 05, page 264.
4. 28-Day gavage toxicity study with AG013736 in dogs ([redacted] (b) (4) study # 6750-143). [redacted] (b) (4) audited draft report. Vol. 06, page 107.

GENETIC TOXICOLOGY

1. Microbial Reverse Mutation Assays. Study # 01-2191-02.
2. *In Vitro* Cytogenetic Assays. Study # 01-2191-03.
3. Mouse Micronucleus Assay. Study # 01-2191-01.

4 Pharmacology

4.1 Primary Pharmacology

Studies in this section were reviewed by Alexander H. Putman, Ph.D.

Study Title: AG-013736-NonclinPharm-001: Biochemical Measurements of Receptor Tyrosine Kinase (RTK) Inhibitory Potencies and Selectivity of AG-013736

AG-013736 was evaluated for its inhibitory activity against its intended target kinases, VEGFRs, and other kinases in the Type III, IV, and V receptor tyrosine kinase family. As shown in the summary table of results below, AG-013736 is an inhibitor of VEGFR-1 (Flt-1), various forms of the kinase VEGFR-2 (FLVK), and PDGFR-β. AG-013736 was more potent against the non-phosphorylated form of VEGFR kinases compared to the phosphorylated form.

Enzyme Inhibitory Activities of AG-013736 against Type III -V Family Kinases

Kinase	Ki (nM)	% Inhibition (axitinib concentration)	Experimental Conditions
VEGFR-2-FLVK	1.10	76 (0.05μM)	10 nM recomb catalytic domain, 40 mM Mg^{2+}, 10 mg/mL PGT, 8 mM ATP (for Ki) or 3 mM ATP (for % Inhition)
Phospho-VEGFR-2 -FLVK	7.20	43 (0.05μM)	40 nM recomb enzyme, 8mM ATP, 20 mM Mg^{2+}, 5.1 mg/mL PGT
VEGFR-2-Kin	0.740	73 (0.05μM)	10 nM recomb intracellular domain, 8 mM ATP, 40 mM Mg^{2+}, 10 mg/mL Activation loop peptide
Phospho-VEGFR-2 -Kin	21.7	24 (0.05μM)	40 nM recomb catalytic domain, 1 mM ATP, 40 mM Mg^{2+}, 10 mg/mL PGT
VEGFR-2/FLK-1	ND	81 (0.05μM)	27.5 nM recomb catalytic domain, 3 mM ATP, 60 mM Mg^{2+}, 2 nM Mn^{2+}, 20 mg/mL PGT
Phospho-VEGFR-2/ FLK-1	ND	40 (0.05μM)	25 nM recomb catalytic domain, 1 mM ATP, 20 mM Mg^{2+}, 5.1 mg/mL PGT
VEGFR-1/Flt-1	2.75	75 (0.05μM)	40 nM recomb catalytic domain, 3 mM ATP, 40 mM Mg^{2+}, 2 mM Mn^{2+}, 20 mg/mL PGT
PDGFR-β	1.27	82 (0.05μM)	76 nM recomb catalytic domain 558-1090aa, GST fusion, 2 mM ATP, 60 mM Mg^{2+}, 2.5 mg/mL PGT
CSF-1R	28.1	20 (0.05μM)	10 nM recomb catalytic domain, GST fusion, 2 mM ATP, 15 mM Mg^{2+}, 1 mM Met2 peptide
FGFR-1	47.6	75 (1μM)	124 nM recomb catalytic domain, 3 mM ATP, 60 mM Mg^{2+}, 15 mg/mL PGT
Phospho-FGFR-1	56.7	75 (1μM)	25 nM recomb catalytic domain, 1 mM ATP, 20 mM Mg^{2+}, 5.1 mg/mL PGT

All tests were conducted at Pfizer La Jolla (PGRD). The tests were performed in the presence of 50 nM recombinant enzymes; the data were fitted with tight-binding kinetic equations. ATP: Adenosine 5'-Triphosphate; CSF-1R: Colony scatter factor receptor-1; FGFR-1 kinase: human recombinant FGF receptor-1 kinase domain; FLVK: fibroblast growth factor (FGF)-like VEGFR-2 kinase; K_i: inhibitory constant; Met2: Receptor for hepatocyte growth factor; PGT: poly(Glu_4Tyr); PDGFR-β: GST fusion protein containing the amino acid residues between 558-1090 of PDGFR-β; Phospho-FGFR-1: phosphorylated human recombinant FGF receptor-1 kinase domain; Phospho-VEGFR-2/FLK-1: phosphorylated mouse VEGFR-2 kinase domain; phospho-VEGFR-2-FLVK: phosphorylated FGF-like VEGFR-2 kinase; phospho-VEGFR-2-K_{in}: phosphorylated intracellular domain of VEGFR2; LJ: La Jolla.

(table excerpted from Applicant's package)

AG-013736 was also evaluated for its inhibitory activity against a variety of other kinases using panels that represent a cross-section of the approximately 500 known kinases. Results from the in-house (Pfizer-RTC, Cambridge, MA and former Agouron Pharmaceuticals/Warner-Lambert, La Jolla, CA) and external vendor (b) (4) kinase screening are summarized in the following two figures, respectively. At the 1 μM concentration, AG-013736 only inhibited 10 non-PDGFR family kinases by more than 50%; Aurora-A (Aurora-2), Abl, Arg (murine), AMPK, Axl, MST2, MAPKAP-K1a, TRK-A, MAP4K4, and MARK1.

Summary of Kinase Selectivity of AG-013736 Measured by Pfizer

Other Kinases	Ki (nM)	%Inhibition (1 μM AG-013736)	Site of Assay	Experimental Conditions
Abl	ND	99.1	RTC /Pfizer	Recomb enzyme, Km-level of ATP
AKT1	ND	-4.05	RTC /Pfizer	Recomb enzyme,, Km-level of ATP
Aurora-A	1.95 (IC50)	93.2	RTC /Pfizer	Recomb enzyme, Km-level of ATP
BTK	ND	0	LJ/Pfizer	250 nM recomb catalytic domain, 3 μM ATP, 60 mM Mg^{2+}, 20 mM PGT
BTK	ND	-13.5	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
CDK1/CyclinB	ND	0	LJ/Pfizer	370 pM recomb enzyme, 25 μM ATP
CDK2/CyclinA	ND	2	LJ/Pfizer	150 nM recomb enzyme, 250 μM ATP
CDK2/CyclinA	ND	-6.73	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
CDK4/CyclinD3	ND	7	LJ/Pfizer	50 nM recomb enzyme, 250 μM ATP
CDK6/cyclinD3	ND	0.65	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
CHK1	ND	0	LJ/Pfizer	10 nM CHK1-KH289 kinase domain, 150 μM ATP, 135 μM Syntide2
CHK1	ND	1.32	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
CHK2	ND	2.43	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
CK2	ND	-15.5	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
CKIδ	ND	16.6	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
CLK1	ND	-6.48	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
cSRC	ND	3	LJ/Pfizer	11nM recomb catalytic domain, 2 mM ATP, 20 mM Mg^{2+}, 10 mM PGT
ECK	ND	0.955	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
EGFR	ND	14.9	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
FAK	ND	12	LJ/Pfizer	100 nM recomb catalytic domain 409, 0.2 mM ATP, 40 mM Mg^{2+}, 1mM FAKtide
FGFR-1	ND	89.1	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
GSK3β	ND	-7.86	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
HGFR-P	2080	11	LJ/Pfizer	25 nM recomb catalytic domain, 75 μM ATP, 500 μM Met2 peptide
IKKβ	ND	0.353	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
IKK_i	ND	6.53	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
INSR	ND	-3.89	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
IRK-P	ND	6	LJ/Pfizer	25 nM recomb catalytic domain, 2 mM ATP, 40 mM Mg^{2+}, 15 mM PGT
JAK3	ND	1.07	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
LCK	ND	7.01	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
LCK_P	ND	9	LJ/Pfizer	113 nM recomb catalytic domain, 1 mM ATP, 40 mM Mg^{2+}, 25 mM PGT
MAP3K9	ND	27.6	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
MAP4K4	ND	51.9	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
MAPK1/ERK2	ND	13.2	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
MAPKAP-K2	ND	-7.52	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
MARK1	ND	50.4	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
MASK	ND	13.3	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
MET	ND	21.6	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
MST2	ND	31.3	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
MYLK2	ND	-15	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
NEK2	ND	-6.45	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
PAK4	ND	6.93	LJ/Pfizer	25 nM recomb catalytic domain, 40 μM ATP, 10 mM Mg^{2+}, 400 μM Peptide7
PAK4	ND	8.25	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
PAK4_S474A	ND	14.5	LJ/Pfizer	100 nM recomb catalytic domain S474A mutant, 555 μM ATP, 10 mM Mg^{2+}, 500 μM Peptide7

Other Kinases	K_i (nM)	%Inhibition (1 µM AG-013736)	Site of Assay	Experimental Conditions
PDK-1	ND	14.3	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
PDK1	ND	-5.94	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
PIM2	ND	5.03	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
PIM2	ND	9.89	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
PKA	ND	7.5	LJ/Pfizer	4 nM bovine heart, 100 µM ATP, 200 µM Kemptide peptide
PKA	ND	3.7	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
PKCα	ND	10.8	LJ/Pfizer	2.4 nM recomb full length enzyme, 100 µM ATP, 200 µM Selectide peptide
PKCβII	ND	1.69	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
PKCζ	ND	6.69	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
PLK	ND	0	LJ/Pfizer	10 nM PLK(37-336), 25 µM ATP
PYK2-P	ND	0	LJ/Pfizer	40 nM recomb enzyme, 1 mM ATP, 40 mM Mg^{2+}, 20 mM PGT
PYK2-P	ND	2	LJ/Pfizer	40 nM recomb enzyme, 1 mM ATP, 40 mM Mg^{2+}, 20 mM PGT
ROCK1	ND	1.08	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
S6K_T389E_D3E	ND	10.7	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
SAPK2A/p38	ND	-0.223	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
SGK	ND	-0.32	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
SYK	ND	10.5	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
TAOK3	ND	7.3	RTC /Pfizer	Recomb enzyme, K_m-level of ATP
TIE2-P	ND	45	LJ/Pfizer	18.2nM recomb catalytic domain, 0.1 mM ATP, 40 mM Mg^{2+}, 250 mM Tietide
TRK-A	ND	56.9	RTC /Pfizer	Recomb enzyme, K_m-level of ATP

[a]All kinases were of human origin.

(table excerpted from Applicant's package)

Summary of Kinase Selectivity AG-013736 Measured by External Vendor Kinase Screening

Enzyme	Source	% Inhibition (1 µM AG-013736)	Enzyme	Source	% Inhibition @ 1 µM
AMPK (r)	(b) (4)	84	MKK1 (rb)	(b) (4)	28
Arg (m)		94	MKK4 (m)		-2
Aurora-A		101	MKK6		14
Axl		76	MKK7ß		-5
Bmx		18	MSK1		23
CDK2/cyclin A		18	MST2		72
CHK1		13	NEK2		27
CK1		16	NEK6		4
CK2		19	p70 S6K		24
CSK		41	PAK2		11
DYRK1α		20	PDGFR-α		101
EGFR		7	PDK1		37
EphB2		-2	PHK		1
FGFR-3		90	PKA		10
Flt-3		39	PKBδ PH		-2
GSK3β		0	PKCa		9
IGF-1R		27	PRAK		38
IKKß		8	ROCK-II		19
IR		5	SAPK2a/p38		21
JNK/SAPK1c		11	SAPK2b/p38ß2		2
MAPK2/ERK2 (m)		2	SAPK3/p38γ		12
MAPKAP-K1a		59	SAPK4/p38∂		-1
MAPKAP-K2		6	SGK		20
			ZAP-70 (rb)		1

[a]All kinases were of human origin except (m) - mouse, (r) - rat, (rb) - rabbit.
Note: Data can be found in Pfizer global database and in the local database "Kinase Selectivity 13736_9-28-2006.xls."
FLVK: fibroblast growth factor (FGF)-like VEGFR-2 kinase; Phospho-FLVK: phosphorylated FGF-like Lck: lymphocyte kinase; c-Src: Sarcoma kinase; FAK: focal adhesion kinase; Phospho-Pyk2: proline-rich tyrosine kinase 2; Phospho-IRK: insulin receptor kinase; BTK: Bruton's tyrosine kinase; CDK4: cyclin-dependent kinase 4; CDK2: cyclin-dependent kinase 2, CDK1: cyclin-dependent kinase 1, PKA: protein kinase A, PKC: protein kinase C, PLK: Polo-like kinase; Chk1: checkpoint kinase 1; Phospho-cMet: HGFR, hepatocyte growth factor receptor; EphB2: epherin B2 receptor; IGF-1R: insulin growth factor receptor-1.

(table excerpted from Applicant's package)

Study Title: AG-013736-NonclinPharm-002: Cellular Target Potency, Selectivity, and Functional Activity of AG-013736 (Axitinib)

To further characterize the activity of AG-013736 against a variety of cellular targets, including the Type III, IV, and V receptor tyrosine kinase family proteins (VEGFR-1, -2, and -3, PDGFR-α and -β, KIT, FGFR-1, Flt-3, and CSF-1R), three types of cellular assays were utilized. The first was an ELISA-based VEGFR-2 phosphorylation assay in transfected porcine aorta endothelial (PAE) cells, in the presence of 0.1% FBS and a low level of bovine serum albumin (BSA, 0.045%). The second was an immunoprecipitation and Western blot-based (IP/IB) assay using human umbilical vein endothelial cells (HUVEC) in the presence of 1% FBS + 2.3% BSA. The third, a VEGF-mediated cell survival assay in HUVEC in the presence of 1% FBS + 2.3% BSA, was based on the necessity of VEGF and bFGF as survival factors for endothelial cells. (b) (4)

(b) (4)

Key findings from cell-based assays and western-blot based assays are summarized in the tables below, respectively. Overall, the results from these studies suggest that AG-013736 inhibits the activity of VEGFR-1, -2, and -3 (in the sub-nanomolar range) and PDGFR and KIT (in the nanomolar range). Specifically, in the cell, AG-013736 inhibited VEGF-mediated autophosphorylation of VEGFR-1, 2 and 3, with IC_{50}s of 0.09-0.12 nM, 0.2±0.06 nM, and 0.1-0.29 nM, respectively. AG-013736 had weaker inhibitory activity against PDGFR-α, PDGFR-β, and KIT, with IC_{50}s of 5.0±1.0 nM, 1.6±0.4 nM, and 1.7±0.6 nM, respectively. AG-013736 was not a potent inhibitor of any other RTKs tested, including CSF-1R, Flt-3, FGFR-1, RET, EGFR, and cMet. Furthermore, in the VEGF-mediated cell survival assay, AG-013736 inhibited VEGF-mediated HUVEC survival with an IC_{50} of 0.24±0.09 nM and demonstrated approximately 1000-fold greater selectivity for VEGFR-2 versus FGFR-1.

Summary of Results from Cell-based Assays

Assay	Cell Line	Stimulant	IC_{50} (nM)	IC_{50} (ng/mL)
Receptor Phosphorylation Assays				
VEGFR-2	VEGFR-2/PAE	VEGF-A	0.20 ± 0.06	0.08 ± 0.02
VEGFR-1	HUVEC	VEGF-A	0.09 - 0.12	0.03 - 0.05
VEGFR-3	VEGFR-3/PAE	VEGF-C	0.10 - 0.29	0.04 - 0.11
PDGFR-β	PDGFR-β/PAE	PDGF-BB	1.60 ± 0.4	0.62 ± 0.2
KIT	KIT/PAE	SCF	1.70 ± 0.6	0.66 ± 0.2
Receptor Phosphorylation Assays in Tumor Cells Lines				
Murine VEGFR-2	mVEGFR-2/ NIH3T3	VEGF-A	0.18 ± 0.03	0.07 ± 0.01
PDGFR-α	PDGFR-α/ NIH3T3	PDGF-AA	5.0 ± 1.0	1.9 ± 0.4
CSF-1R	CSF-1R/NIH3T3	M-CSF	73 ± 18	28 ± 6.8
Flt-3	RS;411	Flt-3 ligand	>1000	> 388
Growth Factor-Mediated Survival Assays				
VEGF-HUVEC[a]	HUVEC	VEGF	0.24 ± 0.09	0.09 ± 0.03
bFGF-HUVEC[a]	HUVEC	bFGF	237.9 ± 88.9	92.2 ± 34.4
VEGF-HUVEC[b]	HUVEC	VEGF	2.4 ± 0.9	0.9 ± 0.3
bFGF-HUVEC[b]	HUVEC	bFGF	2479 ± 1188	961 ± 460
Proliferation Assays in Tumor Cell Lines[c]				
MV522 MTT	MV522	10% FBS	>10,000	NA
LLC MTT	LLC	10% FBS	>10,000	NA
M24met MTT	M24met	10% FBS	>10,000	NA

Note: All assays were performed in the presence of 0.045% BSA unless otherwise noted. IC_{50}s are expressed as average ± standard error of the mean (SEM), or a range if only two assays were performed. IC_{50} = 50% inhibitory concentration; PAE = porcine aorta endothelial cells; SCF = stem cell factor; M-CSF = macrophage colony stimulating factor; HUVEC = human umbilical vein endothelial cells; MTT = 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay; FBS = fetal bovine serum; NA = not applicable.
[a] Assay performed in the presence of 1% fetal bovine serum without bovine serum albumin (BSA).
[b] Assay performed in the presence of 1% fetal bovine serum and 2.3% bovine serum albumin (BSA).
[c] After adjust for protein binding; Assay performed in the presence of 1% fetal bovine serum and 2.3% bovine serum albumin
[d] Measured by the immunoprecipitation and immunoblotting method.

Summary of Results from Western Blot Assays

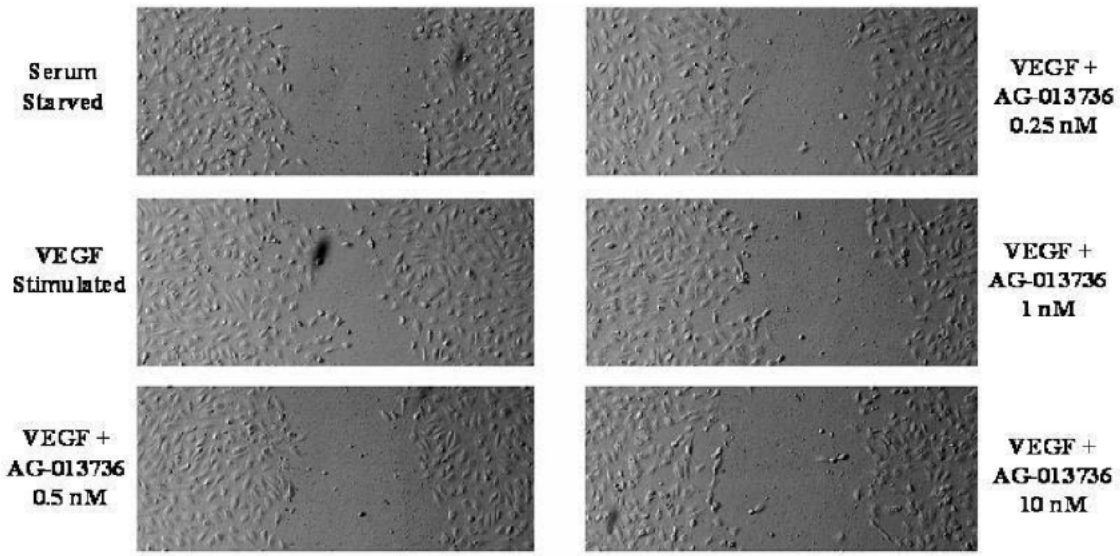
Target	VEGFR-2		VEGFR-1	VEGFR-3	PDGFR-β	KIT
Cells	HUVEC	HUVEC	HUVEC	VEGFR-3/PAE	Hras-NIH/3T3	NCI H526
Condition	1% FBS + 2.3% BSA	1% FBS	1% FBS + 2.3% BSA	0.1% FBS	2.3% BSA	0.5% FBS + 2.3% BSA
Stimuli	VEGF-A	VEGF-A	VEGF-A	VEGF-C	PDGF-BB	SCF
Observed IC_{50} (nM) (95% CI)	4.1 (3.1 - 5.4)	NA	1.0 (0.88 - 1.2)	NA	28.6 (12.6 - 64.7)	9.8 (5.5 - 17.6)
Protein Binding Corrected IC_{50} (nM) (95% CI)	0.4	0.06	0.1	0.1 (0.08 - 0.12) and 0.29	2.9	0.98

FBS = fetal bovine serum; BSA = bovine serum albumin. NA: not applicable. CI: confidence interval

(tables excerpted from Applicant's package)

To determine whether AG-013736 could inhibit growth factor-mediated cell migration on extracellular matrix proteins, a cell migration assay was performed (b) (4) [redacted] As shown below, AG-013736 appeared to partially inhibit HUVEC migration towards the open space in the VEGF-mediated culture conditions. According to the Applicant, AG-013736 had no effect on cell migration in the PDGF-mediated culture conditions (data not shown).

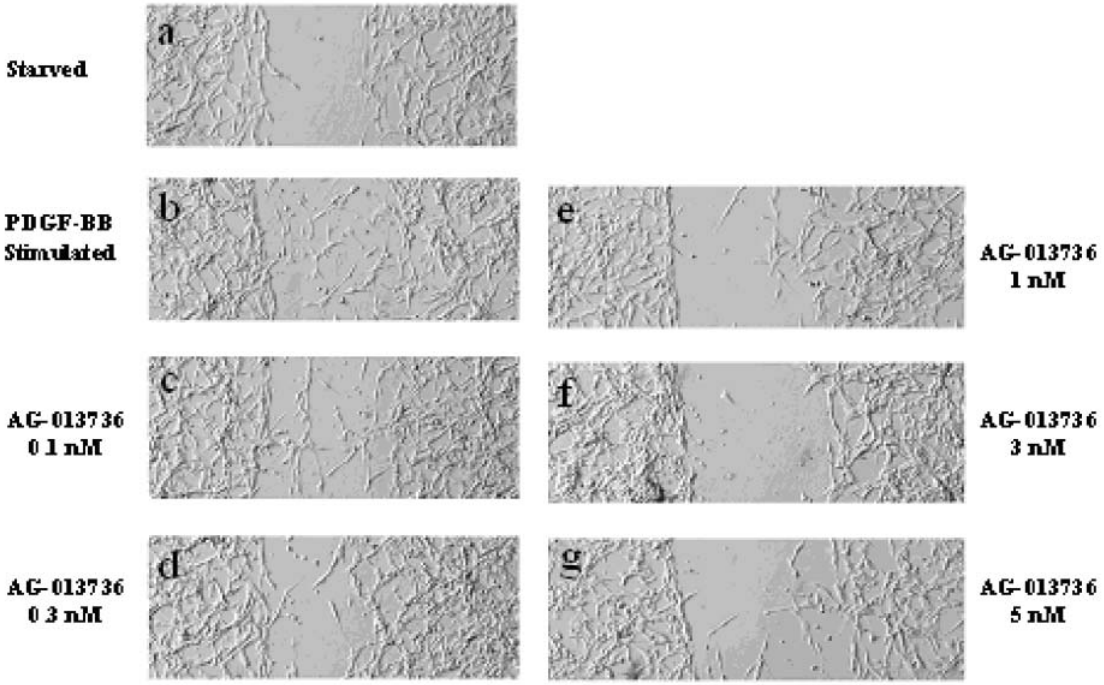
AG-013736 Inhibited VEGF-mediated HUVEC Migration (15 hour visualization)



(figure excerpted from Applicant's package)

Using the same assay with U87MG cells expressing a high level of PDGFR-β, AG-013736 blocked PDGF-BB mediated U87MG migration in a dose-dependent manner (See figure below).

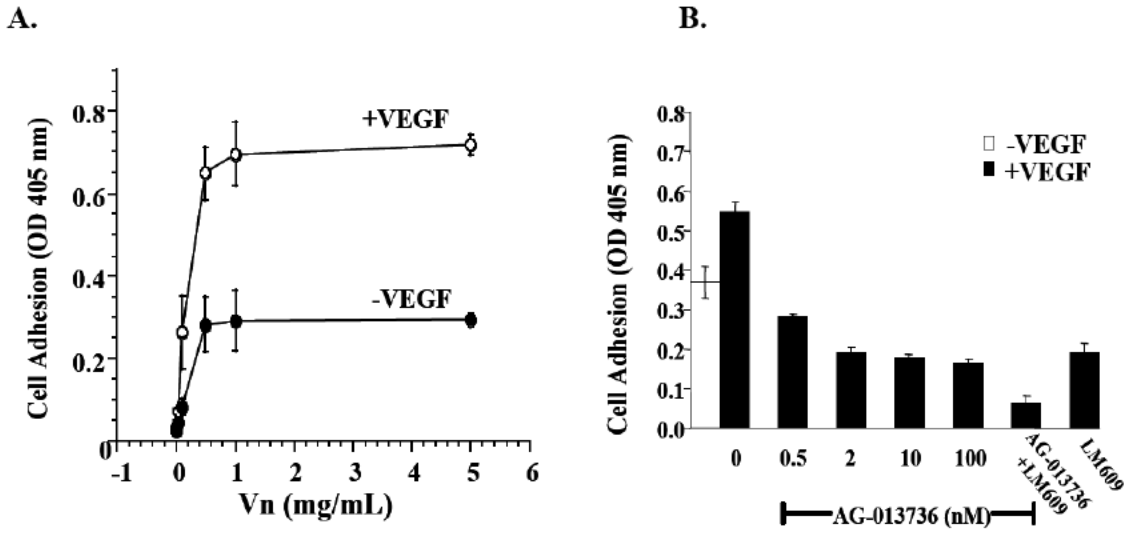
AG-013736 Inhibited PDGF-BB-mediated U87MG Migration (15 hour visualization)



(figure excerpted from Applicant's package)

An additional experiment was conducted to determine whether AG-013736 could inhibit HUVEC adhesion on the extracellular matrix protein vitronectin (Vn), a ligand for integrin avb3 that is highly expressed on endothelial cells. As shown below, the addition of VEGF in the media enhanced HUVEC adhesion to Vn compared to 1% FBS alone (A). According to the Applicant, the increase in cell adhesion was not likely due to the mitogenic function of VEGF because the adhesion event reached a plateau in 2 hours, a timeframe much shorter than the doubling time of the cells (~ 24 hours). AG-013736 dose-dependently inhibited this VEGF-induced HUVEC adhesion to Vn, with a maximal inhibitory effect ~ 68% (B). Combining AG-013736 with LM609 (20 µg/mL), a monoclonal antibody against integrin avb3, reduced cell adhesion by nearly 90% of cell adhesion (B).

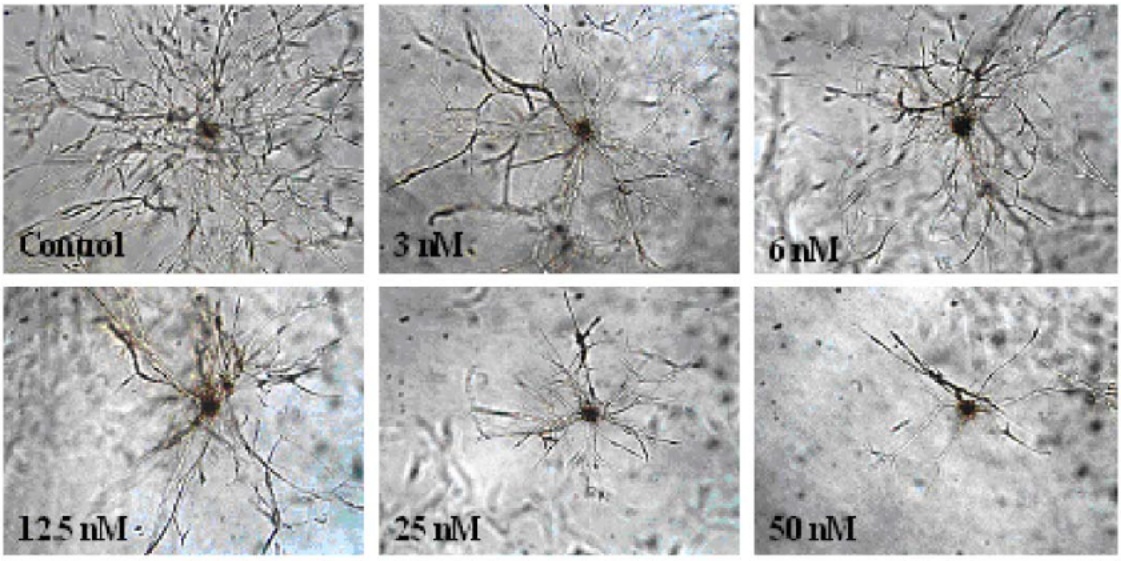
AG-013736 Inhibited VEGF-stimulated HUVEC Adhesion to Vitronectin



(graphs excerpted from Applicant's package)

AG-013736 was also evaluated for its ability to inhibit vascular sprouting and tube formation in an *in vitro* 3-D fibrin system. (b) (4) In this system, AG-013736 demonstrated dose-dependent inhibition of vascular sprouting and tube formation, as shown below.

AG-013736 Inhibited 3-D Tube Formation from Spheroidal Endothelial Cells in the Fibrin Matrix

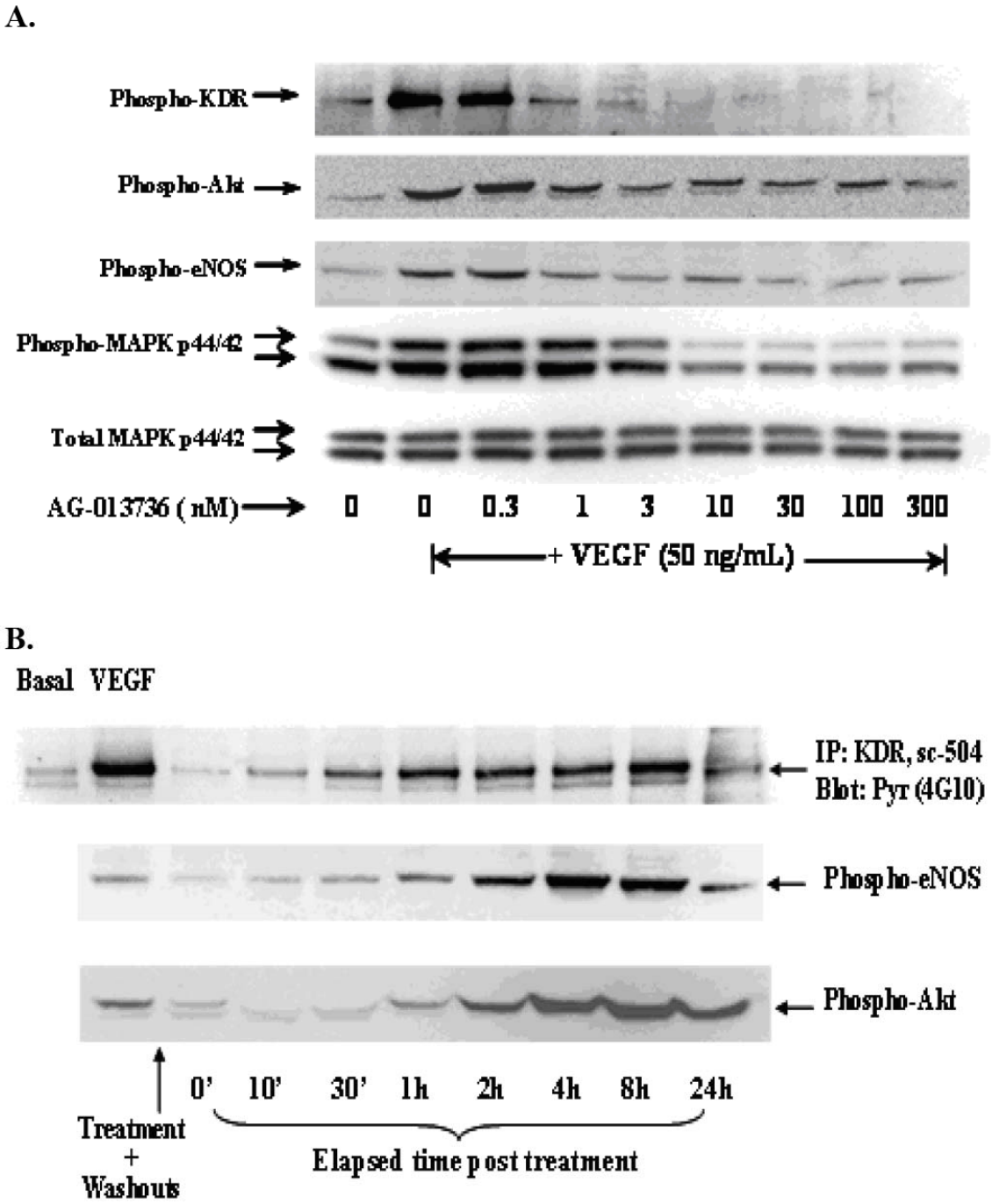


(figure excerpted from Applicant's package)

Immunoprecipitation and Western-blot analysis using HUVEC were utilized to measure the ability of AG-013736 to inhibit signaling molecules downstream of VEGF (Akt,

eNOS, and MAPK$_{44/42}$ (ERK1/2)). As shown in the figure below (A), dose-dependent inhibition of Akt, eNOS, and ERK1/2 phosphorylation by AG-013736 was seen in HUVEC. Equal protein loading was demonstrated by the unchanged total MAPK$_{44/42}$ signal across all samples (A, bottom row). The inhibition of VEGRF-2, eNOS and Akt phosphorylation by AG-013736 appeared to be reversible within ≤ 24 hours (B).

AG-013736 Inhibited Activity of eNOS, AKT, and ERK Downstream of VEGFR-2 (KDR) in HUVEC

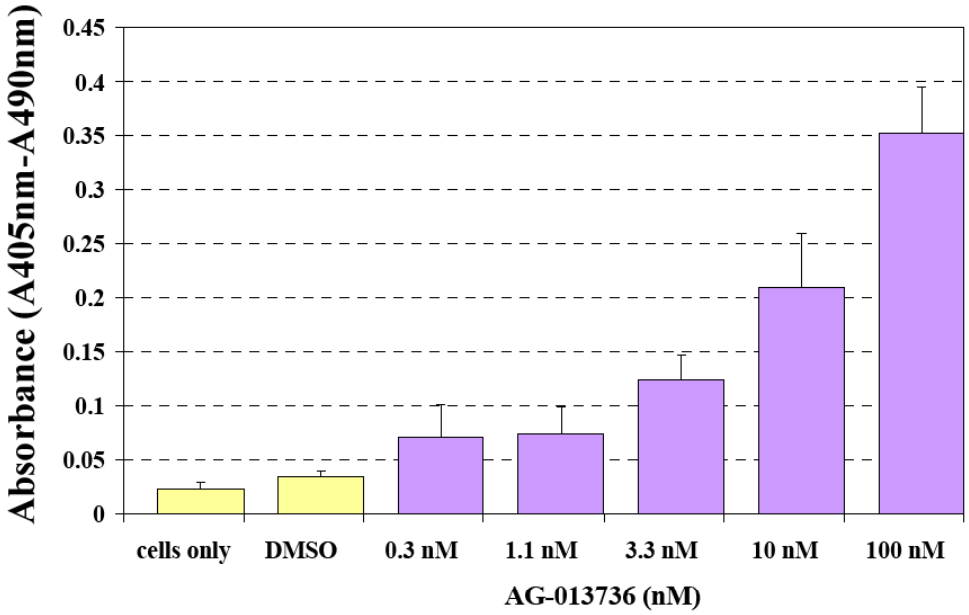


(figures excerpted from Applicant's package)

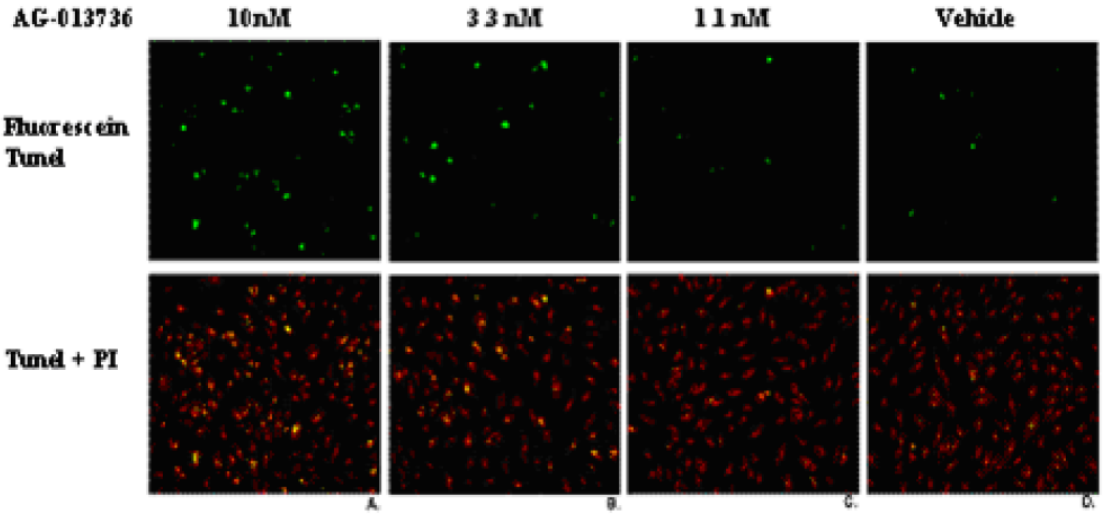
To measure apoptosis, an ELISA was used to detect cytoplasmic histone-associated DNA fragments in cell lysates. As shown below, 24 hour exposure of HUVEC to AG-013736 resulted in a dose-dependent induction of apoptosis, even in the presence of 20 ng/mL VEGF (A). A TUNEL immunocytochemistry analysis confirmed these results (B); the top panels show Apoptotic HUVEC cells displayed as green fluorescent cells, while the bottom panels show the addition of all cell nuclei stained red and double staining, colored yellow.

AG-013736 Induced HUVEC Apoptosis Even in the Presence of VEGF

A.



B.



(figures excerpted from Applicant's package)

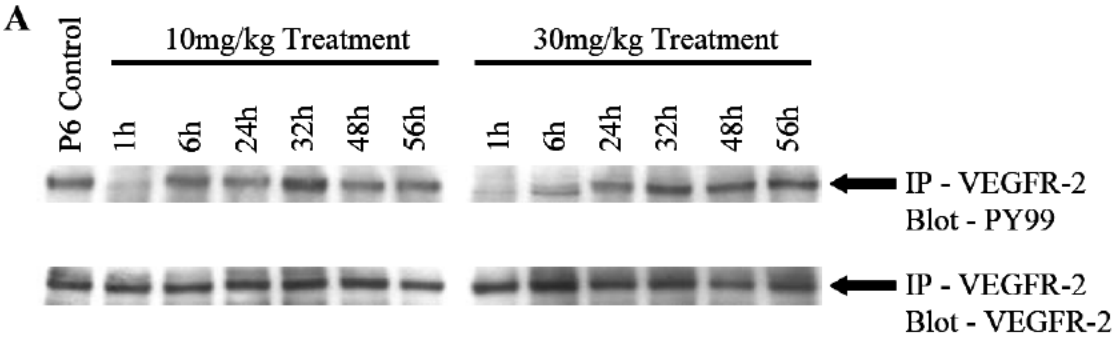
According to the Applicant, tumor cells that do not express the RTK targets were tested for any non-specific anti-proliferative activity or cytotoxic effect associated with AG-013736. The cells lines used were MV522 (human colon carcinoma), M24met (human melanoma), and LLC (murine Lewis lung carcinoma). AG-013736 did not exhibit any significant anti-proliferative activity in the proliferation assay (IC_{50}s >10 μM; data not shown). Therefore, the Applicant concludes that the anti-tumor efficacy observed after AG-013736 treatment in the xenograft tumors models established from these cells is due to anti-angiogenesis of the vessels, not a direct anti-proliferation or cytotoxicity induction of the tumor cells.

Study Title: AG-013736-NonclinPharm-003: AG-013736 In Vivo Target Modulation, Induction of Vascular Phenotypical Changes, Efficacious Plasma Concentrations, and Pharmacokinetic-Pharmacodynamics Correlation

To determine if AG-013736 was able to modulate VEGFR-2 in angiogenic tissues *in vivo*, a series of studies were carried out in angiogenic retinal tissues of newborn rats and in the vasculature of the M24met human xenograft tumors in mice. According to literature, retinal tissues in neonatal rats have abundant angiogenic vessels, allowing relatively robust measurement and quantification of VEGFR-2 phosphorylation.

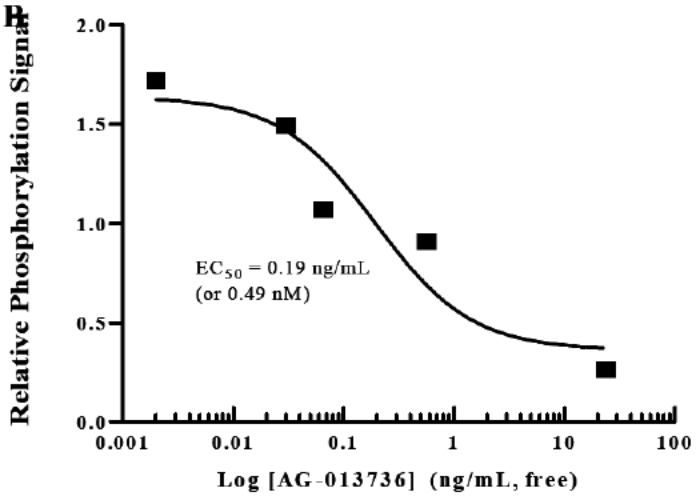
The first study treated newborn rats with two IP injections of AG-013968 (the HCl salt form of AG-013736) of 10 or 30 mg/kg. Immunoprecipitation and Western blot analysis were performed on retinal tissue for the determination of rat VEGFR-2 phosphorylation. As shown below, compared to vehicle-treated tissues, VEGFR-2 phosphorylation in the AG-013736-treated tissues was reduced by 80 – 90% one hour following treatment with either dose levels (A). In the low-dose group, the signal fully rebounded at 6 hours post-dose, when the unbound plasma drug concentration was 0.017 ± 0.02 ng/mL (or 0.04 ± 0.06 nM), well below IC_{50} for VEGFR-2 (0.20 ± 0.04 nM; obtained from cell-based assay in study AG-013736-NonclinPharm-002). In the high-dose group, a 50 – 60% recovery of phospho-VEGFR-2 signal occurred 6 – 24 hours post-dose. This was associated with an unbound plasma drug concentration of 0.57 ± 0.14 ng/mL (6 hour) and 0.066 ± 0.008 ng/mL (24 hour), which translated to 1.5 ± 0.36 nM (6 hour) and 0.17 ± 0.02 nM (24 hour), respectively. The full activity of VEGFR-2 rebounded between 24–32 hours post-dosing. Overall, the degree of target inhibition correlated to the plasma concentration of AG-013968 across the time points. According to the Applicant, a non-linear regression analysis of the 30 mg/kg group using the sigmoidal dose response model in Prizm (Graphpad) revealed an EC_{50} for target inhibition of 0.49 (± 3.1) nM or 0.19 ng/mL (B).

Inhibition of Rat VEGFR-2 Phosphorylation in Retinal Tissues of Neonatal Rats and the Determination of EC50



Elapsed Time	Unbound (2% Free) Plasma Concentration (ng/mL)			
	10 mg/kg		30 mg/kg	
Hour	Average	Stdev	Average	Stdev
1	7.83	1.91	> 20	--
6	0.0172	0.0244	0.572	0.142
24	0.0184	0.0262	0.0662	0.0084
36	0	0	0.0302	0.0276
48	BCR	--	BCR	--
56	BCR	--	BCR	--

BCR: Below the calibration range

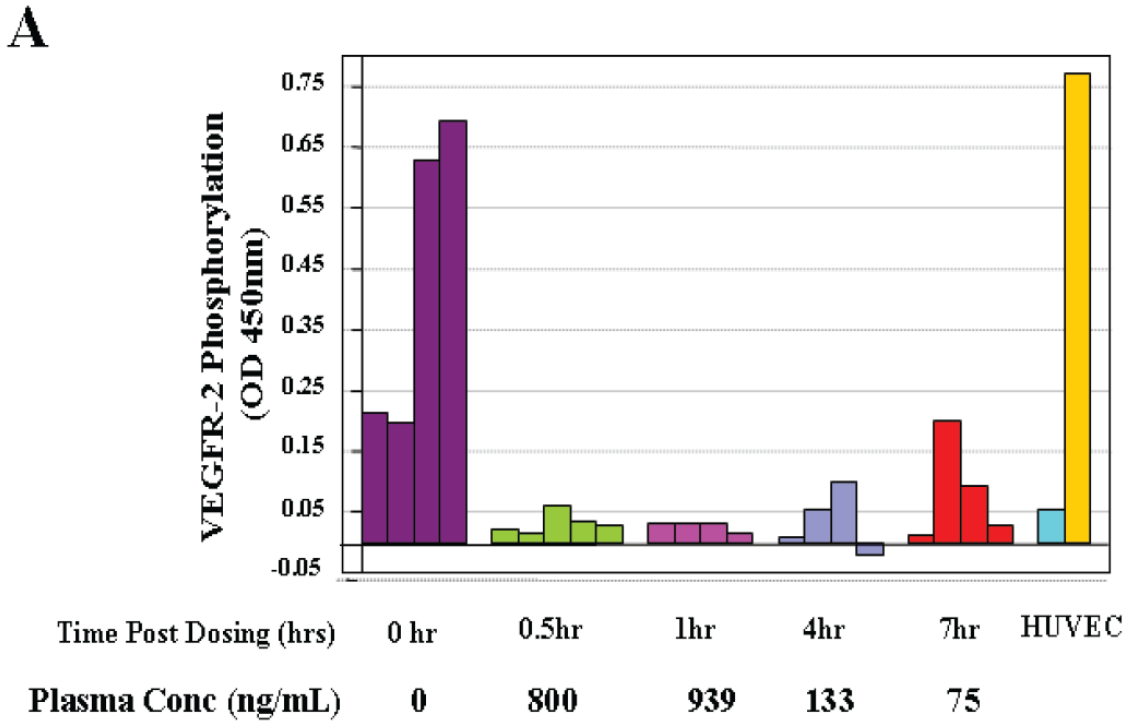


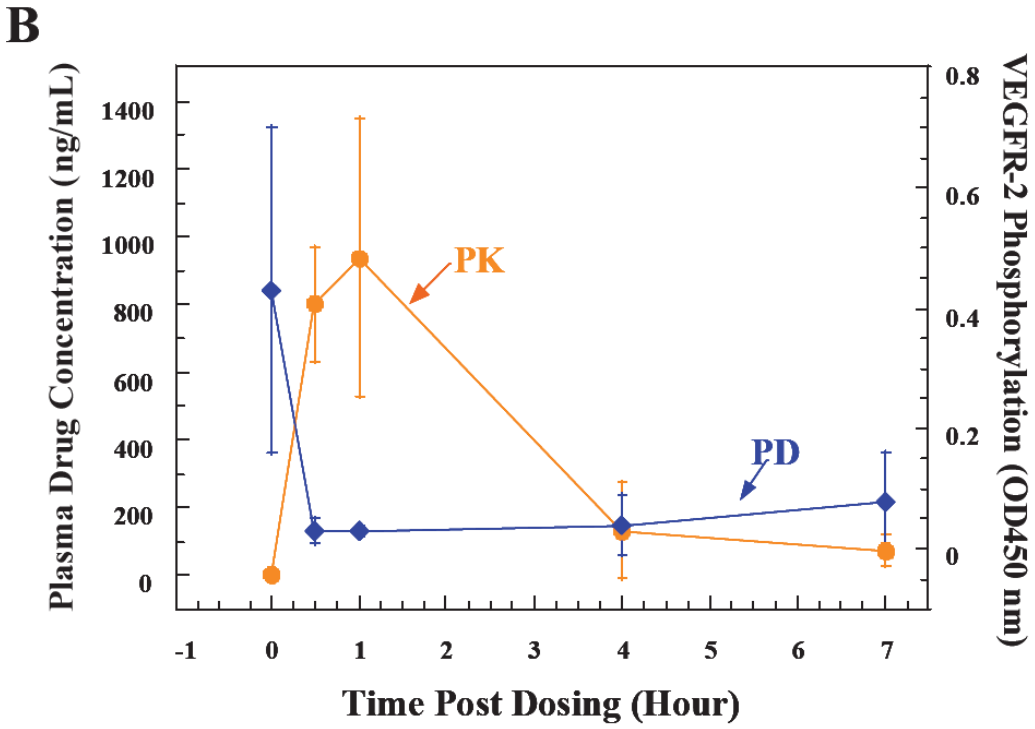
(figures excerpted from Applicant's package)

The second study utilized the M24met human melanoma xenograft model. (b) (4)

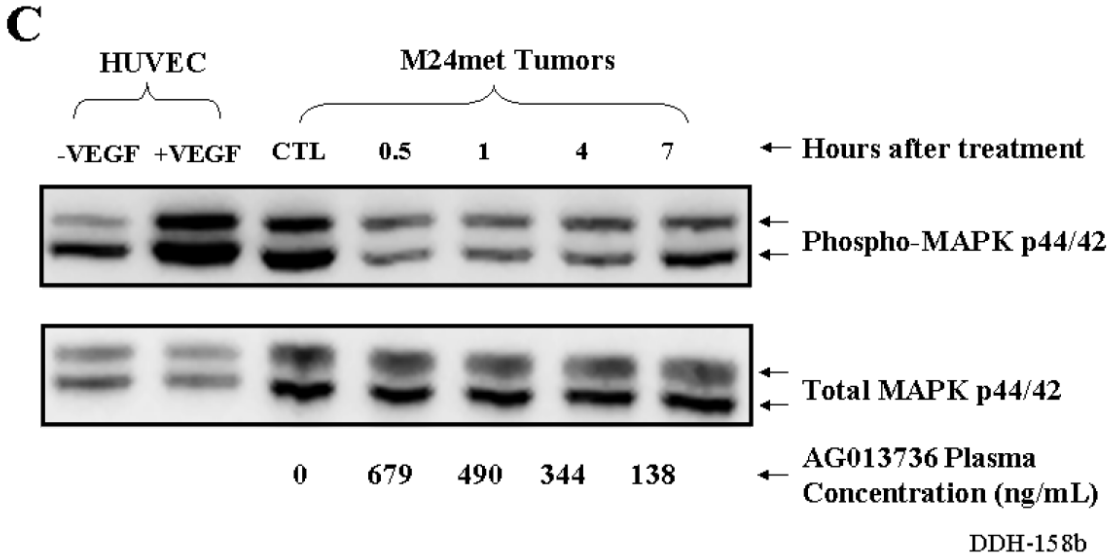
As shown below, the results of this study demonstrate that a single oral dose of AG-013736 (50 mg/kg) strongly suppressed murine VEGFR- 2 phosphorylation in tumor tissues for up to 7 hours compared to the vehicle-treated tumors (A). In the 30 mg/kg dose group, the level of VEGFR-2 phosphorylation in the tumors inversely correlated with AG-013736 plasma concentrations (B). Western blot analyses also showed that AG-013736 inhibited phospho-ERK (MAPK44/42), but not total ERK (C).

Single Dose of AG-013736 Significantly Reduced VEGFR-2 Phosphorylation in M24met Melanoma Tumors in Mice





Single Dose of AG-013736 Significantly Reduced VEGFR-2 Phosphorylation in M24met Melanoma Tumors in Mice (continued)

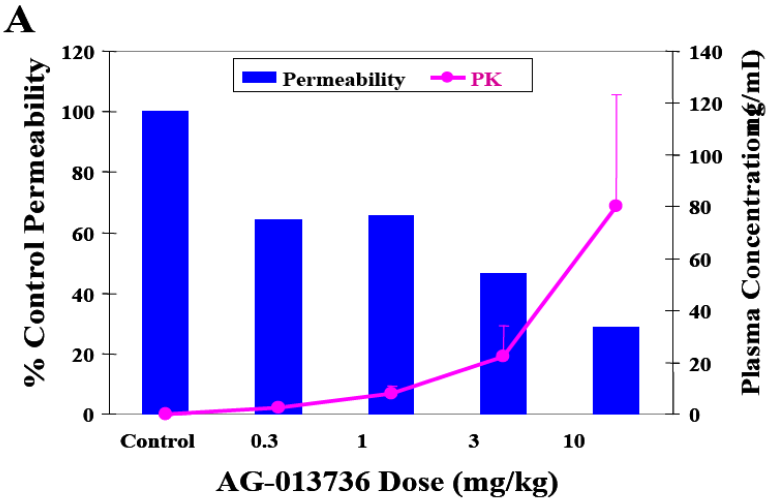


(figures excerpted from Applicant's package)

To measure the effect of AG-013736 on VEGF-mediated vascular permeability (VP), the Applicant utilized the Miles assay, which measures the extravasation of Evan's Blue dye from leaky vessels. As shown below, acute AG-013736 dose-dependently reduced VEGF-mediated vascular permeability in the skin of mice compared to vehicle treated control mice (A). Based on the pharmacokinetics and %VP inhibition, the effective concentration from this study was determined to be 0.46 nM (B). Utilizing an alternate method using FITC-dextran (MW of 250 kDa) as a tracer for assessing vessel permeability, AG-013736 was shown to reduce VP by 88% in MV522 human colon carcinoma tumors, compared to vehicle-treated tumors, following a single 100 mg/kg PO dose in mice (C).

Inhibition of Vascular Permeability in Skin and MV522 Tumor by AG-013736

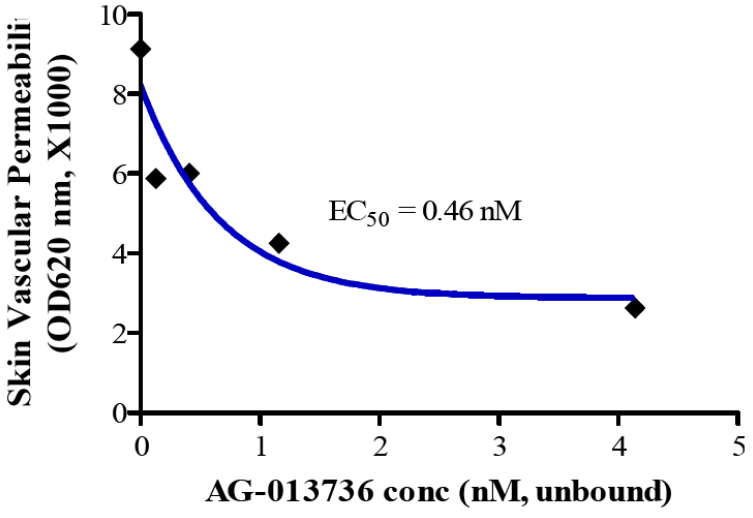
A



Dose (mg/kg)	VP (x1000)	SD (x1000)	Total Plasma Conc (ng/mL)	SD	Free Plasma Conc (ng/mL)	Free Plasma Conc (nM)
control	9.13	4.07	0.00	0.00	0.00	0.00
0.3	5.88	2.81	2.50	1.00	0.05	0.13
1	6.00	3.94	8.00	2.83	0.16	0.41
3	4.25	2.75	22.50	11.47	0.45	1.16
10	2.63	2.31	80.25	42.77	1.61	4.14

VP measured 1 hours post dose

B



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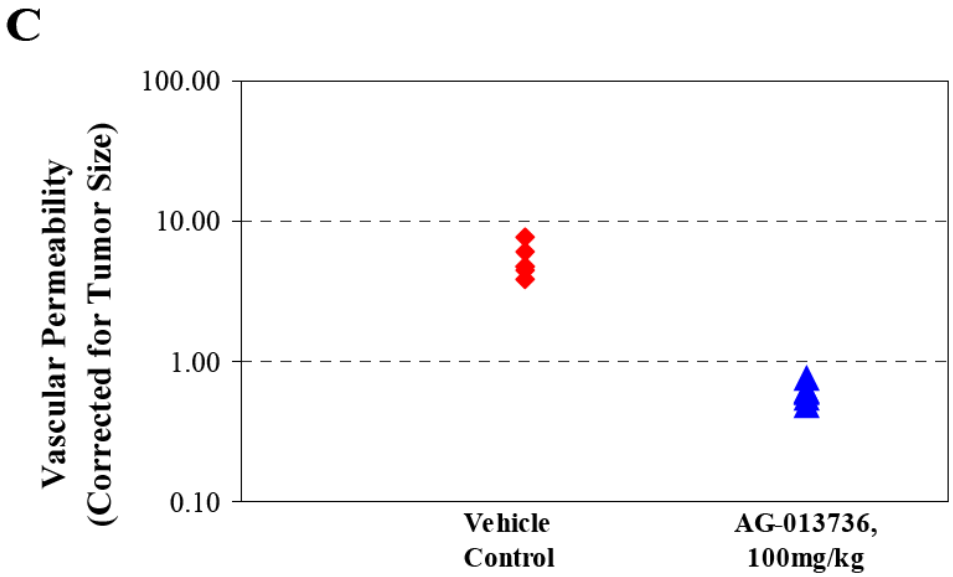
(excerpted from Applicant's package)

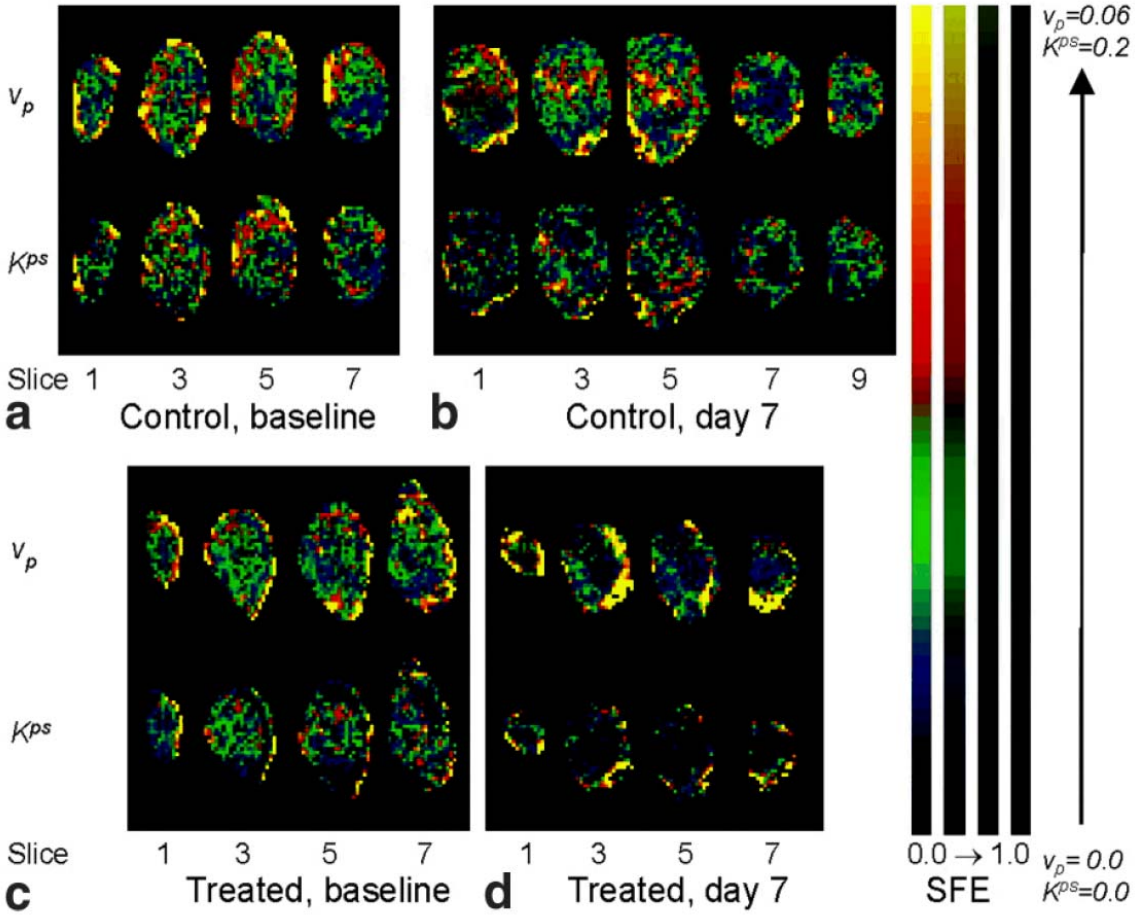
Inhibition of Vascular Permeability in Skin and MV522 Tumor by AG-013736 (continued)



(graph excerpted from Applicant's package)

To evaluate the impact of AG-013736 on angiogenesis, vascular phenotypical changes of the BT474 human breast carcinoma xenograft tumors in mice were measured following 7 days of AG-013736 treatment by dceMRI. Vascular permeability was reported using the MRI parameters K_{trans} or K_{ps} (endothelial volume transfer constant), which are measures of vascular leakage of a contrast enhancement agent, Gd-DTPA or Albumin-Gd- DTPA. As shown below, AG-013736 treatment decreased the overall tumor blood flow. According to the sponsor, changes in vascular K_{trans} correlated with decreased microvessel density, cellular viability, and tumor growth.

Anti-vascular Permeability and Tumor Growth by AG-013736 in the BT474 Model Assessed by dceMRI Analysis and Tumor Volume Assessments

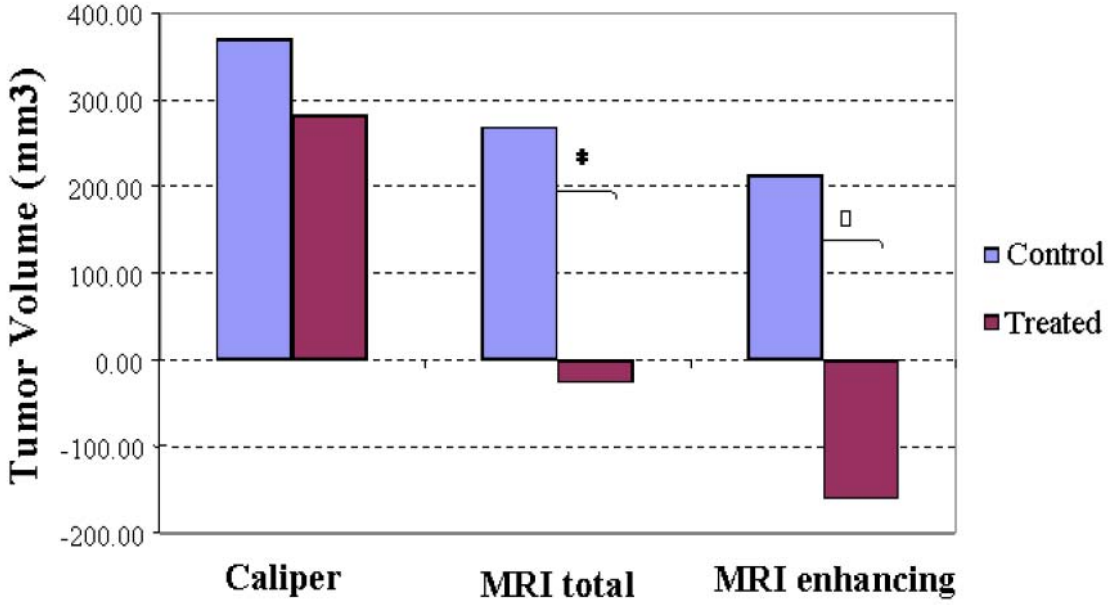


A tumor in the control group is shown at **(a)** baseline and **(b)** seven days after treatment; and a tumor in the AG-013736 treated group is shown **(c)** before, and **(d)** after seven days of AG-013736 treatment. In **a-d**, the upper row represents vp maps, while the lower row shows Kps maps. The color bars illustrate the relationship between hues and the values of the fitted values of Kps and vp, and between brightness and the fitting uncertainty (SFE). Brightness from the highest to the lowest represents the value of SFE from 0.0 to 1.0.

(figure excerpted from Applicant's package)

Within the same study, day 7 tumor volumes were measured using both a conventional digital caliper and dceMRI. The results, shown below, revealed that while the caliper measurements failed to show any significant difference in tumor volume, statistically significant reductions in tumor volume were found using MR measurements (‡, P=0.0335) and MR enhancing volume (□, P=0.0090).

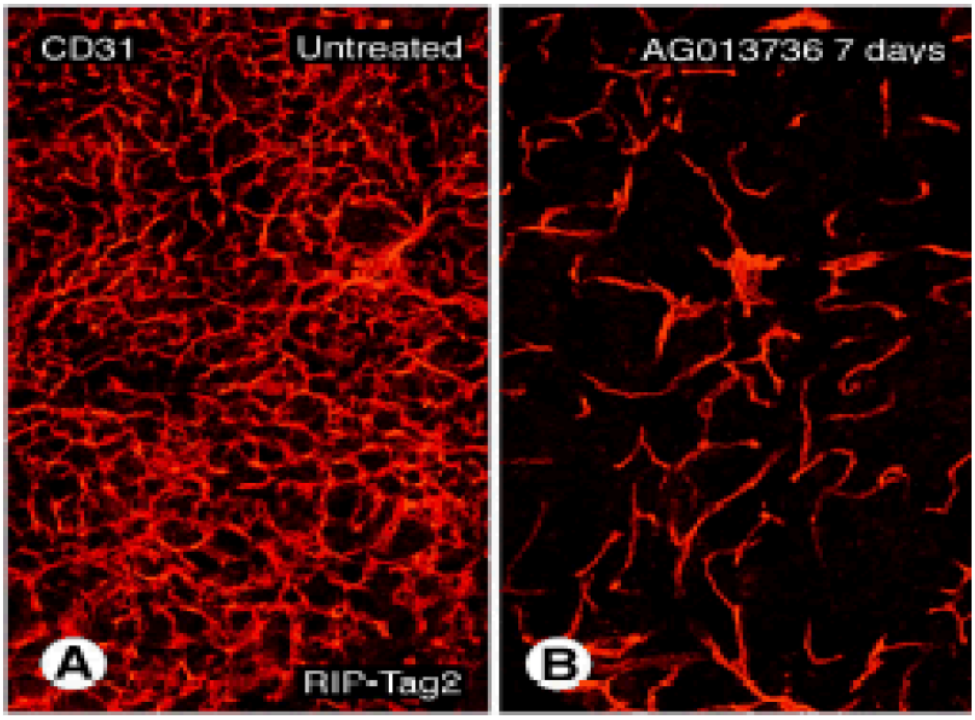
Anti-vascular Permeability and Tumor Growth by AG-013736 in the BT474 Model Assessed by dceMRI Analysis and Tumor Volume Assessments (continued)

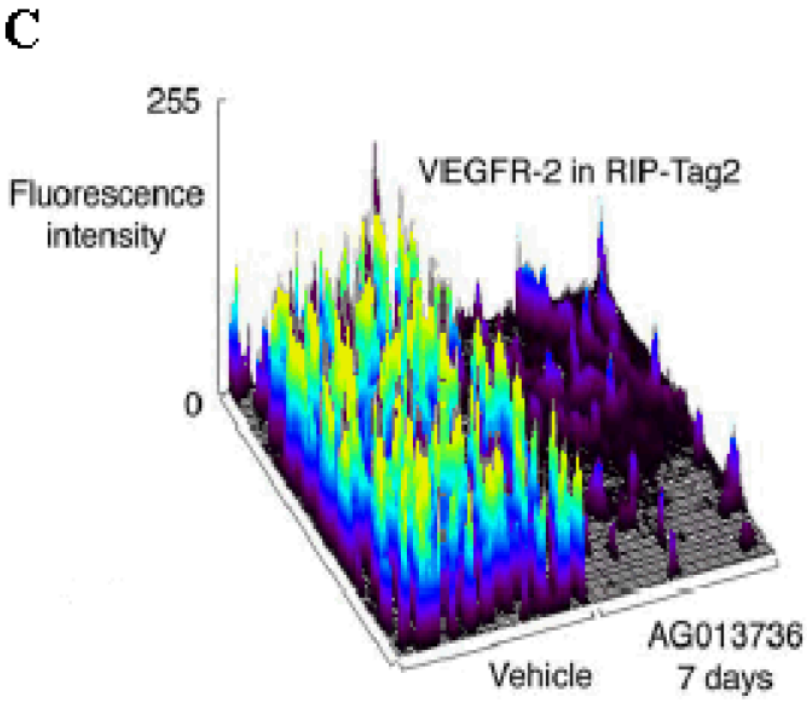


(graph excerpted from Applicant's package)

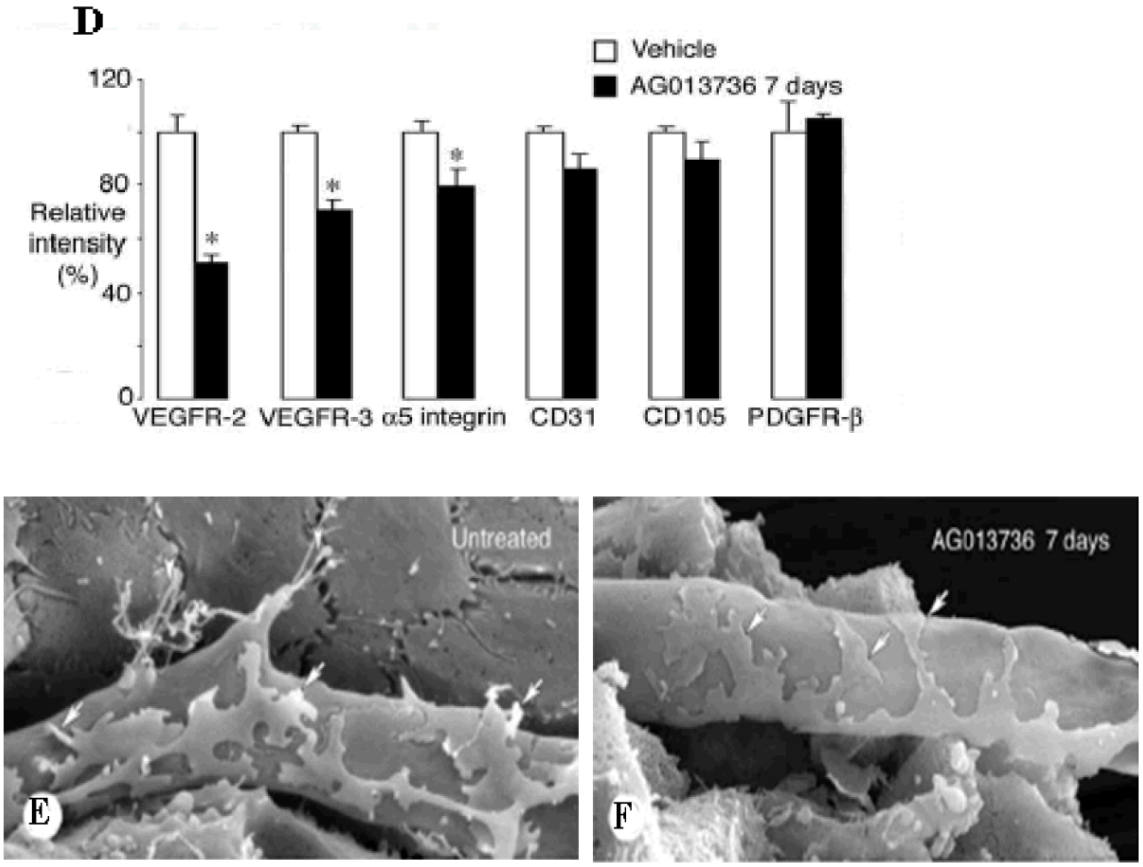
Multiple studies were carried out [redacted] (b) (4) to understand the molecular, cellular, and phenotypical changes in response to AG-013736 treatment by utilizing fluorescent-based (classic immunohistochemistry and confocal) microscopy and electron microscopy (EM). As shown below, utilizing the spontaneous pancreatic islet-cell tumors of RIP-Tag2 transgenic mice, AG-013736 treatment (25 mg/kg, IP or PO, BID, for 7 days) resulted in reduction in the tumor vascular density (A and B). According to the Applicant, by Day 7 of treatment, vascular density decreased more than 70%. Also by Day 7, surviving endothelial cells showed significant reductions in VEGFR-2, VEGFR-3, and α5 integrin immunoreactivities expression (C and D). The Applicant also suggests that while α-SMA immunoreactive pericytes (see arrow in E and F) are loosely attached to CD31-positive endothelial cells, treatment with AG-013736 for 7 days results in more tightly attached α-SMA immunoreactive pericytes (E and F).

Reduction in Tumor Vascular Density and VEGFR-2 Expression in the Pancreatic Islet Tumors of the RIP-Tag2 Transgenic Mice





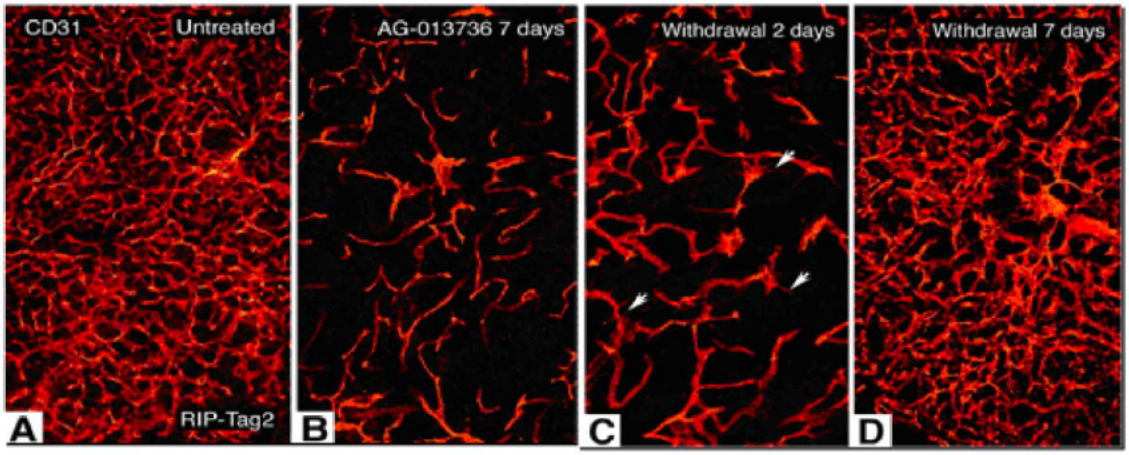
Reduction in Tumor Vascular Density and VEGFR-2 Expression in the Pancreatic Islet Tumors of the RIP-Tag2 Transgenic Mice (continued)



(figures excerpted from Applicant's package)

Following 7 days of withdrawal from AG-013736 treatment, confocal microscopy showed (below) RIP-Tag2 tumors fully revascularized, with tumor vascular density similar to untreated tumors. According to the Applicant, this suggests that best effect of AG-013736 therapy would be produced from uninterrupted daily treatment.

Tumor Blood Vessels Regrowth after Cessation of AG-013736 Treatment



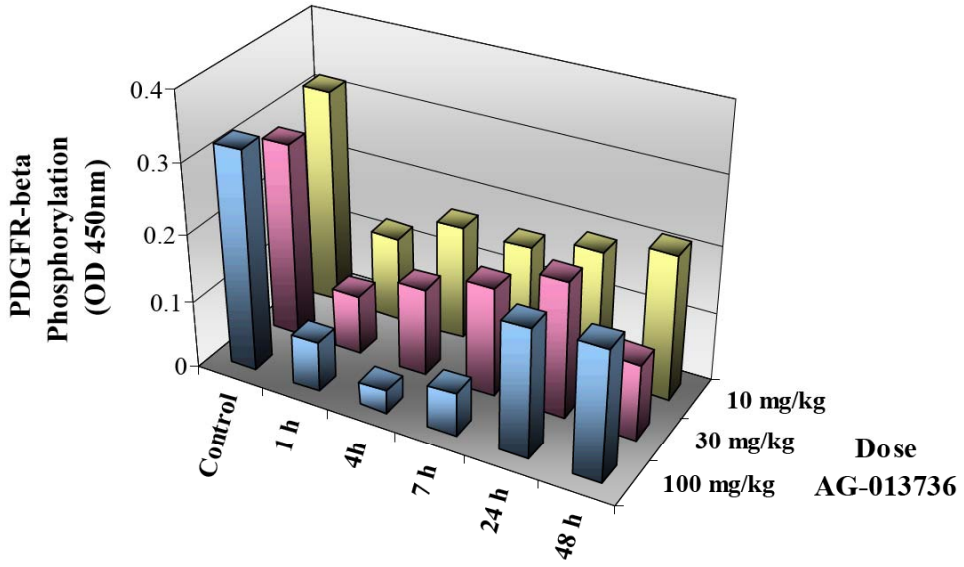
Arrows mark vascular sprouts, i.e. the beginning of vascular regrowth

(figure excerpted from Applicant's package)

Since previous studies showed that AG-013736 is also an inhibitor of PDGFR-β (IC_{50}=1.6 nM, i.e., approximately 10-fold higher than that for VEGFRs), the effect of AG-013736 treatment on PDGFR-β phosphorylation was examined in the C6 rat glioma tumor model grown in mice. (b) (4)

Mice bearing large C6 tumors were treated with AG-013736 at 10, 30, and 100 mg/kg (PO). A total of 3 injections of AG-013736 were given before tumors were collected to assess phospho-PDGFR-β using an ELISA-based assay. As shown below, AG-013736 dose- and time-dependently inhibited PDGFR-β phosphorylation in these tumors. AG-013736 at 100 mg/kg produced significant (90%) and sustained inhibition of PDGFR-β activity (>7 hours). In all dose groups the full activity of PDGFR-β did not return within 48 hours, although the drug concentration in the plasma was no longer detectable at 24 hours.

Dose-Dependent Inhibition of Tumor PDGFR-β Phosphorylation by AG-013736



Time Post Dose	10mg/kg		30mg/kg		100mg/kg	
	% Signal	PK	% Signal	PK	% Signal	PK
0	100.0	0.0	100.0	0.0	100.0	0.0
1	39.1	37.1	30.8	123.7	22.9	586.7
4	52.7	3.5	44.7	18.3	10.6	122.4
7	52.2	2.9	56.3	2.2	19.5	16.7
24	59.1	BDL	69.7	BDL	58.8	BDL
48	66.7	BDL	na	BDL	60.0	BDL

PK are in ng/mL; BDL: Below detection limit (1 ng/mL); na: not available

(excerpted from Applicant's package)

Study Title: AG-013736-NonclinPharm-003: In Vivo Anti-tumor Efficacy, Anti-angiogenesis, and Biomarkers or AG-013736 (Axitinib) in Rodent Models of Cancer.

AG-013736 was evaluated for its in vivo anti-tumor efficacy in a variety of tumor models in rodent animals, including: 1) subcutaneously (sc) implanted human tumor xenograft models; 2) orthotopically implanted and spontaneously metastatic human tumor xenograft models; and 3) murine syngeneic tumor models.

In regard to the single agent anti-tumor efficacy of AG-013736 in sc or orthotopically implanted tumor models, the following table summarizes the experimental design, parameters, receptor tyrosine kinase (RTK) expression when available, and efficacy (% tumor growth inhibition (TGI)) of AG-013736. AG-013736 consistently and significantly demonstrated dose-dependent anti-tumor efficacy in tumor models of breast, colon, lung, pancreas, kidney, brain, skin and liver. According to the Applicant, significant TGI regardless of RTK expression status suggests that in the RTK- negative tumor models (as indicated in the table below), the anti-tumor efficacy was a consequence of in vivo anti-angiogenic activity of AG-013736.

Single Agent Anti-tumor Efficacy of AG-013736 in Mice

Disease Type	Model (Phospho-RTK Expression)	Study Description	Dose (mg/kg)	Regimen	Size at Start of Rx (mm^3)	Treat-ment Period (Days)	TGI (%)	Study ID
Colon Carcinoma	MV522 (None)	Dose-dependent efficacy using AG-013968 (axitinib-HCl salt)	0.3	PO, BID	160 - 180	16	40	DDH-119
			1				50	
			3				57	
			10				66	
			30				81	
			100				96	
		Dose-dependent TGI using axitinib	0.3	PO, BID	130	16	-20	DDH-MG-371
			1				18	
			3				15	
			10				64	
			30				61	
			100				80	
			150				95	
			200				96	
		TGI using Alzet pump delivery of axitinib	1 mg/mL	Continuous infusion	100	14	20	DDH-KA-302
			3 mg/mL				31	
			10 mg/mL				79	
			20 mg/mL				94	
			30 mg/mL				85	
	HT29 (ND)	Dose response TGI	10	PO, QD	101	16	51	HT29-e143
			30				76	
			100				99	
			150				99	
	HCT-116-GFP (ND)	TGI in (orthotopically implantation)	30	PO, BID	One day after implant	17	87	HCT-116 GFP (#3)
			30			28	80	
Breast Carcinoma	MDA-MB-435\ HAL-Luc (None)	TGI via bioluminescent signal and conventional caliper measurements	60	PO, BID	90	18	50	DDH-MG-446
			10	PO, BID	130	11	Dose-dependent reduction in bioluminescent signal	DDH-MG-355
			100					
Lung Carcinoma	LLC (PDGFR-β)	Dose-dependent efficacy using AG-013968 (axitinib·HCl salt)	1	PO, BID	< 50	18	33	DDH-123
			3				49	
			10				75	
			30				68	
			100				69	
			300				Poorly tolerated	
		TGI and anti-metastasis activity assessment	100	PO, BID (trocar implant)	Prophy-lactic dose	38	73	LLC-DCH-201
			100	PO, BID (tail vein implant)	--	survival	~ 11 days median survival	
	NCI-H526 (SCLC) (KIT^+)	Efficacy and dose response	30	PO, BID	180	16	47	DDH-MG-293
			100				61	

Single Agent Anti-tumor Efficacy of AG-013736 in Mice (continued)

Disease Type	Model (Phospho-RTK Expression)	Study Description	Dose (mg/kg)	Regimen	Size at Start of Rx (mm^3)	Rx Period (Days)	TGI (%)	Study ID
Pancreatic Carcinoma	**MiaPaCa-2 (ND)**	Dose dependent TGI	3	PO, BID	100	15	42	MiaPaCa -e208S1
			30				75	
			60				87	
Melanoma	**A375** (VEGFR-2, PDGFR-β)	Efficacy in established A375 melanoma	10	PO, BID	350	14	38	DDH-MG-268
			30				59	
	M24met (None)	Dose-dependent TGI in sc implanted M24met	3	PO, BID	120 - 160	13	60	DDH-163
			10				49	
			30				73	
		Anti-metastasis activity in ID-implanted M24met with either early or late start treatments	50 (early Rx 7 days before surgery)	PO, BID	Primary tumor resection when average tumor size ~ 400 mm^3	23	Significant reduction in l.n. and lung metastasis (both grps). Early dosing had greater effect	DDH-158A
			50 (late dose: 1 day before surgery)			23		
	A2058 (ND; activating bRaf mutation)	Dose-dependent TGI	3	PO, BID	150	15	36	DDH-MY-424
			10				57	
			30				65	
			100				83	
Renal Cell Carcinoma	**SN12C-GFP** (ND)	TGI in orthotopically implanted RCC	10	PO, BID	Two days after implant	41	55	SN12C-GFP
			10			72	61	
			30			41	56	
			30			72	74	
			100			41	63	
			100			72	70	
Glioma	**C6** (pPDGFRs)	Dose-dependent TGI	10	PO, BID	70 - 80	15	37	DDH-GW-247
			30				47	
			100				64	
	U87MG (pPDGFRs)	Dose-dependent TGI	10	PO, BID	46 - 72	17	44	DDH-GW-273
			30				69	
			60				74	
NHL	**Namalwa** (ND)	Efficacy with time course	50	PO, BID	1 day after implant	14	IP tumor burden reduction (days 22 & 23)	DDH-RF-240
						22		
						23		
HCC	**Human Primary tumors LIM-SH050** (ND)	TGI	15	PO, BID	140-160	26	54	PFL-TR-C009.1
			30	PO, BID			45	

BID: twice daily; HCC: hepatocellular carcinoma; IP: intraperitoneal; L.n.: lymph node; ND: not determined; NHL: non-Hodgkin's lymphoma; PO: oral, RCC: renal cell carcinoma; TGI: tumor growth inhibition

(tables excerpted from Applicant's package)

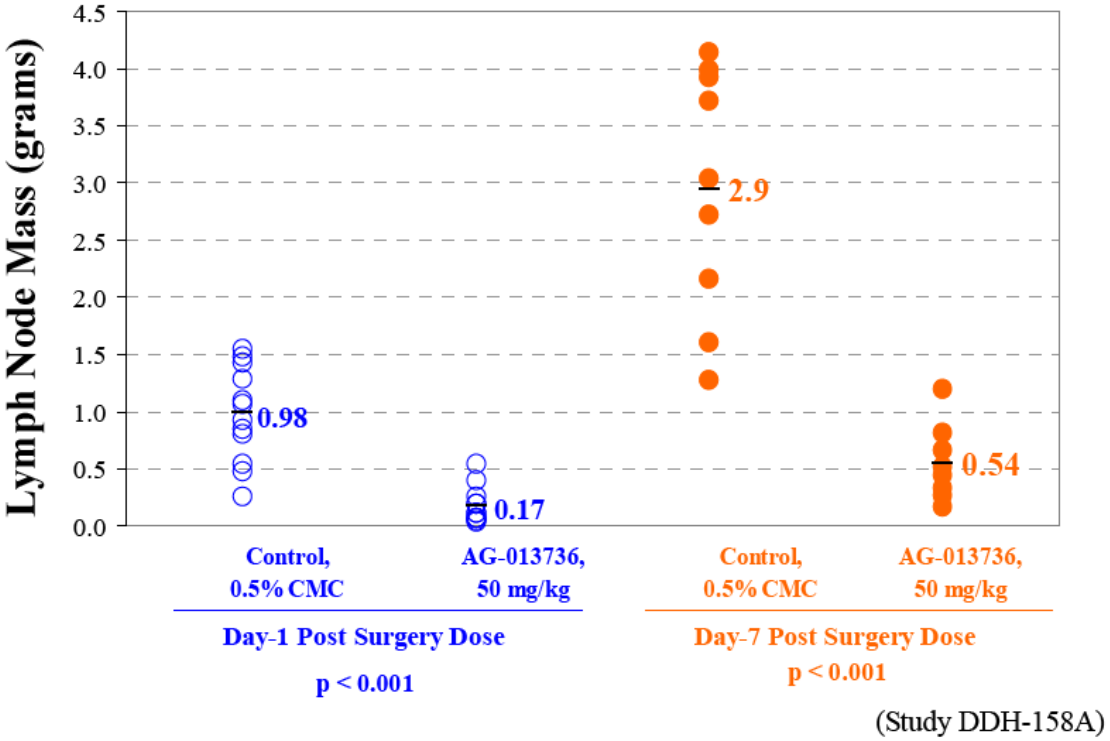
Among the various tumor models in rodents used to evaluate the efficacy of AG-013736, the M24met human melanoma model enabled the evaluation of multiple efficacy end points, including primary tumor growth, metastasis, and survival of animals.

This model is based on an aggressive human melanoma that spontaneously metastasizes to lymph nodes and lungs in mice. According to the Applicant, removing primary tumors promotes the metastasis potential. Also, according to the Applicant, M24met cells express mutant p53 and are reported to express high levels of oncogenic molecules such as N-RAS, ERK, as well as integrin α5β1 that are involved in tumor migration, adhesion and survival. The cells also express a low level of phospho-PDGFRs but not functional VEGFRs. Thus, as previously mentioned, anti-tumor efficacy should primarily be a consequence of AG-013736-induced in vivo anti-angiogenic activity.

Utilizing this tumor model, the first goal was to determine if AG-013736 could inhibit the growth of primary M24met tumors implanted subcutaneously in nude mice (study DDH-163, see table above). At dose levels of 3, 10, and 30 mg/kg, AG-013736 (PO, BID) significantly delayed tumor growth with TGIs of 60%, 49%, and 73%, respectively. According to the Applicant, statistically, TGIs from the 3 and 10 mg/kg AG- 013736 groups were not different from each other (p=0.4), whereas TGI in the group receiving 30 mg/kg AG-013736 was significantly greater than that of the 10 mg/kg dose group (p=0.033).

The second goal was to establish whether AG-013736 could inhibit spontaneous metastasis when M24met cells ($2.5x10^6$) were intradermally implanted in SCID (Balb/c) mice (DDH-158A, see table above). When the primary tumors reached the size of 300–400 mm^3, they were surgically removed to promote distant metastasis. For “Early Treatment” arms, the dosing of AG- 013736 (50 mg/kg, PO BID) started one week prior to primary tumor removal. For “Late Treatment” arms, the treatment started 1 day before the primary tumor removal. The total treatment time was 3 weeks for all groups. As shown below, at the end of the study, metastatic tumors in the lymph nodes (mainly at the ipsilateral and counter-ipsilateral sites, with occasional tumors found at the ingroinal sites) were weighed. AG-013736 significantly reduced metastatic potential in the lymph nodes (p<0.0001) compared to the control, following both “Early Treatment” and “Late Treatment”.

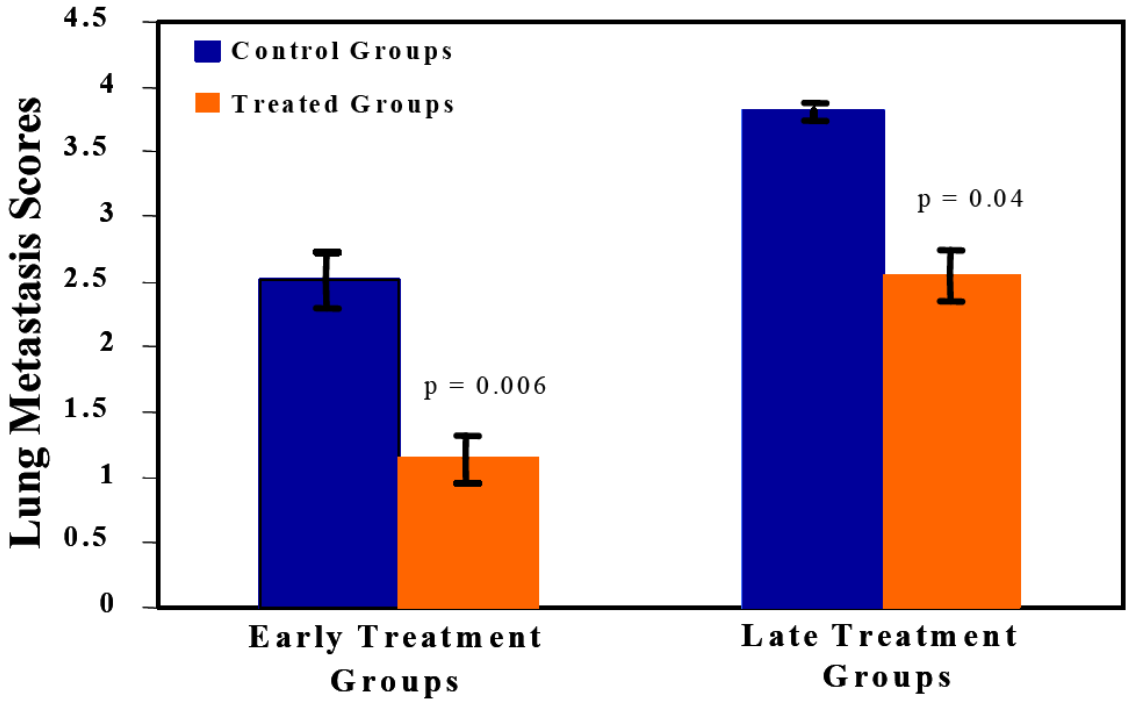
Inhibition of Lymph Node Metastasis by AG-013736 in M24met Model



(graph excerpted from Applicant's package)

Lungs were fixed in Bouin solution and lung metastases were manually counted under the microscope by four independent scientists. As shown below, averaging the results demonstrated that AG-013736 significantly reduced metastatic potential in the lung (p=0.006) for the "Early Treatment" group. For the "Late Treatment" group, the inhibition of lung metastasis was significant (p=0.04) but attenuated when compared with the "Early Treatment" group.

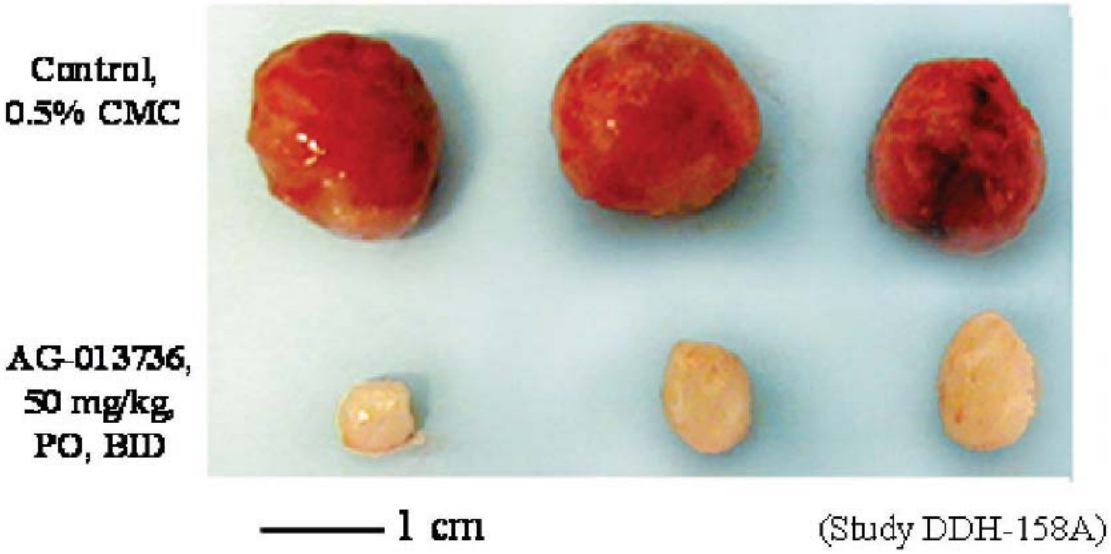
Inhibition of M24met Metastasis to Lungs by AG-013736



(graph excerpted from Applicant's package)

The following tumor images are from the vehicle control and AG-013736-treated lymph nodes and lungs of mice that were in the "Early Rx" groups.

Lymph Node Metastatic Tumors in Control and AG-013736 Treated Groups of M24met Model



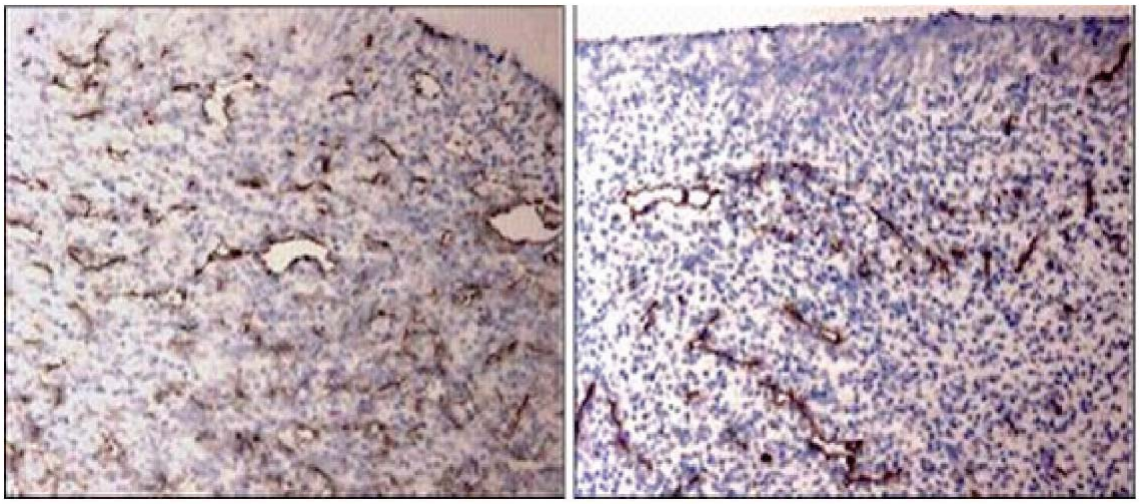
(figure excerpted from Applicant's package)

Reduced Lung Metastasis in AG-013736 "Early Treatment" Group (bottom row) Versus Control Group (top row)

(figure excerpted from Applicant's package)

As shown below, AG-013736 treatment also resulted in decreased microvessel density (MVD) in lymph node tumors following both "Early Treatment" and "Late Treatment", as demonstrated by CD-31 immunohistochemistry staining.

Tumor Vessels in Lymph Node Tumors of Control (left) and AG-013736 (right) Treatment Groups



Magnification = 10x.

(figure excerpted from Applicant's package)

4.2 Secondary Pharmacology

Studies in this section were reviewed by Robeena Aziz, MPH, Ph.D.

Study Title: 1010519: ProfilingScreen Data Report on Test Compound AG-013736 (AGP-58)

Report #:	1010519
Conducting Laboratory and Location:	[redacted] (b) (4)
Date of Study Initiation:	October 2000
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	AG-013736 or AGP-58, 1918709
Concentration used:	10 uM
Methods:	Radioligand Displacement Assay against the following receptors or ion channels :

- adenosine A1 and A2A
- adrenergic $\alpha 1$, $\alpha 2$, $\beta 1$, and $\beta 2$
- bradykinin B2
- calcium channel type L
- dopamine D1 and D2L
- estrogen ERα
- GABAA (agonist and chloride channel); glucocorticoid
- glutamate (*N*methyl *D*-aspartate)
- glutamate (non-selective)
- glycine (strychnine-sensitive)
- histamine H1 and H3
- insulin
- muscarinic M1, M2, and M3
- neuropeptide Y2, nicotinic acetylcholine
- (central)
- opiate (δ, κ, μ)
- phorbol ester
- purinergic (P2X, P2Y)
- serotonin (5-HT1, 5-HT2)
- sigma (non-selective)
- sodium channel (site 2)
- tachykinin NK1
- testosterone

Results and Conclusions:

- Modest binding affinity in the low μM range was observed for the adenosine A_{2A} (Ki of 2.76 μM), muscarinic M_2 (Ki of 2.23 μM), and neuropeptide Y_2 (IC50 of 10 μM) receptors.
- In secondary functional assays, axitinib showed no activity (agonistic or antagonistic) for the A_{2A}, M2, or Y2 receptors at concentrations up to 30 μM.

- Axitinib did not show functional activity for any of the assayed receptors or ion channels at pharmacologically relevant concentrations.
- Summary of results are listed in Figures below.

PT#: 1010519
CODE: AGP-58, AG-013736

September 23, 2003 3:45 PM
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EXPERIMENTAL RESULTS - BIOCHEMICAL ASSAYS

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION % (-100 -50 0 50 100)	IC_{50}	K_i	n_H	R
200510	Adenosine A_1	27482	hum	2	10 µM	36				
♦ 200610	Adenosine A_{2A}	27483	hum	2	10 µM	68				
♦		27701	hum	2	10 µM	67	4.91 µM	2.76 µM	0.975	
				2	1 µM	16				
				2	0.1 µM	6				
				2	10 nM	3				
203500	Adrenergic α_1, Non-Selective	27375	rat	2	10 µM	13				
203900	Adrenergic α_2, Non-Selective	27568	rat	2	10 µM	13				
204010	Adrenergic β_1	27562	hum	2	10 µM	31				
204110	Adrenergic β_2	27561	hum	2	10 µM	2				
212610	Bradykinin B_2	27489	hum	2	10 µM	-5				
214600	Calcium Channel L-Type, Dihydropyridine	27490	rat	2	10 µM	-2				
219500	Dopamine D_1	27508	hum	2	10 µM	4				
219600	Dopamine D_{2L}	27509	hum	2	10 µM	7				
226010	Estrogen ERα	27556	hum	2	10 µM	10				
226500	$GABA_A$, Agonist Site	27418	rat	2	10 µM	1				
226800	$GABA_A$, Chloride Channel,TBOB	27519	rat	2	10 µM	2				
232010	Glucocorticoid	27398	hum	2	10 µM	8				
233000	Glutamate, NMDA, Phencyclidine	27520	rat	2	10 µM	1				
235000	Glutamate, Non-Selective	27491	rat	2	10 µM	1				
239000	Glycine, Strychnine-Sensitive	27399	rat	2	10 µM	-2				
239500	Histamine H_1, Central	27396	gp	2	10 µM	7				
239800	Histamine H_3	27388	rat	2	10 µM	-12				
243000	Insulin	27577	rat	2	10 µM	-13				
252600	Muscarinic M_1	27492	hum	2	10 µM	22				
♦ 252700	Muscarinic M_2	27493	hum	2	10 µM	62				
♦		27707	hum	2	10 µM	69	6.26 µM	2.23 µM	1.66	
				2	1 µM	4				
				2	0.1 µM	2				
				2	10 nM	5				
252800	Muscarinic M_3	27494	hum	2	10 µM	11				

* Batch: Represents compounds tested concurrently in the same assay(s). ‡ Partially soluble in *in vitro* test solvent.
♦ Denotes item meeting criteria for significance
† Results with ≥ 50% stimulation or inhibition are highlighted. (Negative values correspond to stimulation of binding or enzyme activity)
R=Additional Comments
gp=guinea pig; hum=human

Cat. #	TARGET	BATCH*	SPP.	n=	CONC.	†% INHIBITION % (-100 -50 0 50 100)	IC_{50}	K_i	n_H	R
♦ 257110	Neuropeptide Y_2	27564	hum	2	10 µM	52				
		27799	hum	2	10 µM	49	>10 µM			
				2	1 µM	6				
				2	0.1 µM	-17				
				2	10 nM	-3				
258600	Nicotinic Acetylcholine, Central	27497	rat	2	10 µM	-5				
260110	Opiate δ (OP1, DOP)	27498	hum	2	10 µM	17				
260210	Opiate κ (OP2, KOP)	27499	hum	2	10 µM	6				
260410	Opiate µ (OP3, MOP)	27500	hum	2	10 µM	23				
264500	Phorbol Ester	27403	mouse	2	10 µM	2				
268700	Purinergic P_{2X}	27569	rabbit	2	10 µM	-1				
268810	Purinergic P_{2Y}	28599	rat	2	10 µM	10				
271000	Serotonin (5-Hydroxytryptamine) 5-HT_1, Non-Selective	27521	rat	2	10 µM	4				
271600	Serotonin (5-Hydroxytryptamine) 5-HT_2, Non-Selective	27571	rat	2	10 µM	25				
278300	Sigma, Non-Selective	27573	gp	2	10 µM	-1				
279500	Sodium Channel, Site 2	27385	rat	2	10 µM	15				
255510	Tachykinin NK_1	27377	hum	2	10 µM	-4				
285000	Testosterone	27567	rat	2	10 µM	2				

TISSUE ASSAYS

Cat. #	ASSAY NAME	BATCH*	TISSUE, SPECIES	n=	CONC.	CRITERIA	RESP.	AG.	ANT.	R
402020	Adenosine A_{2A}	28887	platelet rich hum	2	30 µM	≥ 50%		5%	0%	
449500	Muscarinic M_2	28678	left atria, gp	2	30 µM	≥ 50%		-19%	0%	
454500	Neuropeptide Y_2	28680	vas deferens, rat	2	30 µM	≥ 50%		4%	4%	

(figures excerpted from Applicant's package)

4.3 Safety Pharmacology

Studies in this section were reviewed by Robeena Aziz, MPH, Ph.D.

Neurological effects:

Study Title: VFF-488: AG13736 - Irwin Dose-Range in Mice Including Temperature and Locomotor Assessment

Key Study Findings:

- No signs of mortality or gross signs of toxicity during the 7-day post-dose observation period.
- No biologically significant effects on behavioral or physiological state, on body temperature and spontaneous locomotor activity at the maximum dose tested (30 mg/kg).

Report #:	VFF-488
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	July 5, 2001
GLP Compliance:	Yes
QA Report:	Yes (X), No ()
Drug, lot #, and % purity:	AG13736, AC3088, 99%
Doses:	0, 3, 10, and 30 mg/kg
Species/strain:	Mice/ CD-1
Number/sex/group or time point:	4 males/group
Route; formulation; volume; and infusion rate:	Oral gavage single dose 0.5% w/v carboxymethylcellulose (CMC) 5 mL/kg/
Age:	Approximately 6 weeks
Weight:	22 to 26 g
Parameters:	• Behavior, • Skeletal muscle tone, • Reflexes, • Body temperatures • Overt autonomic and neurological effects
Methods	Functional Observational Battery measurements taken pre-dosing, and at approximately at 30, 90, 150, and 300 minutes and 24 hours after dosing

<u>Pulmonary effects</u>:

Study Title: VFF-491: AG13736 Evaluation of Respiratory parameters in the conscious rat using whole body bias flow plethysmography

Key Study Findings:

- No remarkable findings.

Report #:	VFF-491
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	September 11, 2001
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	AG13736; AC3088, >98%

Doses:	0, 50, 250, and 500 mg/kg (0, 300, 1500, and 3000 mg/m^2)
Species/strain:	Rat/ Winstar
Number/sex/group or time point:	8 males/group
Route; formulation; volume; and infusion rate:	Oral Gavage single dose 0.5% w/v carboxymethylcellulose (CMC) 5 mL/kg
Age:	7 weeks
Weight:	160 to 213 grams
Parameters:	Respiratory rate, tidal volume, and minute volume.

Experimental conditions:

Group	Oral treatment	Animals/group	Dose (mg/kg)
1	0.5% w/v CMC	8	0
2	AG13736	8	50
3	AG13736	8	250
4	AG13736	8	500
5	Morphine sulphate (reference standard)	8	200
6	AG13736	8	50
7	AG13736	8	250
8	AG13736	8	500

Results and Conclusion:

- No mortalities were observed.
- No effects on respiration rate, tidal volume, and/or minute volume.

Gastrointestinal effects:

Study Title: VFF-489: Charcoal Propulsion Test in Mice Following Oral Administration.

Key Study Findings:

- No remarkable findings.

Report #:	VFF-489
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	July 18, 2001
GLP Compliance:	Yes
QA Report:	Yes (X), No ()
Drug, lot #, and % purity:	AG13736, AC3088, >99%
Doses:	0, 3, 10, and 30 mg/kg

Species/strain:	Mice/ CD-1
Number/sex/group or time point:	10 males/group
Route; formulation; volume; and infusion rate:	Oral gavage single dose 0.5% w/v carboxymethylcellulose (CMC) 5 mL/kg
Age:	Approximately 5 weeks
Weight:	20 to 24 grams
Parameters:	Gastrointestinal irritancy
Methods	▪ 30 minutes after oral administration animals received 0.25 ml of charcoal in water (5% w/v) by gavage. ▪ 30 minutes after charcoal administration, animals were sacrificed and checked for gastrointestinal irritancy

Experimental conditions:

Group	Oral treatment	Animals/group	Dose (mg/kg)
1	0.5% w/v CMC	10	0
2	AG13736	10	3
3	AG13736	10	10
4	AG13736	10	30
5	Morphine sulphate (reference standard)	10	200

Results and Conclusion:

- No mortalities were observed.
- No remarkable changes in terms of gastrointestinal irritancy were observed.

Study Title: VFF-490: Assessment of Effects on Gastric Emptying in Rats Following Oral Administration (Phenol Red Method).

Key Study Findings

- All doses produced increases in gastric emptying; however, statistically significant increases occurred at doses ≥10 mg/kg when compared to controls.

Report #:	VFF-490
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	August 16, 2001
GLP Compliance:	Yes
QA Report:	Yes (X), No ()
Drug, lot #, and % purity:	AG13736, AC3088, >98%

Doses:	0, 3, 10, and 30 mg/kg
Species/strain:	Rat/ Winstar
Number/sex/group or time point:	10 males/group
Route; formulation; volume; and infusion rate:	Oral gavage single dose 0.5% w/v carboxymethylcellulose (CMC) 5 mL/kg
Age:	Approximately 7 weeks
Weight:	172 to 200 grams
Parameters:	Gastrointestinal irritancy
Methods	▪ Phenol red method of testing was used - 30 minutes after oral administration animals received 1.5 ml of marker solution (phenol red) by gavage ▪ 30 minutes later, rats were euthanized and stomach contents were homogenized in NaOH and red on absorbance spectra read (560 nm) for gastrointestinal irritancy

Experimental conditions:

Group	Oral treatment	Animals/group	Dose (mg/kg)
1	Standard (phenol red)	10	-
2	0.5% w/v CMC	10	0
3	AG13736	10	3
4	AG13736	10	10
5	AG13736	10	30
6	Morphine sulphate (reference standard)	10	20

Results and Conclusion:

- No mortalities were observed.
- All doses produced increases in gastric emptying; however, statistically significant increases occurred at doses ≥10 mg/kg when compared to controls. See Applicant's Table 1 below.

TABLE 1

Effects of oral administration of AG13736 on gastric emptying in the rat

Group	Oral treatment	Dose (mg/kg)	% gastric emptying
1	Phenol red only	-	0.0
2	Vehicle	-	58.7
3	AG13736	5	73.9
4	AG13736	10	81.5*
5	AG13736	30	73.9*
6	Morphine sulphate	20	28.8**

Significance of difference from vehicle-treated group: * $p<0.05$, ** $p<0.01$

(table excerpted from Applicant's package)

Cardiovascular effects:

Study Title: AG013736HERG: Assessment of the Potential Effect of AG-013736 on *hERG* Potassium Current Stably Expressed in HEK293 Cells

Report #:	AG013736HERG
Conducting Laboratory and Location:	Pfizer Global Research and Development Safety Pharmacology, Drug Safety Evaluation Agouron Pharmaceuticals, Inc., San Diego, CA 92121
Date of Study Initiation	September 26, 2003
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	AG-013736; AC3273, 99.7%
Vehicle	DMSO
Methods	3 μM
Doses:	
Study Design:	Standard hERG assay using HEK293 cell line

Results and Conclusion:

- AG-013736 was tested at one concentration of 3μM. Higher concentrations could not be tested because 3 μM is the limit of AG-013736 solubility in hERG extracellular recording saline.

- At that nominal concentration of 3μM, AG-013736 inhibited the hERG K+ current by 7 ± 0.6% (mean ± SEM, N=6). See Applicant's Table 1 below.

Table 1. Inhibition of *hERG* potassium currents by AG-013736. Percent inhibition after 5 minutes drug exposure was calculated based on the ratio of the current in the presence of drug relative to the extrapolated control amplitude. Statistical analysis of the data was performed using a paired T-test (two-tailed) of the extrapolated control current amplitudes versus the current amplitudes in the presence of drug, in which p<0.05 is considered significant. See the Data Analysis section for a description of control data correction.

Observation	% Inhibition at 3 μM
1	6.0
2	8.5
3	6.0
4	5.5
5	8.2
6	7.9
Mean ± SEM	**7.0** ± **0.6**, p< 0.05

(table excerpted from Applicant's package)

Study Title: DOF GBLFP1 E-02102006: Ki Determination of AG-028458 (sulfoxide metabolite of AG-013736) in Dofetilide Fluroescence Polorization Binding Assay

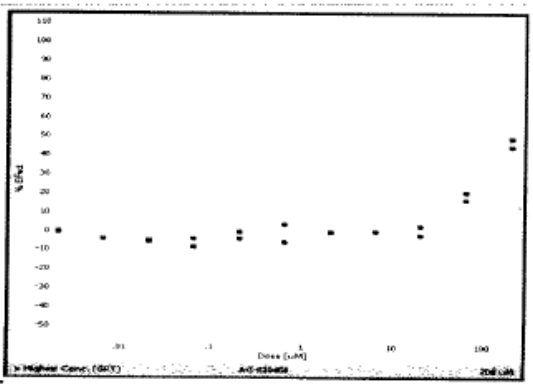
Report #:	DOF GBLFP1 E-02102006
Conducting Laboratory and Location:	Pfizer Global Research and Development Biochemical Pharmacology San Diego, CA 92121
Date of Study Initiation	December 23, 2009
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	AG-028458 (sulfoxide metabolite of AG-013736); no other information was given
Methods Doses:	0.002, 0.006, 0.02, 0.064, 0.201, 0.635, 2.01, 6.34, 20, 63.3, and 200 μM
Study Design:	To determine binding affinity using Cy3B tagged N-desmethyl dofetilide competitively bound to hERG cell HEK293

Results and Conclusion:

- The observed IC_{50} is >200 μM and the K_i is >79 μM. See Applicant's Table below.
- The results suggest that the binding affinity (K_i) of AG-028458 (sulfoxide metabolite of AG-013736) may be predictive of functional hERG channel blockage.

AG-028458

Dose [uM]	% Inhibition Replicate 1	% Inhibition Replicate 2	Average %Inhibition
200	49.0	44.9	46.9
63.3	20.4	16.3	18.4
20	2.7	-2.0	0.3
6.34	0.0	0.0	0.0
2.01	-0.7	-0.7	-0.7
0.635	3.4	-6.1	-1.4
0.201	-4.1	-0.7	-2.4
0.064	-4.1	-8.2	-6.1
0.02	-4.8	-5.4	-5.1
0.006	-4.1	-4.1	-4.1
0.002	0.0	-0.7	-0.3



(table excerpted from Applicant's package)

Study Title: SP107: Assessment of the Effect of PF-04621675 on hERG Potassium Current Stably Expressed in HEK293 Cells

Report #:	SP107
Conducting Laboratory and Location:	Pfizer Global Research and Development Biochemical Pharmacology San Diego, CA 92121
Date of Study Initiation	August 13, 2007
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	PF-04621675-00-0002 (glucuronide conjugate of AG-013736); 118435-102-01,no purity info. provided
Methods	1, 3, 10, and 30 μM.
Doses:	
Study Design:	Standard hERG assay using HEK293 cell line

Results and Conclusion:

- PF-04621675, the glucuronide conjugate of AG-013736 and a primary metabolite of AG-013736 in humans, was tested at concentrations of 1, 3, 10, and 30 μM. The highest concentration tested (30 μM) is predicted to be >90 fold the human free C_{max}.
- PF-04621675 did not significantly inhibit the hERG current at any of the concentrations tested.

- The IC_{50} value could not be determined because 50% inhibition was not achieved at the highest concentration tested (30 µM).
- Therefore the IC_{50} for PF-04621675 is >30 µM. See Applicant's Table 1 below.

Table 1. Inhibition of *hERG* potassium currents by PF-04621675 . Percent inhibition after 4 minutes compound exposure was calculated based on the ratio of the current in the presence of compound relative to the control current amplitude. NS=not significant.

Cell number	% Inhibition 1 µM	% Inhibition 3 µM	% Inhibition 10 µM	% Inhibition 30 µM	Cell IC_{50} µM
1	12.6	18.3	22.3	20.8	>30
2	-0.1	0.2	-3.9	-2.2	>30
3	-	5.4	7.0	11.6	>30
4	-	2.9	4.7	7.3	>30
5	-	-4.5	-8.3	-5.0	>30
6	-	-3.8	-8.9	-12.6	>30
7	-	-2.5	-4.8	-2.1	>30
8	-	1.6	3.0	2.4	>30
9	-	-2.2	-0.4	-4.8	>30
Mean ± SEM	6.2 NS	1.7 ± 2.3 NS	1.2 ± 3.2 NS	1.7 ± 3.4 NS	>30

(table excerpted from Applicant's package)

Study Title: SP4002: Evaluation of Cardiovascular Effects of Oral AG-013736 in Mice

Report #:	SP4002
Conducting Laboratory and Location:	Pfizer Global Research and Development Safety Pharmacology, Drug Safety Evaluation San Diego, CA 92121
Date of Study Initiation	July, 2001
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	AG-013736, AG-013736, 99.8%
Doses:	0, 10, 30, and 100 mg/kg
Species/strain:	Mice/ C57/BL6
Number/sex/group or time point:	9 male/group
Route; formulation; volume; and infusion rate:	Oral gavage twice daily (6 hours apart) for 4 consecutive days followed by 3 days recovery 0.5% w/v carboxymethylcellulose (CMC) 10 mL/kg

Age:	Approximately 5 weeks
Weight:	20 to 24 grams
Parameters:	Clinical signs (pre-dose on dosing days) Body weights Heart rate Systemic arterial blood pressures (systolic, diastolic, and mean pressure) In addition, plasma samples (measuring AUC) were collected on Days 1 and 4 at al doses

Results and Conclusion:

- No mortalities were observed.
- Due to change in blood pressure observed in vehicle animals, the results (statistical analysis) were inconclusive.
- TK data showed AUC was 63, 1020, and 3720 ng*h/mL at 3, 30, and 100 mg/kg, respectively.

Study Title: SP4002-2: Evaluation of Cardiovascular Effects of Oral AG-013736 in Mice

Key Study Findings:

- Study was repeated due to inconclusive results from Study No. SP4002.

Report #:	SP4002-2
Conducting Laboratory and Location:	Pfizer Global Research and Development Safety Pharmacology, Drug Safety Evaluation San Diego, CA 92121
Date of Study Initiation	July, 2001
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	AG-013736, AG-013736, 99.8%
Doses:	0 and 30 mg/kg
Species/strain:	Mice/ C57/BL6
Number/sex/group or time point:	3 male/group for control; 6/male/group for AG-013736-treated
Route; formulation; volume; and infusion rate:	Oral gavage twice daily (6 hours apart) for 4 consecutive days followed by 3 days recovery 0.5% w/v carboxymethylcellulose (CMC)

	10 mL/kg
Age:	Approximately 5 weeks
Weight:	20 to 24 grams
Parameters:	Clinical signs (pre-dose on dosing days) Body weights Heart rate Systemic arterial blood pressures (systolic, diastolic, and mean pressure) In addition, plasma samples were collected at 0.25, 1.5, 3, 6, 8, and 24 hours at 30 mg/kg

Results and Conclusion:

- No mortalities were observed.
- At 30 mg/kg and baseline, ↑ systolic 9-16%, ↑ diastolic 13-20%, and ↑ mean arterial 11-18%, compared to control.
- At 30 mg/kg and 1-8 hours post-dose, ↓ Heart rate; -10 to -13%.
- At 30 mg/kg and 16-24 hours post-dose, ↑ Heart rate; 10-14%.
- Heart rate and blood pressure remained ↑ 10-20% at times during the first 2 days of recovery and began to return to baseline values on recovery Day 3.
- Most systemic arterial blood pressure changes were the result of AG-013736 .

Study Title: SP0304: Evaluation of Chronic AG13736 Treatment in Conscious Telemeterized Wistar Rats

Key Study Findings:

- Temporal patterns of systolic blood pressure and heart rate showed changes in daily peak time and increased fluctuations, respectively at ≥300 mg/kg/day.
- Following withdrawal, systolic blood pressure and heart rate returned to vehicle-treated values within 48 hours.

Report #:	SP0304
Conducting Laboratory and Location:	Pfizer Global Research and Development Safety Pharmacology, Drug Safety Evaluation San Diego, CA 92121
Date of Study Initiation	July, 2009
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	AG-013736, AC3088, 100%
Doses:	0, 100, 300, and 500 mg/kg/day

Species/strain:	Rat/Wistar
Number/sex/group or time point:	9 M (vehicle); 11M (300 and 500 mg/kg/day)
Route; formulation; volume; and infusion rate:	Oral gavage twice daily (6 hours apart) for 7 days 0.5% w/v carboxymethylcellulose (CMC) 10 mL/kg
Age:	Approximately 7 weeks
Weight:	225-250 grams
Parameters:	Clinical signs (pre-dose on dosing days) Body weights Heart rate Systemic arterial blood pressures (systolic, diastolic, and mean pressure) In addition, plasma samples were collected at 2, 6, 8, and 24 hours post dose

Experimental conditions:

Group	Oral treatment	Animals/group	Dose (mg/kg/day)
1	0.5% w/v CMC	9	0
2	AG13736	9	100
3	AG13736	11	300
4	AG13736	11	500

Results and Conclusion:

- No mortalities were observed.
- A modest dose-dependent elevation in systolic blood pressure (SBP) was observed in all treated animals compared to controls (See Applicant's Table 1)
- Doses of 300 and 500 mg/kg/day resulted in statistically significant increases in SBP on Days 2-4 of treatment (See Applicant's Table 1).
- SBP was significantly increased on Days 5-7 of treatment at 500 mg/kg/day only (See Applicant's Table 1).

Table 1. Systolic Blood Pressure

	Vehicle	AG13736 (mg/kg/day)		
		100	300	500
Day 0 (mmHg)	117±2	114±2	114±2	115±2
Day 4 (mmHg)	116±1	117±1	118±2*	122±2*
Day 7 (mmHg)	116±1	116±2	118±2	120±2*
Day 14 (mmHg)	113±1	113±2	111±2	111±2

SBP, systolic blood pressure.
* $p<0.05$ vs. Vehicle, Data represented as mean ± SEM.
n=9 (vehicle, 100 mg/kg groups), n= 11 (300, 500 mg/kg groups)

(table excerpted from Applicant's package)

- Lower HR was observed in the 300 mg/kg/day group only.

Table 2. Heart Rate

	Vehicle	AG13736 (mg/kg/day)		
		100	300	500
Day 0 (bpm)	428±10	433±6	418±7	423±5
Day 4 (bpm)	428±6	430±5	405±5*	416±5
Day 7 (bpm)	421±5	425±4	398±7*	416±5
Day 14 (mmHg)	405±5	411±4	400±6	409±4

SBP, systolic blood pressure.
* $p<0.05$ vs. Vehicle, Data represented as mean ± SEM.
n=9 (vehicle, 100 mg/kg groups), n= 11 (300, 500 mg/kg groups)

(table excerpted from Applicant's package)

- Following withdrawal, SBP and heart rate returned to vehicle-treated values within 48 hours.
- AUC and C_{max} increased with increasing dose. See Applicant's Table 3 Plasma PK Table below.

Table 3. Plasma Pharmacokinetics

	AG13736 (mg/kg/day)		
	100	300	500
T max (hr)	6.5±2.8	6.7±2.4	6.4±2.8
C max total (ng/mL)	101.8±46	294.4±96	458.9±218
C max free (ng/mL)	3.66±1	10.80±3	19.26±7
AUC total (ng*hr/mL)	1080±573	3180±1060	4790±1060
AUC free (ng*hr/mL)	38.88±19	110.40±29	199.22±66.3

n=9 (100 mg/kg group), n= 11 (300, 500 mg/kg groups)
Data represent mean values ± STDEV
- plasma protein binding in rat = 96.4%

(table excerpted from Applicant's package)

Study Title: VFF/492: Telemetric Evaluation of Cardiovascular Effects in the Conscious Dog (oral administration)

Key Study Findings:
- No remarkable changes in ECG waveform intervals (PR, QRS, QT, QTcB, QTcF)

Report #:	VFF/492
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	July 17, 2001
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	AG-013736, AC3088, >98%
Doses:	0, 3, 10, and 30 mg/kg/day
Species/strain:	Dog/Beagle
Number/sex/group or time point:	1 M and 1 F
Route; formulation; volume; and infusion rate:	Oral gavage once with a 7 day wash out period 0.5% w/v carboxymethylcellulose (CMC) 2 mL/kg
Age:	297 days
Weight:	10.5-13.1 kg
Parameters:	Behavior observations Arterial blood pressure (systolic,

diastolic, and mean)
Heart rate
ECG intervals (PR, QRS, QT, QTcB, and QTcF)

In addition, plasma samples were collected at pre-dose and 2 hours post-dose

Experimental conditions:

Group	Oral treatment	Animals/group	Dose (mg/kg/day)
1	0.5% w/v CMC	1	0
2	AG13736	1	3
3	AG13736	1	10
4	AG13736	1	30

Results and Conclusion:

- No mortalities were observed.
- No remarkable changes in ECG waveform intervals (PR, QRS, QT, QTcB, QTcF)
- No remarkable behavioral changes
- Mean combined plasma concentration at 30 mg/kg was 223.4 ng/mL.

Study Title: SPT04-029: Assessment of the Effects of AG-013736 on Blood Pressure and Heart Rate in Conscious, Telemetered Beagle Dogs

Key Study Findings:

- It is inconclusive whether the increase in arterial blood pressure and decrease in heart rate are test article related or a result of normal biological variation due both (1) the variability in baseline hemodynamic data between the treatment groups on Day 1 vs. Day 3 and (2) the high degree of inter-animal variability in the plasma concentrations.

Report #:	SPT04-029
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	October 24, 2004
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	AG-013736, 2737-C-1-P, >98%
Doses:	0, 10, 50, and 150 mg/kg/day
Species/strain:	Dog/Beagle
Number/sex/group or time point:	6M
Route; formulation; volume; and	Oral gavage bid (6 hours apart) for 3

infusion rate:	days 0.5% w/v carboxymethylcellulose (CMC) 5 mL/kg
Age:	297 days
Weight:	10.5-13.1 kg
Parameters:	Clinical observations Body weights Arterial blood pressure (systolic, diastolic, and mean) Heart rate In addition, plasma samples were collected at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 24 hours after drug administration on Days 1 and Day 5 to access exposure on AG-013736 (drug) and AG-028458 (sulfoxide metabolite)

Experimental conditions:

Group	Oral treatment	Animals/group	Dose (mg/kg/day)
1	0.5% w/v CMC	6	0
2	AG13736	6	10
3	AG13736	6	50
4	AG13736	6	150

Results and Conclusion:

- No mortalities were observed.
- Arterial blood pressure parameters increased during treatment when compared to vehicle or baseline during study Days 1 and 3.
- Heart rate tended to decrease over time.
- Both arterial blood pressure and heart rate trends were dose-related; however, the variability in baseline hemodynamic data between the treatment groups on Day 1 vs. Day 3 and the high degree of inter-animal variability in the plasma concentrations preclude a definitive conclusion on whether the trends noted in arterial blood pressure and heart rate are test article related or a result of normal biological variation.
- Mean pharmacokinetic parameter estimates for AG-013736 and AG-028458 are shown in the Tables below. Pharmacokinetic analysis could not be conducted for treatment group 1 (5 mg/kg/dose), since all plasma concentrations were below the limit of quantitation of 4.00 ng/mL (BLQ).

Summary of Pharmacokinetic Parameter Estimates for AG-013736 on Study Day 5 after Repeat Oral Gavage Dose Administration of AG-013736

Pharmacokinetic Parameter	5 mg/kg/dose AG-013736		25 mg/kg/dose AG-013736		75 mg/kg/dose AG-013736	
	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
C_{max} (ng/mL)	BLQ	BLQ	81.5 ($n = 6$)	172 ($n = 6$)	124 ($n = 6$)	787 ($n = 6$)
AUC_{last} (ng· h/mL)	NA	NA	171 ($n = 6$)	394 ($n = 6$)	246 ($n = 6$)	5230 ($n = 6$)
t_{max}[b] (h)	NA	NA	2 ($n = 3$)	2[a] ($n = 3$)	2 ($n = 4$)	3[a] ($n = 6$)
t_{last}[b] (h)	NA	NA	3 ($n = 3$)	4[a] ($n = 3$)	5 ($n = 4$)	4[a] ($n = 6$)
$t_{1/2}$ (h)	NE	NE	0.39 ($n = 1$)	NE	NE	1.8 ($n = 1$)

BLQ = Below the Quantitation Limit of 4.00 ng/mL
NA = Not Applicable
NE = Not Estimated
[a]= Relative to the time of administration of Dose 2.
[b] = Median for t_{max} and t_{last}

Summary of Pharmacokinetic Parameter Estimates for AG-028458 on Study Day 5 after Repeat Oral Gavage Dose Administration of AG-013736

Pharmacokinetic Parameter	5 mg/kg/dose AG-028458		25 mg/kg/dose AG-028458		75 mg/kg/dose AG-028458	
	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
C_{max} (ng/mL)	BLQ	BLQ	26.9 ($n = 6$)	37.3 ($n = 6$)	21.0 ($n = 6$)	111 ($n = 6$)
AUC_{last} (ng· h/mL)	NA	NA	62.0 ($n = 6$)	96.4 ($n = 6$)	51.4 ($n = 6$)	238 ($n = 6$)
t_{max}[b] (h)	NA	NA	1.5 ($n = 4$)	3[a] ($n = 5$)	2 ($n = 4$)	3[a] ($n = 6$)
t_{last}[b] (h)	NA	NA	3 ($n = 4$)	4[a] ($n = 5$)	3.5 ($n = 4$)	4[a] ($n = 6$)
$t_{1/2}$ (h)	NE	NE	0.63 ($n = 1$)	NE	NE	NE

BLQ = Below the Quantitation Limit of 4.00 ng/mL
NA = Not Applicable
NE = Not Estimated
[a]= Relative to the time of administration of Dose 2.
[b] = Median for t_{max} and t_{last}

(tables excerpted from Applicant's package)

5 Pharmacokinetics/ADME/Toxicokinetics

Study title: Determination of plasma levels in rats following oral administration (study # VFF/526). See Attachment 1.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: Acute Oral Toxicity Study in Mice with AG13736. Study # 22337-D-800. See Attachment 1

Study title: Single Dose Oral Gavage Toxicity and Toxicokinetic Study with AG-013736 in Dogs with a 14-Day Observation Period.

Study no.:	(b) (4) – 6348-505 Pfizer – 08LJ017
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	15 May 2008
GLP compliance:	No
QA statement:	Yes
Drug, lot #, and % purity:	AG-0137736, lot # AXM-00-004A, 99.8% purity

Key Study Findings

- Single dose administration of AG-013736 up to 2000 mg/kg did not cause any mortality in dogs.
- There were no drug-related changes in body weight, food consumption or clinical pathology parameters.
- Combined male and female C_{max} and AUC_{0-24} were approximately 499 ng/mL and 5070 ng*hr/mL, respectively

Methods

Doses:	0, 500, 1000, and 2000 mg/kg
Frequency of dosing:	single dose
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% carboxymethylcellulose
Species/Strain:	dog / Beagle
Number/Sex/Group:	3
Age:	8 to 9 months
Weight:	7.3 to 8.2 kg for males 7.1 to 8.8 kg for females
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

- None
-

Clinical Signs (Twice daily)

- 500 mg/kg – Abnormal fecal excretion in one male
- 2000 mg/kg – Vomiting and mucoid and discolored feces

Body Weights (predose, dosing day and weekly after dosing)

- No difference

Feed Consumption (Daily)

- No difference

Ophthalmoscopy Not conducted

ECG Not conducted

Hematology

- No significant difference (large variations and small number of animals)

Clinical Chemistry

- No difference

Urinalysis Not conducted
Gross Pathology Not conducted
Organ Weights Not conducted
Histopathology Not conducted

Toxicokinetics (1, 4, 8, and 24 hours after dosing)

Mean toxicokinetic parameters

Dose group	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng*hr/mL
2	M	347±175	3.0±1.7	1950±1470
	F	574±407	4.3±3.5	4740±4460
3	M	334±381	16±13	2580±3170
	F	177±151	16±13	1680±1880

Dose group	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng•hr/mL
4	M	295±490	2.0±1.7	3420±5780
	F	702±887	11±12	6720±9130

6.2 Repeat-Dose Toxicity

Study title: 14-Day Gavage Toxicity Study with AG13736 in Mice: Study # 6750-144. See Attachment 1

Study title: 28-Day Gavage Toxicity Study with AG13736 in Mice: Study # 6750-145. See Attachment 1

Study title: 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with AG13736 in Mice with a 13-Week Interim Sacrifice and 4-Week Recovery Period.

Study no.: (b) (4) study # 6750-148
Study report location: (b) (4)
Conducting laboratory and location: (b) (4)

Date of study initiation: 24 August 2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: AG13736, lot # AC3088, 98% purity

Key Study Findings

- Oral administration of AG13736 induced poor health, broken and/or missing tooth/teeth and malocclusion in a dose-dependent manner.
- STD_{10} was 30 mg/kg/day.
- Spleen, testes and uterus weights were reduced in a dose-related manner and correlated with microscopic findings.
- AUC increase was slightly greater than dose proportional.

Methods

Doses: 0 (control), 5, 15, 50 and 125 mg/kg/dose
Frequency of dosing: twice daily (see table below for details)
Route of administration: Oral gavage
Dose volume: 5 mL/kg/dose
Formulation/Vehicle: 0.5% carboxymethylcellulose
Species/Strain: Crl:CD-1®(ICR)BR mice

Number/Sex/Group: 40
Age: 6 to 7 weeks
Weight: 25 to 34 g for males
20 to 28 g for females
Satellite groups: 100 animals/sex/group for PK
Unique study design: None
Deviation from study protocol: None

Group	Number of Animals Male	Number of Animals Female	Dose Level mg/kg/day	Dose Level mg/kg/BID	Concentration mg/mL
			Main Study Mice		
1 (Control)[a,b]	40	40	0	0	0
2 (Low)[a]	30	30	10	5	1
3 (Mid)[a,b]	40	40	30	15	3
4 (Mid-High)[a,b]	40	40	100	50	10
5 (High)[a,b]	40	40	250	125	25
			Toxicokinetic Mice		
6 (Low)	100	100	10	5	1
7 (Mid)	100	100	30	15	3
8 (Mid-High)	100	100	100	50	10
9 (High)	100	100	250	125	25

a The first surviving 15 mice/sex/group (based on accession numbers) were sacrificed after at least 13 weeks of treatment.

b The subsequent first surviving 10 mice/sex/group (based on accession numbers) were sacrificed following at least 13 weeks of treatment and 4 weeks of recovery, except for the 250 mg/kg/day dose group where 8 males and 9 females were sacrificed.

c The remaining animals were sacrificed following 26 weeks of treatment (Terminal sacrifice).

(excerpted from the Applicant's submission)

Observations and Results

Mortality: Scheduled and unscheduled sacrifices or deaths

Group/ Treatment	Interim Sacrifice		Recovery Sacrifice		Terminal Sacrifice		Mortality	
	M	F	M	F	M	F	M	F
1 (Control)	15	15	10	10	15	14	0	1
2 (Low - 10 mg/kg/day)	15	15	0	0	14	15	1	0
3 (Mid - 30 mg/kg/day)	15	15	10	10	13	14	2	1
4 (Mid High - 100 mg/kg/day)	15	15	10	10	11	11	4	4
5 (High - 250 mg/kg/day)	15	15	8	9	6	3	11	13

M = Males/ F = Females

(excerpted from the Applicant's submission)

Clinical Signs: (twice daily)

- 100 and 250 mg/kg/day - Treatment-related increases in malocclusion, broken and/or missing tooth/teeth, poor health, hypoactivity, hunched posture, thinness, and/or rough hair coat

Body Weights: (weekly)

Mean body weight Change (g)

Week	Sex	Group				
		1	2	3	4	5
		Dose (mg/kg/dose)				
		0	5	15	50	125
1-13 (interim phase)	M	6.3±2.4	6.4±2.3	5.0±3.1	6.0±1.8	1.8*±5.1
	F	4.4±1.6	4.4±1.5	4.3±1.6	3.9±1.5	2.5±2.8
14-17 (recovery phase)	M	1.0±0.8	0	1.5±1.2	1.1±0.8	4.9*±3.6
	F	-0.4±1.0	0	2.5*±1.4	1.2±0.5	2.6*±2.1
14-26 (terminal phase)	M	0.9±0.7	1.2±1.1	1.1±1.4	-0.5±3.1	0
	F	0.1±1.8	1.0±1.1	0.9±1.1	1.4±1.9	0

Statistically significant from control; P ≤ 0.05

Feed Consumption: (weekly)

Mean Food consumption (g)

Week	Sex	Group				
		1	2	3	4	5
		Dose (mg/kg/dose)				
		0	5	15	50	125
1-13 (interm phase)	M	498±30	502±40	494±41	482±23	458±28
	F	452±27	458±25	441±21	430±20	426±24
14-17 (recovery phase)	M	157±10	0	156±11	161±9	160±12
	F	158±12	0	163±15	157±11	154±13
14-26 (terminal phase)	M	478±27	484±46	472±29	479±37	0
	F	457±33	471±31	453±31	442±37	0

Ophthalmoscopy: No treatment related ocular findings during weeks 13, 17, 22 and 26.
ECG: Not conducted
Hematology: (Weeks 14, 18, 22, and 27).
No dose-related effects
Clinical Chemistry: No drug-related effects
Urinalysis: No drug-related effects
Gross Pathology:

Interim sacrifice: 27-28 November 2001 (13 weeks)
Recovery sacrifice: 26-27 December 2001 (13 +4 weeks)
Terminal sacrifice (250 mg/kg/day): 22 January 2002
Terminal sacrifice (all remaining animals): 25-26 February 2002 ((26 weeks)

- Broken incisors in a dose-related manner, soft testes and small uteri of high dose animals.

Organ Weights:
- Reduced mean organ weight (relative and absolute) of spleen, testes, and uterus of mid and high dose animals.

Histopathology:
Adequate Battery: Yes
Peer Review: Yes
Histological Findings

Interim Sacrifice

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Organ/finding Dose (mg/kg/day)	0	10	30	100	250	0	10	30	100	250
Adrenal, cortex, hyperplasia, subcapsular						4	0	0	1	7
hemorrhage						0	0	0	1	0
necrosis						0	0	0	1	0
Bone, femur, thickened growth plate	0	0	3	0	9	0	0	1	2	9
Bone, tibia, thickened growth plate	0	0	0	1	4	0	0	0	0	4
Cecum, hyperplasia, epithelial	0	0	0	3	10					
inflammation	0	1	1	1	13					
crypt gland necrosis	0	0	0	0	1					
Colon, hyperplasia, epithelial	1	0	0	0	3	0	0	0	0	1
crypt gland necrosis	0	0	0	0	2					
inflammation	0	0	1	0	1	0	0	0	0	2
Duodenum, hyperplasia					1					
crypt gland necrosis					1					
Epididymis, sperm, abnormal morphology	0	0	1	6	16					
Esophagus, necrosis						0	0	0	0	1
Gallbladder, inflammation						0	0	0	0	1
Heart, peri/epicardium, inflammation and foreign material						0	0	0	0	1
Ileum, hyperplasia					1					
Incisor tooth, odontoblast,										

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Organ/finding Dose (mg/kg/day)	0	10	30	100	250	0	10	30	100	250
degeneration/necrosis/drop out	0	0	6	5	15	0	0	1	10	10
dentin, degeneration/ malformation	0	12	16	14	19	0	6	14	17	17
ameloblast, degeneration	0	0	8	5	2	0	0	2	4	6
periodontal inflammation	0	0	0	0	5					
pulp cavity, inflammation/necrosis/	0	0	0	1	11	0	0	0	0	2
fibroplasia, pulp cavity, fibroplasia	0	0	0	0	4					
odontopathy	0	12	16	14	20	0	6	14	17	17
Kidney, pyelonephritis					2					
abscess				1	1	0	0	0	1	0
Liver, necrosis					2					
pigment			1	1	10	0	0	0	1	5
extramedullary hematopoiesis, increased					1	0	0	0	1	0
Lung, alveolus, macrophages, vacuolated	7				13	4	0	0	1	6
congestion	0	0	2	0	6	0	0	0	1	2
hemorrhage					1					
Marrow, femur, hyperplasia, myeloid	0	0	1	0	1	0	0	0	1	0
Marrow, rib, , hyperplasia, myeloid	0	0	1	0	1	0	0	0	1	0
Nerve, sciatic, degeneration						0	0	0	0	1
Ovary, bursa, cyst						0	0	0	1	1
Ovary, decreased corpora lutea						1	3	7	13	17
Seminal vesicle, abscess	0	0	0	1	1					
inflammation, suppurative	0	0	0	0	1					
Spleen, extramedullary hematopoiesis, increased	5	14	8	9	5	8	8	3	13	7
pigment	0	0	1	2	7	1	0	0	2	3
depletion, lymphoid	0	0	2	2	7	0	0	0	3	6
Stomach, hyperkeratosis				1	2					
acanthosis					1					
Testis, sperm, abnormal morphology	0	0	0	0	1					
hypospermia	0	0	1	7	15					
Thyroid, inflammation						0	0	0	0	2
Thymus, depletion, lymphoid	0	0	2	0	5	1	0	0	2	4
perithymic, inflammation	0	0	1	0	0	0	0	0	0	1
Thyroid, follicle, cyst					1					
Trachea, peritracheal inflammation					1	0	0	0	0	1
Uterus, atrophy						0	0	0	1	10

Recovery Sacrifice

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Organ/finding Dose (mg/kg/day)	0	10	30	100	250	0	10	30	100	250
Cecum, hyperplasia, epithelial	0	0	0	2	3					
inflammation	0	0	0	0	1					
Colon, hyperplasia, epithelial						0	0	0	0	2
Epididymis, sperm, abnormal morphology	0	0	1	2	3					
Eye, retina, degeneration	0	0	0	0	1					
cornea, mineralization						0	0	0	0	1
Gallbladder, inflammation						0	0	0	0	1
Incisor tooth, odontoblast, degeneration/necrosis/drop out	0	0	0	0	1					
dentin, degeneration/ malformation	0	0	0	3	6	0	0	0	1	4
ameloblast, degeneration	0	0	2	2	0					
periodontal inflammation	0	0	0	0	1	0	0	0	0	1
odontopathy	0	0	0	3	7	0	0	0	1	4

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Organ/finding Dose (mg/kg/day)	0	10	30	100	250	0	10	30	100	250
Kidney, pyelonephritis						0	0	0	0	2
Liver, necrosis						0	0	0	0	1
extramedullary hematopoiesis, increased						0	0	0	0	1
Lung, alveolus, macrophages, vacuolated	1	0	0		3	0	0	0	0	1
congestion	0	0	0	0	1					
pneumonia, interstitial	0	0	0	0	1	0	0	0	0	2
Marrow, rib, histiocytic infiltrate	0	0	0	0	1					
Ovary, follicle cyst						0	0	4	4	3
decreased corpora lutea						o	o	2	3	1
Spleen, pigment						0	0	0	0	1
Testis, hypospermia	1	0	0	1	2					
syncytial cells	0	0	0	1	0					
Uterus, atrophy						0	0	0	1	2
edema						0	0	0	1	0

Terminal Sacrifice

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Organ/finding Dose (mg/kg/day)	0	10	30	100	250	0	10	30	100	250
Adrenal, medulla, unilaterally examined	0	0	0	0	2					
Bone, femur, thickened growth plate	0	0	0	0	2	1	1	1	0	5
Bone, tibia, thickened growth plate	0	0	0	0	1	1	0	1	0	1
Cecum, hyperplasia, epithelial	0	0	0	7	7	0	0	3	8	8
inflammation	0	2	3	9	8	0	0	5	11	9
crypt gland necrosis	0	0	0	0	1					
Colon, hyperplasia, epithelial	0	0	0	0	2	0	0	0	1	1
crypt gland necrosis	0	0	0	0	1	0	0	0	0	1
inflammation	0	0	0	0	2	0	0	0	1	1
Epididymis, sperm, abnormal morphology	0	1	0	2	7					
Eye, cornea, mineralization						0	0	0	0	1
Gallbladder, dilatation						0	0	0	0	1
erosion/inflammation/fibrosis						0	0	0	0	2
Incisor tooth, odontoblast, degeneration/necrosis/drop out	0	0	3	9	10	0	0	1	5	10
dentin, degeneration/ malformation	0	11	12	112	10	0	8	10	9	10
ameloblast, degeneration	0	1	0	2	3	0	4	0	2	4
periodontal inflammation	0	0	0	0	3	0	0	0	0	3
pulp cavity, inflammation/necrosis	0	1	0	2	3	0	0	1	0	2
pulp cavity, fibroplasia	0	0	0	1	0					
odontopathy	0	11	12	11	10	0	10	9	9	10
Kidney, abscess	0	0	0	0	1					
tubule vacuolation	0	0	0	0	1					
tubule, microconcretion						0	0	0	0	1
tubules, pigment						0	0	0	0	1
amyloidosis						0	0	0	0	1
Lacrimal gland, inflammation	2	1	0	1	5					
Liver, pigment	0	1	0	4	6	1	3	6	7	8
Lung, alveolus, macrophages, vacuolated	3	0	0	1	7	3	0	0	0	1
congestion	0	3	0	1	3	0	0	0	0	5
Optic nerve, degeneration						0	0	0	0	1
Ovary, decreased corpora lutea						1	4	9	11	10

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Organ/finding Dose (mg/kg/day)	0	10	30	100	250	0	10	30	100	250
Rectum, hyperplasia, epithelial	0	0	0	0	1					
inflammation						0	0	0	0	1
Spleen, extramedullary hematopoiesis, increased	4	6	4	6	7					
pigment	0	0	1	5	2	1	1	4	6	7
depletion, lymphoid	0	1	0	1	5	1	0	1	0	7
Stomach, erosion	0	0	0	0	2					
Testis, hypospermia	0	1	1	5	8					
Thymus, depletion, lymphoid	0	1	0	3	4	1	0	1	0	4
Urinary bladder, hemorrhage	0	0	0	1	0					
necrosis	0	0	0	1	0					
vacuolar change						0	0	0	0	2
uterus, atrophy						0	2	1	8	5

Special Evaluation: None

Toxicokinetics: (prior to dosing and 0.25, 0.5, 1, 2, 6, 6.5, 7, 8, 12 and 24 hours after dosing on day 1, week 13, and week 26)

Group mean AG13736 toxicokinetics in mice following oral administration

AG13736 Dose (mg/kg/day)	Sex*	Group Mean AG13736 Toxicokinetic Parameters On Day 1, Week 13, and Week 26								
		Day 1			Week 13			Week 26		
		C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0-\infty}$ (ng.hr/mL)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24h} (ng.hr/mL)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24h} (ng.hr/mL)
	M	190.90	6.5	496.97	94.50	0.5	427.44	108.13	6.5	342.74
10	F	239.33	0.5	724.58	719.67	0.3	664.73	166.77	6.5	457.04
	M + F	**179.12**	**6.5**	**609.65**	**372.70**	**0.3**	**546.09**	**137.45**	**6.5**	**401.07**
	M	699.67	6.5	2011.78	345.33	0.5	1837.22	436.33	1.0	1885.68
30	F	1137.00	6.5	3747.39	802.33	0.5	2955.85	817.67	0.5	2643.32
	M + F	**918.33**	**6.5**	**2879.55**	**573.83**	**0.5**	**2436.42**	**559.53**	**0.5**	**2262.19**
	M	2011.00	8.0	10996.08	1643.67	7.0	8067.28	2346.67	1.0	11297.13
100	F	3560.00	1.0	13715.85	2490.00	0.5	15035.76	2460.00	8.0	15200.02
	M + F	**2666.67**	**1.0**	**12353.66**	**1618.20**	**7.0**	**11508.58**	**1842.17**	**8.0**	**13562.81**
	M	7090.00	6.5	32579.60	7853.33	8.0	32236.45	2460.00	7.0	14394.15
250	F	7590.00	7.0	52525.57	5561.67	7.0	21511.68	3437.00	2.0	13800.88
	M + F	**6230.00**	**8.0**	**42114.54**	**5585.17**	**8.0**	**27150.32**	**3437.00**	**2.0**	**16834.43**

*Values were group means for n = 3 M or 3 F, or n = 6 M + F mice
Group mean values were calculated only if >50% of values were above the LLOQ (4 ng/mL)
Samples reported as BLQ (below limit of quantitation) were set equal to zero for TK calculation purposes.

(excerpted from the Applicant's submission)

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Study title: 14-Day Gavage Toxicity Study with AG13736 in Dogs: Study # 6750-142. See Attachment 1

Study title: 28-Day Gavage Toxicity Study with AG13736 in Dogs: Study # 6750-143. See Attachment 1

Study title: 26-Week Oral Gavage Chronic Toxicity and Toxicokinetic Study with AG13736 in Dogs with a 4-Week Recovery Period.

Study no.:	(b) (4) 6750-150
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 22, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AG13736, lot # AC3088

Key Study Findings

- Two females and one male at 10 mg/kg/day were euthanized during weeks 12, 17 and 18, respectively.
- Thymic lymphoid atrophy was the major microscopic finding.

Methods

Doses:	0.5, 1.5, 3.0, and 5.0 mg/kg/dose
Frequency of dosing:	Twice daily, at least 6 hours apart
Route of administration:	Oral gavage
Dose volume:	5 mL/kg/dose
Formulation/Vehicle:	0.5% carboxymethylcellulose
Species/Strain:	Beagle dogs
Number/Sex/Group:	See table below
Age:	7 to 10 months
Weight:	7.5 to 12.4 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Group	No. of Animals Male	No. of Animals Female	Dose Level (mg/kg/day)	Dose Level[a] (mg/kg/BID)	Concentration[a] (mg/mL)
1 (Control)[b,c,d]	10	10	0	0	0
2 (Low)[c]	8	8	1	0.5	0.1
3 (Mid)[c]	8	8	3	1.5	0.3
4 (Mid-High)[c,d]	11	11	6	3	0.6
5 (High)[c,d]	11	11	10	5	1.0

a The dose volume was 5 mL/kg/dose.
b Group 1 animals received the control article only.
c Four animals/sex in Groups 1 through 4 and four males and three females in Group 5 were sacrificed after at least 13 weeks of treatment.
d Two animals/sex in the control group and three animals/sex in Groups 4 and 5 were sacrificed following at least 13 weeks of treatment and 4 weeks of recovery.

(excerpted from the Applicant's submission)

Observations and Results:

Mortality (Twice daily)

- 10 mg/kg/day – Two females and one male were euthanized during weeks 12, 17 and 18, respectively.

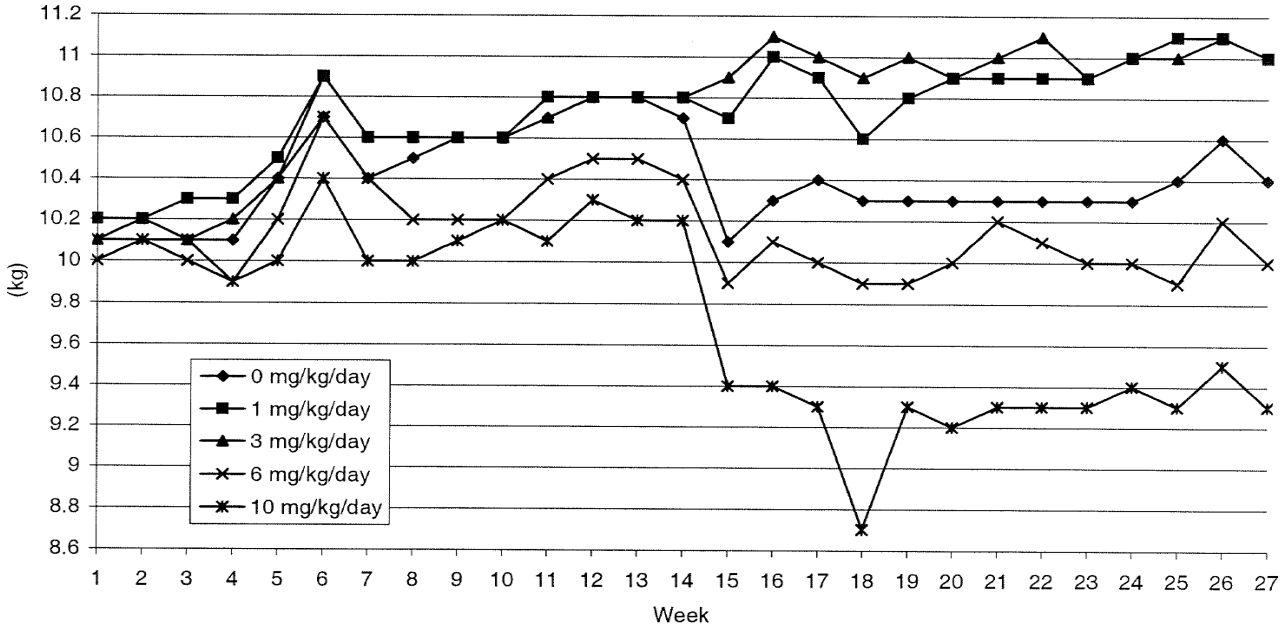
Clinical Signs (Twice daily)

- 10 mg/kg/day – Dose dependent fecal abnormalities (colored feces, mucoid feces, liquid feces),

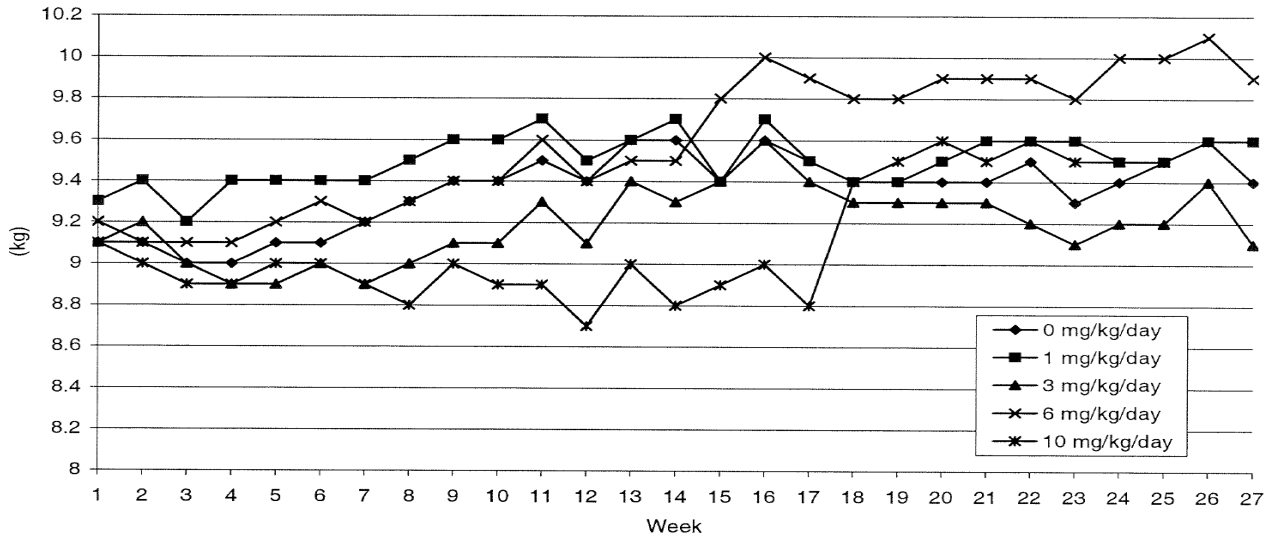
Body Weights (Weekly)

- 10 mg/kg/day – 12% decreased relative to control

Mean Male body weight



Mean female body weight



(excerpted from the Applicant's submission)

Feed Consumption (Weekly)

- 10 mg/kg/day - decreased

Ophthalmoscopy (Once before treatment and once during weeks 13, 17 and 26)

Ophthalmic observations

Sex		Male					Female				
Group		1	2	3	4	5	1	2	3	4	5
Ophthalmic observations	Dose (mg/kg/day)	0	1	3	6	10	0	1	3	6	10
Week 13	Lens, posterior capsular, cataract, eye right	0	0	0	0	0	0	0	0	1	1
	left	0	0	0	0	0	0	0	0	1	0
	Vitreous floaters, eye-left	0	0	0	0	0	0	0	0	1	0
Week 26	Lens, posterior capsular ,cataract, eye-right	0	0	1	0	0	0	0	0	0	0
	Vitreous floaters, eye-left	0	0	0	0	0	0	0	0	1	0
Recovery	Lens, posterior capsular cataract, eye-right	0			0	0	0			0	1

ECG (Once before treatment and once during weeks 13, 17 and 26)

Changes in electrocardiographic data compared to baseline (%)

Week	Parameter	Male					Female				
		Group 1	Group 2	Group 3	Group 4	Group 5	Group 1	Group 2	Group 3	Group 4	Group 5
13	Heart rate (beats/min)	4↓	7↓	3↑	6↓	2↑	5↑	25↑	9↓	0	6↑
	P-R Int (second)	0	11↓	0	11↑	0	0	0	0	0	11↑
	QRS Int (second)	0	0	0	20↓	0	25↑	0	0	0	0
	Q-T Int (second)	6↑	0	5↓	5↑	5↑	5↓	11↓	0	6↓	5↓

Week	Parameter	Male					Female				
		Group 1	Group 2	Group 3	Group 4	Group 5	Group 1	Group 2	Group 3	Group 4	Group 5
26	Heart rate (beats/min)	3↓	8↓	0	0	4↑	38↑	22↑	5↓	2↑	12↑
	P-R Int (second)	0	0	11↑	22↑	0	0	0	11↑	11↑	11↑
	QRS Int (second)	25↑	25↑	25↑	0	0	25↑	25↑	25↑	0	0
	Q-T Int (second)	6↑	0	0	12↑	0	6↓	0	0	12↑	12↓

Hematology Prior to treatment, during weeks 6 (end of week), 14 (beginning of week), 17 (end of week), and 27 (beginning of week).

- No dose-related hematological changes

Clinical Chemistry

- No dose-related changes

Urinalysis

- No dose-related changes

Gross Pathology

Interim sacrifice: Day 91 of the study
Recovery sacrifice: Day 123 of the study
Terminal sacrifice: Day 183 of the study

Histopathology

Adequate Battery: Yes
Peer Review: Yes

Histological findings (interim sacrifice)

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day	0	1	3	6	10	0	1	3	6	10
Organ and findings Number of animals	4	4	4	4	4	4	4	4	4	3
Epididymides, inflammation, chronic-active	0			2	0					
abnormal, sperm forms	1			1	1					
Kidney, infiltrate, lymphohistiocytic	0	1	2	0	0	0	0	0	1	0
Liver, necrosis, individual hepatocyte	0	0	0	0	0	0	0	1	0	0
Lung, inflammation, chronic	0	1	4	1	2	2	1	0	3	0
infiltrate, macrophage, alveolar	0	0	0	0	0	1	0	0	0	0
Ovary, inflammation, vascular/perivascular						0	0	0	0	1

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day	0	1	3	6	10	0	1	3	6	10
Organ and findings Number of animals	4	4	4	4	4	4	4	4	4	3
Spleen, pigment, increased	0			0	0	0			0	1
Thymus, involution	0	1	0	1	0	1	1	1	1	0
atrophy, lymphoid	0	0	0	0	0	0	0	0	0	3

- There were no microscopic findings considered to be related to the administration of test article at the recovery sacrifice.

Histological findings (terminal sacrifice)

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day	0	1	3	6	10	0	1	3	6	10
Organ and findings Number of animals	4	4	4	4	3	4	4	4	4	3
Kidney, infiltrate, lymphohistiocytic	0	1	0	0	0	1	1	0	0	1
Liver, necrosis, individual hepatocyte	0	0	0	0	0	0	0	0	1	0
Spleen, pigment, increased	0	0	0	0	0	0	0	0	0	1
Thymus, involution	2	2	3	3	2	1	2	3	2	1

Toxicokinetics

Prior to dosing (days 85 and 176) and approximately 0.25, 0.5, 1, 2, 6, 6.5, 7, 8, 12, and 24 hours on days 1, 85 and 175.

Mean Toxicokinetics

AG13736 Dose (mg/kg/day)	Sex	Mean AG13736 Toxicokinetic Parameters On Day 1, 84, and 175								
		Day 1[a]			Day 84[a]			Day 175[b]		
		C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0-\infty}$ (ng.hr/mL)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24h} (ng.hr/mL)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24h} (ng.hr/mL)
	M	1.67 (3.14)	2.8 (3.3)	2.25 (2.03)	4.14 (4.72)	2.2 (2.4)	9.83 (8.73)	1.29 (0.53)	19.8 (8.5)	11.02 (5.87)
1	F	6.02 (11.00)	2.4 (2.3)	14.30 (17.87)	3.79 (8.37)	8.9 (9.8)	24.46 (53.97)	1.85 (1.90)	8.5 (2.4)	6.08 (2.28)
	M + F	3.99 (8.36)	2.6 (2.7)	8.67 (14.15)	3.96 (6.57)	5.5 (7.7)	17.15 (38.10)	1.57 (1.33)	14.1 (8.3)	8.55 (4.90)
	M	6.66 (6.35)	2.5 (3.1)	22.20 (19.55)	2.26 (1.94)	4.7 (4.6)	11.88 (6.94)	7.28 (11.22)	4.3 (3.8)	12.30 (14.24)
3	F	5.34 (2.65)	7.8 (0.5)	16.92 (6.70)	21.88 (31.94)	7.3 (3.0)	68.85 (86.27)	17.03 (19.30)	5.8 (2.5)	48.65 (39.85)
	M + F	6.00 (4.75)	5.1 (3.5)	19.56 (14.38)	12.07 (24.07)	6.0 (4.0)	40.36 (56.04)	12.15 (15.52)	5.0 (3.1)	30.47 (33.84)
	M	22.89 (17.64)	4.3 (3.4)	94.16 (80.16)	41.78 (80.23)	5.6 (3.3)	138.66 (249.29)	29.53 (24.09)	4.3 (3.2)	97.57 (78.81)
6	F	15.76 (13.84)	3.9 (3.3)	52.20 (44.19)	50.12 (119.63)	6.4 (2.9)	158.23 (380.06)	56.54 (82.70)	6.0 (3.4)	176.37 (259.19)
	M + F	19.32 (15.89)	4.1 (3.3)	73.18 (66.72)	45.95 (99.49)	6.0 (3.0)	148.45 (313.81)	43.03 (58.21)	5.1 (3.2)	136.97 (182.28)
	M	127.06 (114.70)	2.4 (1.9)	319.89 (206.34)	89.01 (84.25)	5.6 (3.4)	350.72 (378.78)	286.37 (267.71)	5.7 (3.2)	959.09 (926.29)
10	F	46.95 (20.81)	2.4 (1.9)	187.97 (110.09)	60.32 (44.54)	7.4 (1.9)	216.88 (139.11)	8.59 (5.34)	7.7 (0.6)	29.43 (13.68)
	M + F	87.01 (90.28)	2.4 (1.9)	253.93 (174.94)	75.35 (68.24)	6.5 (2.9)	286.99 (291.78)	147.48 (227.66)	6.7 (2.3)	494.26 (776.25)

Mean (SD) values were calculated only if >50% of values were above the LLOQ (0.2 ng/ml)
Samples reported as BLQ (below limit of quantitation) were set equal to zero for PK calculation purposes.
[a]Mean (SD) values calculated from 8M and 8F dogs for the 1 and 3 mg/kg/day dose groups and from 11M and 11F dogs for the 6 and 10 mg/kg/day dose groups.
[b]Mean (SD) values calculated from 4M and 4F dogs for the 1, 3, 6 mg/kg/day dose groups and from 3M and 3F dogs for the 10 mg/kg/day dose group.
Statistical analysis (Student's paired t-test) resulted in no significant differences for M + F TK parameters between Days 1 and 84 or between Days 1 and 175 at all doses. However, at the 1 mg/kg dose, Tmax was significantly different between Days 1 and 175, and at 10 mg/kg, Tmax was significantly different between Days 1 and 84.

(excerpted from the Applicant's submission)

Study title: 9-Month Oral Gavage Toxicity and Toxicokinetic Study of AG-013736 in Dogs with 8-Week Recovery.

Study no.: (b) (4) – 6348-470
Sponsor – 07LJ134

Study report location: electronic submission

Conducting laboratory and location: (b) (4)

Date of study initiation: January 17, 2008

GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AG-013736, lot # AXM-00-0004A (GR01037), 99.93% purity

Key Study Findings

- Administration of AG-013736 twice daily (approximately 6 hours apart) via oral gavage at 0.5, 1.5,or 3 mg/kg/dose twice daily for 39 weeks to male and female dogs did not cause any mortality.
- Treatment of AG-013736 did not affect the mean body weight, food consumption, ophthalmic or electrocardiographic evaluation.
- Treatment-related decreased testis weight of mid and high dose animals correlated with microscopic findings.
- Systemic exposure (C_{max} and AUC_{0-24}) indicated the slight accumulation.

Methods

Doses:	0.5, 1.5 or 3.0 mg/kg/dose
Frequency of dosing:	Twice daily (~6 hours apart)
Route of administration:	Oral gavage
Dose volume:	2.5 mL/kg/dose or 5 mL/kg/day
Formulation/Vehicle:	0.5% CMC
Species/Strain:	Beagle dogs
Number/Sex/Group:	See table below
Age:	7-8 months
Weight:	6.8 to 9.4 kg for males 5.6 to 7.3 kg for females
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Group[a]	No. of Animals[b]		Dose Level[c]		Dose Concentration
	Male	Female	(mg/kg/day)	(mg/kg/dose)	(mg/mL)
1 (Control)	6	6	0	0	0
2 (Low)	4	4	1	0.5	0.2
3 (Mid)	7	7	3	1.5	0.6
4 (High)	7	7	6	3.0	1.2

a Group 1 received control article only.
b Animals designated for recovery sacrifice (the last two animals/sex in Group 1; the last three animals/sex in Groups 3 and 4) in ascending order underwent 8 weeks of recovery following final dose administration.
c Animals were dosed at a volume of 2.5 mL/kg/dose or 5 mL/kg/day.

(excerpted from the Applicant's submission)

Observations and Results

Mortality (Twice daily)

- None

Clinical Signs

- Treatment related fecal abnormalities (no feces, liquid, mucoid, and nonformed feces, discolored feces) in a dose-response related manner.

Body Weights (Weekly)

- No drug-related effects

Feed Consumption (Daily)

- No dose- or duration of exposure-related effects

Ophthalmoscopy (Predose and on Days 106 and 259)

- No visible dose-related lesions

ECG (Predose and on Days 112 and 274)

- No clear test article-related effect on the PR, QRS or QT intervals as shown below.

Mean electrocardiographic data - Males

			Dosing Day 274 -Predose						Dosing Day 274 - 1 - 2 Hours Postdose					
Group	Dose Level (mg/kg/day)		RR Int (msec)	Heart Rate (Beats/minute)	QRS Int (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)	RR Int (msec)	Heart Rate (Beats/minute)	QRS Int (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)
1	0	Mean	500	124	36	84	189	240	508	125	36	84	192	243
		SD	102.6	23.7	1.9	8.2	8.2	11.0	142.3	29.1	2.3	14.1	11.7	8.2
		N	6	6	6	6	6	6	6	6	6	6	6	6
2	1	Mean	562	110	35	96	192	233	571	106	37	98	192	232
		SD	124.8	21.8	2.0	19.2	11.9	10.4	60.4	12.0	1.5	18.5	5.8	6.4
		N	4	4	4	4	4	4	4	4	4	4	4	4
3	3	Mean	536	117	35	85	186	230	559	113	36	87	188	229*
		SD	112.4	26.8	2.2	7.8	10.1	5.9	121.0	31.0	2.0	10.9	12.9	6.4
		N	7	7	7	7	7	7	7	7	7	7	7	7
4	6	Mean	535	114	35	87	189	234	576	107	36	87	194	234
		SD	71.4	19.8	2.1	10.4	7.1	3.9	106.9	20.0	1.8	11.9	8.8	7.0
		N	7	7	7	7	7	7	7	7	7	7	7	7

* P< or =0.05

(excerpted from the Applicant's submission)

Mean electrocardiographic data - females

			Dosing Day 274 -Predose						Dosing Day 274 - 1 - 2 Hours Postdose					
Group	Dose Level (mg/kg/day)		RR Int (msec)	Heart Rate (Beats/minute)	QRS Int (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)	RR Int (msec)	Heart Rate (Beats/minute)	QRS Int (msec)	PR Int (msec)	QT Int (msec)	QTc Int (msec)
1	0	Mean	442	139	34	86	179	235	429	144	34	83	180	239
		SD	69.2	23.1	2.0	8.6	12.5	8.5	83.8	23.5	1.9	7.3	18.4	9.7
		N	6	6	6	6	6	6	6	6	6	6	6	6
2	1	Mean	502	120	34	97	183	230	564	111	35	97	190	231
		SD	37.4	8.9	1.3	3.7	1.8	4.7	140.0	24.0	2.0	7.3	9.3	13.3
		N	4	4	4	4	4	4	4	4	4	4	4	4
3	3	Mean	554	110	35	89	187	228	671*	94*	36	87	198	227
		SD	79.3	16.9	2.7	10.3	10.2	9.3	149.8	22.2	2.7	8.1	14.1	7.0
		N	7	7	7	7	7	7	7	7	7	7	7	7
4	6	Mean	518	118	36	88	193	240	606	103*	37	90	201	238
		SD	72.2	16.5	1.7	10.5	11.4	5.7	126.2	22.6	2.2	8.7	13.2	7.3
		N	7	7	7	7	7	7	7	7	7	7	7	7

* P< or =0.05

(excerpted from the Applicant's submission)

Hematology (predose and approximately 0.25, 0.5, 1, 2, 6 (immediately before second dose), 6.5, 7, 8, 12, and 24 hours postdose on Days 1, 36, 90, and 272 of the dosing phase)

- No treatment-related hematological changes

Clinical Chemistry

- Group 4 (6 mg/kg/day) – ↑ Cholesterol (1.3x control group mean) in females only on day 275 and reversed during the recovery period.

Urinalysis

- No drug-related effects

Gross Pathology First 4 animals/sex in groups 1-4 were sacrificed and necropsied after 39 weeks of treatment. Remaining animals were sacrificed and necropsied after 39 weeks of administration followed by 8 weeks of recovery.

Organ Weights

- Groups 3 and 4 – Dose-related decreased testis weights

Histopathology

Adequate Battery: Yes
Peer Review: No

Final phase sacrifice (39 week sacrifice)

Sex	Males				Females			
Dose group	1	2	3	4	1	2	3	4
Dose (mg/kg/day)	0	1	3	6	0	1	3	6
Tissue with diagnoses / No. in group	4	4	4	4	4	4	4	4
Brain, infiltrate, lymphocytes/macrophages	0	0	0	1	0	0	0	2
mineralization	0	0	0	0	0	0	0	1
Epididymis, hypospermia	0	0	0	4				
debris, cellular, lumen	0	0	1	2				
infiltrate, lymphocytes/macrophages	1	1	0	0				
cribriform change, epithelium	2	1	2	0				
Testis, degeneration/atrophy	1	0	3	4				
syncytial cells	0	0	3	4				

Recovery Phase sacrifice

Sex	Males			Females		
Dose group	1	3	4	1	3	4
Dose (mg/kg/day)	0	3	6	0	3	6
Tissue with diagnoses / No. in group	2	3	3	2	3	3
Epididymis, hypospermia	0	0	0			
debris, cellular, lumen	1	1	0			
infiltrate, lymphocytes/macrophages	1	1	0			
cribriform change, epithelium	1	0	2			
Prostate, infiltrate, lymphocytes/macrophages	0	0	1			
Testis, degeneration/atrophy	0	0	0			
syncytial cells	1	1	0			
hypospermatogenesis	1	2	2			
hypoplasia	1	2	2			
spermatocele	0	0	1			
Ovary, atresia, follicle				2	2	3

Toxicokinetics

Summary of mean combined male and female toxicokinetics

Group	dose (mg/kg/day)	Cmax (ng/mL)				Tmax (hr)				AUC 0-24 (ng*hr/mL)			
		Day 1	Week 6	Week 13	Week 39	Day 1	Week 6	Week 13	Week 39	Day 1	Week 6	Week 13	Week 39
2	0.89	0.07	2.1± 3.2	1.7± 2.6	3.3± 6.5	1.3	1.0	1.0± 0.5	1.1± 0.4	0.25	3.9± 5.4	3.8± 4.1	6.9± 9.8
3	2.56	2.4± 4.8	28.0± 27.3	14.1± 20.9	9.5± 17.0	0.8± 0.4	2.6± 4.5	2.5± 4.5	1.2± 0.6	6.5± 12.5	83.9± 90.8	46.2± 65.6	25.5± 49.0
4	6.0	23.8± 36.4	51.1± 42.9	70.2± 65.9	31.8± 28.8	0.9± 0.5	1.1± 0.6	1.6± 0.6	1.6± 0.5	74.2± 125	133± 118	231± 226	114± 121

Histopathology inventory:

Study	6750-148	6750-150	6348-470
Species	Mouse	Dog	Dog
Adrenals	X*	X*	X*
Aorta			X
Bone Marrow smear	X	X	X
Bone (femur)	X	X	X
Brain	X*	X*	X*
Cecum	X	X	X
Cervix			X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*	X	X
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube			
Gall bladder	X*	X	X
Gross lesions	X	X	X
Harderian gland			
Heart	X*	X*	X*
Ileum	X	X	X
Injection site			
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lachrymal gland	X		
Larynx			X
Liver	X*	X*	X*
Lungs	X*	X*	X
Lymph nodes, cervical			
Lymph nodes mandibular	X	X	
Lymph nodes, mesenteric	X	X	X
Mammary Gland	X	X	X
Nasal cavity			
Optic nerves	X	X	X
Ovaries	X*	X*	X
Pancreas	X	X	X
Parathyroid	X*	X*	X
Peripheral nerve			
Pharynx			

Study	6750-148	6750-150	6348-470
Species	Mouse	Dog	Dog
Pituitary	X*	X*	X
Prostate	X*	X*	X
Rectum	X	X	
Salivary gland	X	X	X
Sciatic nerve	X	X	X
Seminal vesicles	X		
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord	X	X	X
Spleen	X	X*	X*
Sternum	X	X	X
Stomach	X	X	X
Testes	X	X*	X*
Thymus	X*	X*	X*
Thyroid	X*	X*	X
Tongue	X	X	X
Trachea	X	X	X
Upper incisors			
Urinary bladder	X	X	X
Uterus	X	X*	X
Vagina	X*	X	X
Zymbal gland			

X, histopathology performed
*, organ weight obtained

7 Genetic Toxicology

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

Study title: Microbial Reverse Mutation Assays: Study # 01-2191-02, see Attachment 1.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: *In Vitro* Cytogenetic Assays: Study # 01-2191-03, see Attachment 1.

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

Study title: Mouse Micronucleus Assay: Study # 01-2191-01, see Attachment 1.

8 Carcinogenicity: None conducted

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Combined Oral (Gavage) Fertility and Developmental Toxicity Study in Mice Evaluating Twice Daily Administration of AG-013736.

Study no.:	(b) (4) – LIA00238 Sponsor – 06GR118
Study report location:	electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 12, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AG-013736, lot # EO10003889

Key Study Findings

- Toxicity was observed in both sexes at 250 mg/kg/day.
- In the present study, NOAEL for general toxicity was 100 and 250 mg/kg/day for females and males, respectively.
- NOAEL for reproductive toxicity was 30 mg/kg/day in males; no NOAEL for reproductive toxicity was established in females.
- Duration of cohabitation in 250 mg/kg females was increased (4.4 days) as compared to vehicle treated females (2.4 days).
- Fertility index of treated female mice decreased in a dose-related manner.
- The cauda epididymal sperm count of high dose males was reduced by 15% as compared to the vehicle control group value.
- Litter averages for corpora lutea and implantation among the four dose groups did not change significantly.

Methods

Doses:	Males – 0, 5, 15 or 50 mg/kg/dose (0, 10, 30 or 100 mg/kg/day) Females – 0, 15, 50 or 125 mg/kg/dose (0, 30, 100 or 250 mg/kg/day
Frequency of dosing:	Twice daily, approximately 6 hours apart
Dose volume:	5 mL/kg/dose
Route of administration:	Oral gavage

Formulation/Vehicle:	0.5% Carboxymethylcellulose, lot # 052K0203
Species/Strain:	Crl:CD1(ICR) mouse
Number/Sex/Group:	22
Age:	70-80 days
Weight:	30-37 g for males 26-32 g for females
Study design:	Cohabitation – one treated male mouse per untreated female mouse per cage, and consecutively one treated female mouse per untreated male mouse per cage.
Deviation from study protocol:	None
Dose period	Males – 70 days before cohabitation and continued (maximum of 14 days) till sacrifice. Females – 15 days before cohabitation and continued through DG 7.

Observations and Results

Mortality (twice daily)

- One male mouse in each of the 30 and 100 mg/kg/day dose groups was sacrificed due to intubation accident.
- One female mouse in the vehicle control group and one in the 30 mg/kg/day dose group were sacrificed and found dead, respectively.

Clinical Signs (twice daily)

- No drug-related observation

Body Weight (daily)

- Males - No affect on body weights and body weight gains.
- Females – Maternal body weight gain at 100 and 250 mg/kg/day was reduced relative to the vehicle control values.

Toxicokinetics (Day of study (DS) 12 or 13 at 0.5, 1 and 6.5 hours after the first dose of the day from group 1 females and 0 (prior to dose), 0.5, 1.0, 2, 6, 6.5, 7, 12, and 24 hours after the first dose from group 2 to 4 females)

Mean toxicokinetics parameters of AG-013736 after oral administration of AG-013736 on day 12

Dose (mg/kg/day)	Sex	C_{max} (ng/mL)		T_{max} (h)	$AUC_{(0-24)}$ (ng•h/mL)
		Mean	SD	Mean	Mean
10	Male	175	79.3	7.0	947
30	Male	853	208	6.5	3180
	Female	602	245	6.5	2850
	Overall	728	245	6.5	3010
100	Male	2860	633	6.5	15200
	Female	4450	2350	6.5	15200
	Overall	3660	1770	6.5	15200
250	Female	7950	3420	7.0	47900

Mean toxicokinetics parameters of PF-03482595 (metabolite) after oral administration of AG-013736 on day 12 of study.

Dose (mg/kg/day)	Sex	Cmax (ng/mL)		Tmax (h)	AUC(0-24) (ng•h/mL)
		Mean	SD	Mean	Mean
10	Male	125	14.4	6.5	629
30	Male	597	82.4	6.5	2150
	Female	571	160	7.0	2720
	Overall	478	225	1.0	2430
100	Male	1870	889	1.0	9740
	Female	2590	922	6.5	9880
	Overall	2070	822	6.5	9830
250	Female	3710	2010	2.0	22500

(excerpted from Applicant's submission)

Stability and Homogeneity

- Samples were found within specification and stable.

Necropsy

- All male mice were sacrificed after the completion of the cohabitation. Right testis, left testis, left Epididymis (whole and caudal), right epididymis, seminal vesicles (with and without fluid) and prostate were individually weighed and fixed.
- Surviving female mice were sacrificed on DG 14. The number and distribution of corpora lutea were recorded. The uterus of each mouse was excised and examined for pregnancy, number and distribution of implantation site and viable and nonviable embryos.
- No drug-related gross lesions.

Terminal body weights and organ weights of males

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0 (VEHICLE)	10	30	100
MICE TESTED	N	22	22	22	22
INCLUDED IN ANALYSES	N	22	22	21b	21b
TERMINAL BODY WEIGHT	MEAN±S.D.	37.9 ± 2.4	38.6 ± 2.8	38.4 ± 2.3	37.0 ± 1.8
EPIDIDYMIS LEFT	MEAN±S.D.	0.0529 ± 0.0052	0.0550 ± 0.0056	0.0540 ± 0.0045	0.0526 ± 0.0047
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	0.0186 ± 0.0024	0.0205 ± 0.0029*	0.0200 ± 0.0023	0.0205 ± 0.0023*
TESTIS LEFT	MEAN±S.D.	0.1242 ± 0.0125	0.1256 ± 0.0173	0.1184 ± 0.0119	0.0967 ± 0.0132**
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	0.3520 ± 0.0576	0.4117 ± 0.0839*	0.3602 ± 0.0721 [20]c	0.3637 ± 0.0712
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.1274 ± 0.0178	0.1451 ± 0.0184** [21]c	0.1380 ± 0.0229 [20]c	0.1344 ± 0.0200 [20]c
EPIDIDYMIS RIGHT	MEAN±S.D.	0.0501 ± 0.0046	0.0528 ± 0.0055	0.0532 ± 0.0055*	0.0494 ± 0.0028
TESTIS RIGHT	MEAN±S.D.	0.1300 ± 0.0132	0.1314 ± 0.0170	0.1298 ± 0.0189	0.1016 ± 0.0139**
PROSTATE	MEAN±S.D.	0.0247 ± 0.0077 [21]c	0.0220 ± 0.0068 [21]c	0.0221 ± 0.0067 [18]c	0.0225 ± 0.0052

ALL WEIGHTS WERE RECORDED IN GRAMS (G).

a. Dosage occurred twice daily.

b. Excludes values for mice that were sacrificed due to dosing accidents.

c. Excludes values for mice that had abnormal organs (weight affected), organs damaged (weight affected) or organ weights that appeared incorrectly recorded.

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

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

(excerpted from the Applicant's submission)

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

- All mating and fertility parameters (days in cohabitation, fertility index) of treated male mice mated with untreated female mice were unaffected.

Sperm motility, count and density of treated male mice

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)		0 (VEHICLE)	10	30	100
MICE TESTED	N	22	22	22	22
INCLUDED IN ANALYSES	N	22	22	21a	21a
VAS DEFERENS SPERM MOTILITY					
NUMBER MOTILE	MEAN±S.D.	579.5 ± 300.7	713.2 ± 378.3	594.3 ± 277.6	674.4 ± 366.6
MOTILE PERCENT	MEAN±S.D.	88.2 ± 10.7	89.8 ± 16.9**	93.0 ± 4.2	90.6 ± 13.6*
STATIC COUNT (NONMOTILE)	MEAN±S.D.	66.7 ± 46.2	49.2 ± 38.5	48.0 ± 35.7	55.3 ± 65.0
TOTAL COUNT c	MEAN±S.D.	646.3 ± 310.4	762.4 ± 369.5	642.4 ± 298.0	729.7 ± 359.3
CAUDA EPIDIDYMAL SPERM COUNT					
SPERM COUNT d	MEAN±S.D.	80.0 ± 20.6 [21]b	78.0 ± 26.3 [21]b	72.1 ± 19.9	67.9 ± 19.2 [20]b
SPERM DENSITY e	MEAN±S.D.	2491.27 ± 565.65 [21]b	2196.35 ± 613.40 [21]b	2099.14 ± 575.28*	1914.26 ± 502.29** [20]b

a. Excludes values for mice that were sacrificed due to dosing accidents.

b. Excludes values that appeared incorrect due to possible sampling error.

c. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.

d. Sperm count used in the calculation of sperm density. Ten fields were evaluated.

e. The sperm density was calculated by dividing the sperm count by the volume in the image area (34.3×10^{-6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table A15 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8%

(excerpted from the Applicant's submission)

Estrous cycling, mating and fertility of treated female mice

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0 (VEHICLE)	30	100	250
MATING OBSERVATIONS:					
MICE IN COHABITATION b,c,d	N	21	22	22	22
DAYS IN COHABITATION e	MEAN±S.D.	2.4 ± 2.1	2.7 ± 2.3	2.8 ± 2.5	4.4 ± 3.5
MICE THAT MATED	N(%)	21(100.0)	22(100.0)	22(100.0)	22(100.0)
FERTILITY INDEX f	N/N	19/ 21	15/ 22	7/ 22**	4/ 22**
	(%)	(90.5)	(68.2)	(31.8)	(18.2)
MICE WITH CONFIRMED MATING DATES	N	20	22	22	22
MATED BY MALE e					
DAYS 1-7	N(%)	18(90.0)	20(90.9)	20(90.9)	19(86.4)
DAYS 8-14	N(%)	2(10.0)	2(9.1)	2(9.1)	3(13.6)
MICE PREGNANT/MICE IN COHABITATION	N/N	19/ 21	15/ 22	7/ 22**	4/ 22**
	(%)	(90.5)	(68.2)	(31.8)	(18.2)

a. Dosage occurred twice daily on day 1 of study through day 7 of presumed gestation.

b. Excludes values for mouse 908, which was sacrificed on day 9 of study due to adverse clinical observations.

c. All treated female mice were inadvertently cohabited with treated male mice on the first night of cohabitation. As a result, 5, 7, 8 and 4 treated female mice in the 0, 30, 100 and 250 mg/kg/day groups, respectively, had evidence that mating occurred.

d. Includes treated female mice that were inadvertently placed into cohabitation with treated male mice on the first night of cohabitation.

e. Restricted to mice with a confirmed mating date.

f. Number of pregnancies/number of mice that mated.

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

Caesarean sectioning and litter observation of treated female mice

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY) a		0 (VEHICLE)	30	100	250
MICE TESTED b,c	N	21d	22	22	22
PREGNANT	N(%)	19(90.5)	15(68.2)	7(31.8)**	4(18.2)**
FOUND DEAD	N(%)	0(0.0)	1(6.7)	0(0.0)	0(0.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 14 OF GESTATION	N	19e	14	7	4
CORPORA LUTEA	MEAN±S.D.	13.3 ± 1.4	13.8 ± 3.2	15.3 ± 6.5	12.5 ± 3.5
IMPLANTATIONS	MEAN±S.D.	13.0 ± 1.3	13.5 ± 3.1	13.7 ± 6.2	11.2 ± 2.8
VIABLE EMBRYOS	N	235	103	17	0
	MEAN±S.D.	12.4 ± 2.2	7.4 ± 4.4	2.4 ± 4.1	0.0 ± 0.0*
NONVIABLE EMBRYOS	N	13	86	79	45
	MEAN±S.D.	0.7 ± 1.3	6.1 ± 4.4**	11.3 ± 7.6**	11.2 ± 2.8**
MICE WITH ANY NONVIABLE EMBRYOS	N(%)	6(31.6)	12(85.7)**	6(85.7)*	4(100.0)**
MICE WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	4(57.1)**	4(100.0)**
MICE WITH VIABLE EMBRYOS	N(%)	19(100.0)	14(100.0)	3(42.8)**	0(0.0)**
PLACENTAE APPEARED NORMAL f	N(%)	19(100.0)	14(100.0)	3(100.0)	--------
% NONVIABLE EMBRYOS/LITTER f	MEAN±S.D.	5.8 ± 11.6	45.2 ± 29.9**	54.9 ± 47.9	--------

a. Dosage occurred twice daily on day 1 of study through day 7 of gestation.
b. All treated female mice were inadvertently cohabited with treated male mice on the first night of cohabitation for the treated females. As a result, 5, 7, 8 and 4 treated female mice in the 0, 30, 100 and 250 mg/kg/day groups, respectively, had evidence that mating occurred.
c. Includes treated female mice that were inadvertently placed into cohabitation with treated male mice on the first night of cohabitation.
d. Excludes values for mouse 908, which was sacrificed on day 9 of study due to adverse clinical observations.
e. Includes values for mouse 919, which did not have a confirmed mating date.
f. Excludes values for litters with no viable embryos.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

(excerpted from the Applicant's submission)

9.2 Embryonic Fetal Development

Study title: Oral (Gavage) Dosage-Range Finding Embryo-Fetal Development Toxicity Study in Mice Evaluating Twice Daily Administration of AG-013736.

Study no: (b) (4) – LIA00236
Sponsor – 06GR116

Study report location: electronic submission

Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: June 22, 2006
GLP compliance: None
QA statement: None
Drug, lot #, and % purity: AG-013736, lot # E010003889,

Key Study Findings

- AG-013736 did not cause any mortality up to 250 mg/kg/dose (500 mg/kg/day) from days 6 thorough 17 of presumed gestation in mice.
- Postimplantation loss (early resorptions) was increased at ≥3 mg/kg/day.
- 3 mg/kg/dose caused fetal gross findings.

Methods

Doses:	1.5, 15, 125, 250 mg/kg/dose 3, 30, 250, and 500 mg/kg/day
Frequency of dosing:	Twice daily, approximately 6 hours apart
Dose volume:	5 mL/kg
Route of administration:	oral gavage
Formulation/Vehicle:	0.5% carboxymethylcellulose (high viscosity)
Species/Strain:	Crl:CD1(1CR) mice
Number/Sex/Group:	8 presumed pregnant mice / group
Satellite groups:	33 animals at 125 mg/kg/dose for Toxicokinetics
Study design:	Dosed from DGs 6 through 17
Deviation from study protocol:	None

Observations and Results

Mortality (Twice daily)

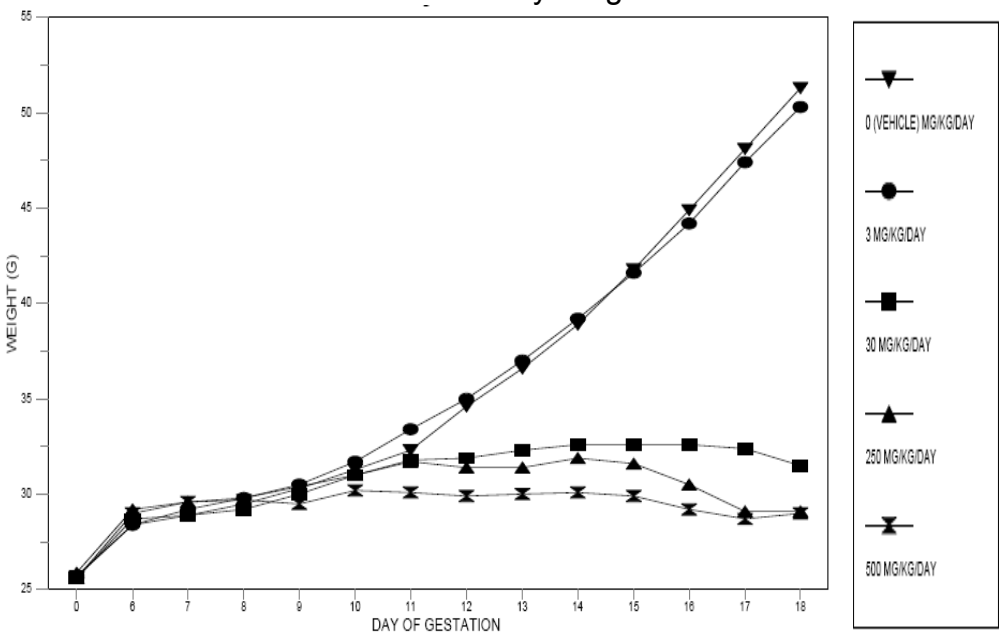
- No mortality

Clinical Signs (Hourly for the first 4 hours after dosing)

- No clinical signs attributed to drug administration.

Body Weight (Daily)

Maternal body weight



(excerpted from the Applicant's submission)

Feed Consumption

- No effects

Toxicokinetics (DGs 17 or 18 at 0, 0.5, 1, 2, 6, 6.5, 7, 12, and 24 hours after the first dose).

Mean toxicokinetic parameters of AG-013736 (parent compound) and PF-03482595 (main metabolite)

Analyte	Dose (mg/kg/day)	Cmax (µg/mL)	Tmax (h)	AUC_{0-24} (µg.h/mL)
AG-013736	250	5.3	7	33.4
PF-03482595	250	3.2	2	21.4

Stability and Homogeneity

- Within specification 90-110%

Necropsy

- No gross lesions

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

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DOSE)a		0 (VEHICLE)	1.5	15	125	250
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	3	30	250	500
MICE TESTED	N	8	8	8	8	8
PREGNANT	N(%)	8(100.0)	8(100.0)	8(100.0)	8(100.0)	8(100.0)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 18 OF GESTATION	N	8	8	8	8	8
CORPORA LUTEA	MEAN±S.D.	12.5 ± 1.9	13.2 ± 1.0	12.5 ± 1.5	14.4 ± 2.3	14.0 ± 1.5
IMPLANTATIONS	MEAN±S.D.	12.4 ± 1.8	12.9 ± 1.0	11.9 ± 1.6	13.8 ± 1.9	13.2 ± 1.0
LITTER SIZES	MEAN±S.D.	12.0 ± 1.9	11.4 ± 1.6	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
LIVE FETUSES	N	96	90	1	0	0
	MEAN±S.D.	12.0 ± 1.9	11.2 ± 1.6	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
DEAD FETUSES	N	0	1	0	0	0
	MEAN±S.D.	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.4 ± 0.5	1.5 ± 1.2	11.8 ± 1.4	13.8 ± 1.9	13.2 ± 1.0
EARLY RESORPTIONS	N	2	12	94	110	106
	MEAN±S.D.	0.2 ± 0.5	1.5 ± 1.2	11.8 ± 1.4	13.8 ± 1.9	13.2 ± 1.0
LATE RESORPTIONS	N	1	0	0	0	0
	MEAN±S.D.	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
MICE WITH ANY RESORPTIONS	N(%)	3(37.5)	7(87.5)	8(100.0)	8(100.0)	8(100.0)
MICE WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	7(87.5)	8(100.0)	8(100.0)
MICE WITH VIABLE FETUSES	N(%)	8(100.0)	8(100.0)	1(12.5)	0(0.0)	0(0.0)
PLACENTAE APPEARED NORMAL b	N(%)	8(100.0)	8(100.0)	1(100.0)	0(0.0)	0(0.0)

(excerpted from the Applicant's submission)

Offspring (Malformations, Variations, etc.): Fetal gross external alterations

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DOSE)a		0 (VEHICLE)	1.5	15	125	250
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	3	30	250	500
LITTERS EVALUATED	N	8	8	1	0	0
FETUSES EVALUATED	N	96	91	1	0	0
LIVE	N	96	90	1	0	0
DEAD	N	0	1b	0	0	0
EYE: BULGE DEPRESSED						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(100.0)		
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(100.0)c		
PALATE: CLEFT						
LITTER INCIDENCE	N(%)	0(0.0)	1(12.5)	1(100.0)		
FETAL INCIDENCE	N(%)	0(0.0)	2(2.2)	1(100.0)c		
BODY: EDEMA						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(100.0)		
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(100.0)c		
BODY: GASTROSCHISIS						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(100.0)		
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(100.0)c		
FORE AND/OR HINDLIMBS: ROTATED						
LITTER INCIDENCE	N(%)	0(0.0)	2(25.0)	0(0.0)		
FETAL INCIDENCE	N(%)	0(0.0)	2(2.2)	0(0.0)		
FORE AND/OR HINDLIMBS: DIGITS ABSENT						
LITTER INCIDENCE	N(%)	0(0.0)	0(0.0)	1(100.0)		
FETAL INCIDENCE	N(%)	0(0.0)	0(0.0)	1(100.0)c		

a. Dosage occurred twice daily (approximately six hours apart) on days 6 through 17 of gestation.
b. Dead fetus was excluded from summarization.
c. Fetus 4019-6 had other gross external alterations.

(excerpted from the Applicant's submission)

Study title: Oral (Gavage) Developmental Toxicity Study in Mice Evaluating Twice Daily Administration of AG-013736.

Study no:	Sponsor – 06GR117 Conducting Lab. – LIA00237
Study report location:	electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	13 September 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AG-013736, lot # E010003889,

Key Study Findings

- AG-013736 at ≤ 1.5 mg/kg/dose did not cause any maternal toxicity.
- The developmental NOAEL was 0.15 mg/kg/dose.
- 1.5 mg/kg/dose group had increased occurrence of cleft palate.
- 0.5 mg/kg/dose group had increased common variations in skeletal ossification.

Methods

Doses:	0.15, 0.5 and 1.5 mg/kg/dose (0.3, 1.0 and 3 mg/kg/day)
Frequency of dosing:	Twice daily approximately 6 hours apart
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% carboxymethylcellulose (high viscosity)
Species/Strain:	Crl:CD1(1CR) mice
Number/Sex/Group:	22, see table below for details
Satellite groups:	yes
Study design:	Animals were dosed on days 6 through 17 of presumed gestation (DG). Animals were sacrificed on DG 18.
Deviation from study protocol:	None mentioned

Dosage Group	Dosage[a] (mg/kg/dose)	Total Dosage[b] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Mice
I	0	0	0	5	22 + 9[c]
II	0.15	0.3	0.03	5	22 + 27[c]
III	0.5	1	0.1	5	22 + 27[c]
IV	1.5	3	0.3	5	22 + 27[c]

a. Dosages were administered twice daily, approximately six hours apart.
b. The test article was considered 100% active/pure for the purpose of dosage calculations.
c. Nine mice in Group I and 27 mice in each of Groups II through IV assigned for use in toxicokinetic sample collection.
d. Mouse 3933 was removed from study before initiation of dosage administration on DG 6 due to body weight loss and was replaced with mouse 3989.
e. Mouse 4069 was removed from study before initiation of dosage administration on DG 6 due to body weight loss and was replaced with mouse 4091.

(excerpted from the Applicant's submission)

Approximate age at arrival: 62 days
Weight on arrival day: 20-29 g
Weight at study assignment (DG 0): 21-29 g

Observations and Results

Mortality (one and 2 hours after each dosage)

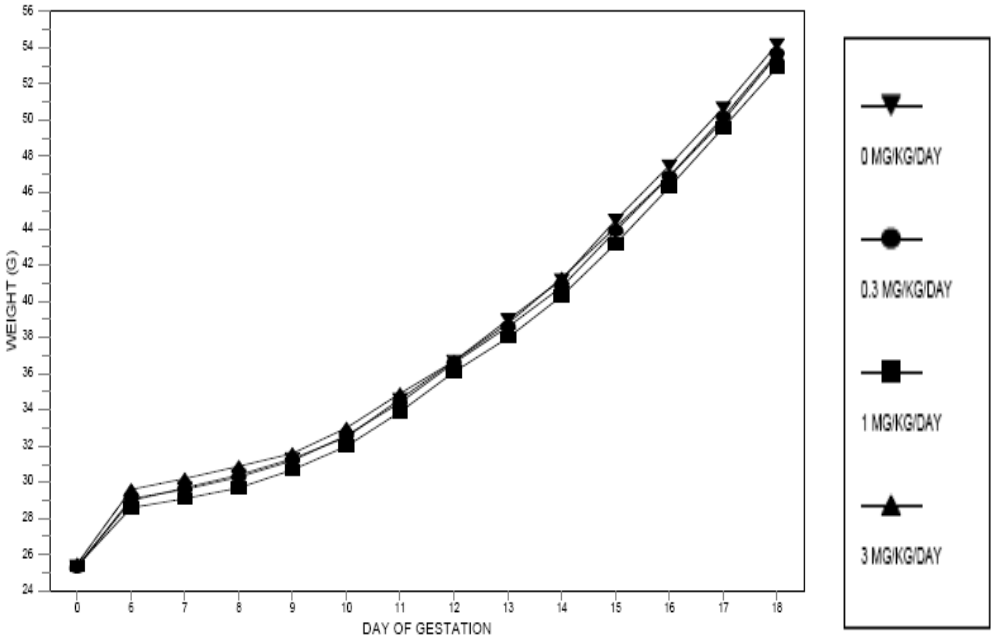
- None

Clinical Signs (one and 2 hours after each dosage)

- No dose-related clinical changes

Body Weight (Daily)

Mean Body Weights in Mice



(excerpted from the Applicant's submission)

Feed Consumption (Daily)

- No apparent effect

Toxicokinetics At 0, 0.5, 1, 2, 6, 6.5, 7, 12, and 24 hours after the first dosage on DG 17 or 18

Mean toxicokinetic parameters of AG-013736 (parent compound) and PF-03482595 (main metabolite)

Analyte	Dose (mg/kg/day)	Cmax (µg/mL)	Tmax (h)	AUC_{0-24} (µg.h/mL)
Axitinib	0.3	3.5±3.1	7	11.1
	1	16.8*	0.5	39.5
	3	36±11.6	6.5	142
PF-03482595	0.3	0.0	0.0	0.0
	1	6.4*	0.5	6.0
	3	15.9*	1.0	76.0

Standard deviation (SD) not calculated due to an n≤2.

Stability and Homogeneity

- ± 10% of intended concentration

Necropsy

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

- The litter averages for corpora lutea, implantation, litter sizes, live fetuses, early and late resorptions, the percentage of resorbed conceptuses, and percentage of live male fetuses were comparable among the four dosage groups.

Cesarean sectioning observation

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DOSE) a		0	0.15	0.5	1.5
DOSAGE (MG/KG/DAY) a		0	0.3	1	3
MICE TESTED	N	22	22	22	22
PREGNANT	N(%)	21(95.4)	19(86.4)	19(86.4)	18(81.8)
MICE PREGNANT AND CAESAREAN-SECTIONED ON DAY 18 OF GESTATION	N	21	19	19	18
CORPORA LUTEA	MEAN±S.D.	13.7 ± 2.3	12.9 ± 1.6	13.4 ± 1.6	13.8 ± 2.5
IMPLANTATIONS	MEAN±S.D.	13.3 ± 2.0	12.7 ± 1.4	12.8 ± 1.6	13.3 ± 2.4
LITTER SIZES	MEAN±S.D.	12.6 ± 2.1	11.9 ± 2.0	12.4 ± 1.5	12.3 ± 2.2
LIVE FETUSES	N	264	226	235	221
	MEAN±S.D.	12.6 ± 2.1	11.9 ± 2.0	12.4 ± 1.5	12.3 ± 2.2
DEAD FETUSES	N	0	1	0	0
	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.7 ± 0.8	0.7 ± 1.2	0.5 ± 0.6	1.0 ± 1.2
EARLY RESORPTIONS	N	12	13	7	19
	MEAN±S.D.	0.6 ± 0.7	0.7 ± 1.1	0.4 ± 0.5	1.0 ± 1.2
LATE RESORPTIONS	N	3	1	2	0
	MEAN±S.D.	0.1 ± 0.5	0.0 ± 0.2	0.1 ± 0.3	0.0 ± 0.0
MICE WITH ANY RESORPTIONS	N(%)	11(52.4)	7(36.8)	8(42.1)	11(61.1)
MICE WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
MICE WITH VIABLE FETUSES	N(%)	21(100.0)	19(100.0)	19(100.0)	18(100.0)
PLACENTAE APPEARED NORMAL	N(%)	21(100.0)	19(100.0)	19(100.0)	18(100.0)

a. Dosage occurred twice daily (approximately 6 hours apart) on days 6 through 17 of gestation.

Litter observations

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DOSE) a		0	0.15	0.5	1.5
DOSAGE (MG/KG/DAY) a		0	0.3	1	3
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	1.27 ± 0.08	1.31 ± 0.07	1.27 ± 0.08	1.22 ± 0.10
MALE FETUSES	MEAN±S.D.	1.29 ± 0.08	1.33 ± 0.06	1.28 ± 0.09	1.26 ± 0.09
FEMALE FETUSES	MEAN±S.D.	1.24 ± 0.10	1.28 ± 0.09	1.25 ± 0.07	1.17 ± 0.10*

(excerpted from the Applicant's submission)

Offspring (Malformations, Variations, etc.)

Group		1	2	3	4
Dose (mg/kg/dose)		0	015	0.5	1.5
Dose (mg/kg/day)		0	0.3	1.0	3.0
Litters evaluated	N	21	19	19	19
Fetuses evaluated	N	264	227	235	221
Live	N	264	226	235	221
Fetuses with any alteration observed	N(%)	67 (25)	53 (23)	61 (26)	79 (36)**
Palate: cleft litter incidence	N (%)	0	0	0	6 (33)
fetal incidence	N (%)	0	0	0	6 (3)
Skull: frontals, contain an interfrontal					
litter incidence	N (%)	9 (42)	10 (53)	13 (68)	15 (83)
fetal incidence	N (%)	15 (11)	19 (16)	27 (22)*	34 (30)**
Skull: supraoccipitals, incomplete ossification					
litter incidence	N (%)	0	0	0	6 (33)**
fetal incidence	N (%)	0	0	0	12 (11)**
Vertebrae, caudal	Mean±SD	8.0±1.6	8.5±0.9	7.3±0.9	6.3±0.9**
Hind limb b tarsals	Mean±SD	0.9±0.4	0.9±0.3	0.7±0.4	0.2±0.3**
phalanges	Mean±SD	11±1.3	11.6±0.9	11.1±1.2	9.9±1.1**

a. Dosage occurred twice daily (approximately 6 hours apart) on days 6 through 17 of gestation
* Significantly different from the control group (p≤0.05)
** Significantly different from the control group value (p≤0.01)

Study title: Oral Dose Range-Finding Study of AG-013736 in Pregnant Rabbits.

Study no: Sponsor- 06GR178
Study report location: electronic submission
Conducting laboratory and location: Pfizer Global Research & Development
Drug Safety Research & Development
Eastern Point Road
Groton, CT USA
Date of study initiation: 08 Oct 2006
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: AG-013736, lot # E10003889, purity 99.2%

Key Study Findings

- Maternal and developmental toxicity was observed at ≥10 mg/kg/day.
- Complete postimplantation loss occurred in all ≥ 30 mg/kg/day animals.

Methods
Doses: 5, 15, 50 and 150 mg/kg/dose

	(10, 30, 100 and 300 mg/kg/day)
Frequency of dosing:	BID, ~ 6 hours apart
Dose volume:	3 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% Carboxymethyl cellulose
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	6 females/group
Age	4.5 to 6.0 months
Weight	2.7 to 3.5 kg
Satellite groups:	None
Study design:	Animals were dosed from GD 7-19 and cesarean sections were performed on GD 29.
Deviation from study protocol:	None

Observations and Results

Mortality (Twice daily)

Group	Dose (mg/kg/day)	Death		
		Number	GD	Status
2	10	1	26	Aborted & euthanized
3	30	1	17	Aborted & euthanized
		1	25	Euthanized
4	100	6	24-26	Euthanized
5	300	3	13	Euthanized
		3	12-13	Died

Clinical Signs (Twice daily)

- 30 mg/kg – One animal had red fluid in/under the cage on GD 25
- 100 mg/kg – All animals had red fluid in/under the cage, one animal had loose stool on GD 25.

Body Weight (Daily)

Body weight loss (%) vs. control

Group	Dose (mg/kg/day)	GD		
		10	20	29
2	10	-		
3	30	-	12	18
4	100	4	16	-
5	300	10	-	-

Feed Consumption (Daily)

- Food consumption was reduced in a dose-related manner compared to the control animals.

Toxicokinetics On GD 19 at 0, 1, 2, 4, 6, 7, 8, and 10 hours post first dose

Mean toxicokinetics parameters of AG-013736 (parent compound) and PF-03482595 (metabolite) in pregnant rabbits

Analyte	Dose (mg/kg/day)	Cmax (ng/mL)	Tmax (h)	AUC $_{0-24}$ (ng.h/mL)
AG-013736	10	5.6±6.2	7	54.4
PF-03482595	10	8.3±5.8	7	60.7

Necropsy

- Groups 4 and 5 – No animals survived to scheduled cesarean section.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):
- Group 2 – 1 fetus from 1 animal had a short tail and 5/5 fetuses from another animal had bilateral local edema in the hind limbs.
- Groups 3, 4, 5 -- No litters were available for examination.

10 Special Toxicology Studies

Study title: Local Vascular Tissue Irritation Study of AG-013736 in Female New Zealand White Rabbits.

Study no.: 05VEG009
Study report location: electronic submission
Conducting laboratory and location: Pfizer Global Research & Development
Safety Sciences
10777 Science Center Dr
La Jolla, CA, USA
Date of study initiation: May 9, 2005
GLP compliance: No
QA statement: Yes
Drug, lot #, and % purity: AG-013736, batch # 37806-ALL-109A

Key Study Findings
- Single intravenous and perivascular administration of AG-013736 at 1 mg/mL did not cause any local vascular irritation in female New Zealand White rabbits.

Methods
Doses: See table below
Frequency of dosing: Single dose
Route of administration: Intravenous and perivascular

Dose volume: See table below for details
Formulation/Vehicle: 65% polyethylene glycol 400:35% H_2O with 0.025 mg/mL butylated hydroxyanisole (BHA)
Species/Strain: Female New Zealand White rabbits
Number/Sex/Group: 3 females/group
Age: 10-12 weeks
Weight: ~2.5 kg
Satellite groups: None
Unique study design: None
Deviation from study protocol: None

Group Number	IV Dose (mg)	IV Dose Volume (uL)	PV Dose Volume (uL)	Dose Concentration, AG-013736 (mg/ml)	Number of Animals Females
1: Saline	0	1000	50	0	3
2: Vehicle: (65%PEG400:35%H2O (w/w) with 0.025 mg/mL BHA	0	1000	50	0	3
3: AG-013736	1	1000	50	1	3

Observations and Results

Mortality

- None

Clinical Signs (0, 1, 4 hours post dose on day 1, twice on day 2, and once prior to necropsy on day 3)

- Intravenous injection – No signs of erythema or edema
- Perivascular injection – Very slight to well defined erythema in groups 2 and 3 animals.
- Slight edema in group 3 animals

Body Weights

Mean Body Weights (kg)

Treatment	Dose	Day 1	Day 3	% Change
Saline	0	2.90±0.04	2.91±0.07	0.24
Vehicle	0	2.90±0.0.09	2.92±0.05	1.93
AG-013736	1	2.86±0.13	2.93±0.11	2.36

Histological Findings

- Minimal to moderate hemorrhage, subcutaneous edema and mixed inflammatory cell infiltrate at the intravenous or perivascular injections sites in all treated animals.

Study title: Determination of the Phototoxic Potential of AG-013736 in the 3T3 Neutral Red Uptake (NRU) Phototoxicity Assay.

Study no.:	05228
Study report location:	electronic submission
Conducting laboratory and location:	Pfizer Global Research & Development Amboise, Safety Sciences Europe Route des Industries, Pocé-sur-Cisse 37400 Amboise, France
Date of study initiation:	September 26, 2005
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	AG-013736, batch # NGMP2737C1P, active moiety 100%

Key Study Findings

- The cellular viability was significantly decreased (below the threshold of 80%) between 50 and 150 µg/mL of AG-013736 in the presence of UVA light.
- AG-013736 may have phototoxic potential *in vitro*.

Methods: Balb/c 3T3, clone 31 mouse fibroblasts were treated with different concentrations of AG-013736 for about 1 hour and then irradiated with 5 joules/cm^2 of UV. The cell viability was measured approximately 24 hours after the irradiation. Chlorpromazine (positive) and sodium Lauryl Sulfate (negative) were used as controls. The IC_{50} (-Irr), IC_{50} (+Irr), PIF, and MPE value were calculated using SoftMaxPro 4.3, Molecular devices (software).

	Stock solution		Working solutions	
Items	Concentration (mg/mL)	Vehicle	Concentrations tested (µg/mL)	Vehicle
CPZ	10	Water	200 – 50 – 12.5 – 3.125 – 0.781 – 0.195 – 0.049 – 0.012	EBSS
SLS	0.7	EBSS	70 – 35 – 17.5 – 8.75 – 4.375 – 2.188 – 1.094 – 0.547	EBSS
AG-013736	15	DMSO	150– 50– 16.667– 5.556 – 1.852– 0.617– 0.206– 0.069	1% DMSO in EBSS

Observations and Results

Products	IC50 (-Irr) µg/mL	IC50 (+Irr) µg/mL	PIF	MPE
Chlorpromazine	10.5	0.836	12.5	0.162
Sodium Lauryl Sulfate	15.2	15.7	0.96	0.004
AG-013736	> 150	> 150	a	b

[a]: the PIF value cannot be determined for AG-013736 since no IC50 values could be determined
[b]: MPE value for AG-013736 is considered not valid due to software limitations

(excerpted from the Applicant's submission)

Study title: Single Dose Oral (Gavage) Phototoxicity Study of AG-013736 In Hairless Mice.

Study no.: (b) (4) - RSA00087
Sponsor – 09LJ055
Study report location: electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: September 28, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: AG-013736, lot # AXM-00-0004A, 100% purity

Key Study Findings

- Oral administration of AG-013736 up to 100 mg/kg to female Crl:SKH1-hr hairless mice did not induce any skin reactions indicative of phototoxicity.

Methods: The test article, comparator article or vehicle treated animals were placed 1.2 meters from UVR source. Each treated mouse received UVR exposure over a period of 30±5 minutes.

Doses: See table below
Frequency of dosing: Single dose
Route of administration: Oral
Dose volume: See table below
Formulation/Vehicle: Carboxymethylcellulose, lot # VL0190
8-Methoxypsoralen as a positive control
Species/Strain: Crl:SKH1-*hr* hairless female mice
Number/Sex/Group: See table below
Age: ~55 days
Weight: 19-26 g
Satellite groups: yes

Unique study design: None
Deviation from study protocol: None

Group	Mice per Group	Formulation				UVR Exposure	
		Descriptor	Dosage (mg/kg)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Instrumental UVR Dose (MED)	Interval[a] (Minutes)
1	6 + 6[b]	Vehicle	0	0	10	0.5	60 ± 10
2	6 + 12[b]	AG-013736	3	0.3	10	0.5	60 ± 10
3	6 + 12[b]	AG-013736	30	3	10	0.5	60 ± 10
4	6 + 12[b]	AG-013736	100	10	10	0.5	60 ± 10
5	3	8-MOP	50	5	10	0.5	60 ± 10

a. Interval between formulation administration and UVR exposure, based on the median dosing time for each group.
b. Mice assigned to toxicokinetic phase were not restrained or exposed to UVR.

c. Phototoxicity phase animals were placed 1.2 meters from the UVR source at the time of the exposure.

(Excerpted from the Applicant's submission)

Observations and Results

Mortality

- None

Clinical Signs (0.25, 1, 4, 24, 48, and 72 hours after UVR exposure)

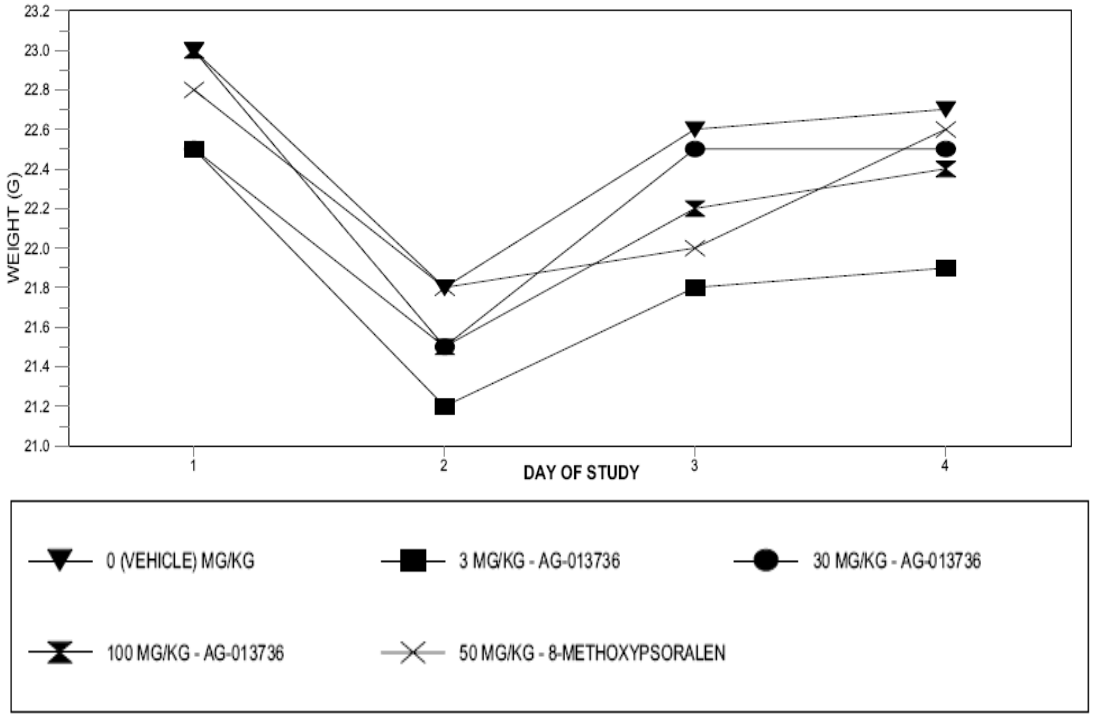
GROUP	1	2	3	4	5
DESCRIPTOR	VEHICLE	AG-013736	AG-013736	AG-013736	8-METHOXYPSORALEN
CONCENTRATION (MG/ML)	0	0.3	3	10	5
DOSAGE (MG/KG)a	0	3	30	100	50
UVR EXPOSURE (MED)	0.5	0.5	0.5	0.5	0.5
INTERVAL (MINUTES)b	60	60	60	60	60
MICE TESTED	6	6	6	6	3
MORTALITY	0	0	0	0	0
SKIN REACTION OBSERVATIONS:					
SITE 1 - ERYTHEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	6/ 3
SITE 1 - EDEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	8/ 3
SITE 1 - SCAB(S)	0/ 0	0/ 0	0/ 0	0/ 0	6/ 3
CLINICAL OBSERVATIONS:					
SEDATED	1/ 1	4/ 4	5/ 5	4/ 4	1/ 1

N/N = Total number of observations/number of animals with observation
MED = Minimal erythema dose
a = Formulation administration and UVR exposure occurred on day 1 of study
b = Approximate interval between formulation administration and UVR exposure

(excerpted from the Applicant's submission)

Body Weights (Daily)

Mean Body Weights (g)



(excerpted from the Applicant's submission)

Toxicokinetics (1, 2, 4, and 24 hours postdose)

Dose Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-24} (ng•hr/mL)
2	3	13.3	1.0	17.9
3	30	91.9	1.0	318
4	100	382	1.0	1080

(excerpted from the Applicant's submission)

Study title: AG-013736: Report for the In Vitro Compatibility of the Parenteral Formulation with Human Blood.

Study no.:	SA1000
Study report location:	electronic submission
Conducting laboratory and location:	Pfizer Inc. 10646 Science Center Dr. San Diego, CA 92121
Date of study initiation:	April 11, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	AG-013736, lot # 00000704,

Key Study Findings

- AG-013736 did not cause hemolysis of human blood at 1:10, 1:6 and 1:3 ratios in PEG400.
- Hemolysis was observed at 1:2 ratios

Methods

Doses:	See below
Formulation/Vehicle:	65%/35% PEG 400/water for injection
Unique study design:	Samples were incubated, centrifuged, erythrocytes lysed and hemoglobin concentration determined at 450 nm.
Deviation from study protocol:	None

Tube Number	Whole Blood	Test Article or Vehicle	Dilution
1	500 µL	50µL	1:10
2	500 µL	100µL	1:6
3	500 µL	200µL	1:3
4	500 µL	500 µL	1:2
Tube Number	**Whole Blood**	**Saline**	**Dilution**
5	500 µL	50µL	1:10
6	500 µL	100µL	1:6
7	500 µL	200µL	1:3
8	500 µL	500 µL	1:2

(table excerpted from Applicant's submission)

(b) (4)

(b) (4)

[redacted]

Observations and Results

Donor	Formulation tested	Formulation or vehicle			
		1:10	1:6	1:3	1:2
A	AG-013736	0	4	0	21
	Vehicle	4	11	11	49
B	AG-013736	0	1	1	19
	Vehicle	12	5	1	27
C	AG-013736	1	0	0	12
	Vehicle	2	4	7	34
Hemolysis (Mean percent)	AG-013736	0	0	0	17
	Vehicle	6	7	7	37

11 Integrated Summary and Safety Evaluation

Pharmacology: Pharmacology studies identified that axitinib inhibits the activity and function of Vascular Endothelial Growth Factor Receptors (VEGFR-1, VEGFR-2 and VEGFR-3), *in vitro* and *in vivo*. VEGFR inhibition is the likely mechanism leading to inhibition of angiogenesis and tumor progression in mouse models of cancer. Axitinib also inhibits other receptor tyrosine kinases including platelet-derived growth factor receptor (PDGFR)-α and -β, and stem cell factor receptor (KIT). Axitinib inhibited VEGF-stimulated endothelial cell survival, vascular tube formation, adhesion, migration, and induced endothelial cell apoptosis *in vitro*. Axitinib was also shown to decrease vascular permeability and density, phosphorylation of VEGFR-2 and PDGFR-β, and tumor growth in mouse xenograft models.

Safety Pharmacology: In safety pharmacology studies, axitinib administered orally to mice and rats did not demonstrate signs of neurotoxicity or adverse effects on the respiratory system when administered as single doses. Axitinib induced increase gastric emptying (stomach) in rats, but did not affect charcoal propulsion in mice. There were no significant effects of axitinib or a major metabolite on the *hERG* channel *in vitro*

or on cardiac function (e.g., PR, QRS, QT, QTcB, QTcF) in dogs. Cardovascular effects were demonstrated in mice (30 mg/kg), rats (≥ 300 mg/kg) and dogs including increased systolic blood pressure and decreased heart rate.

General Toxicology: In order to better characterize the general toxicity of axitinib, the sponsor performed chronic toxicity studies in both mice (14 days, 28 days, and 26 weeks) and dogs (14 days, 28 days, 26 weeks and 9 months).

Male and female mice were dosed at 25, 125 or 250 mg/kg/dose twice daily (75, 375 or 750 mg/m^2/dose BID) at a dose volume of 10 mL/kg for 14 days. Axitinib at 250 mg/kg/dose caused mortalities (5/138) and was associated with hypoactivity, rough hair coat, hunched posture, labored respiration, red nasal discharge and swelling in the axillary region. There were no changes in body weight, food consumption and ophthalmology. Decreased absolute thymus and testis/epididymis weights were observed in high dosed animals. Toxicokinetic and histopathology data were not submitted.

Axitinib administered twice daily to mice by oral gavage for 28 days at 125 mg/kg/dose (375 mg/m^2/dose BID) resulted in 3% mortality. There were no effects on body weights, food consumption, hematology and serum chemistry. Microscopic findings were seen in the femur (thickened physeal cartilage) of males and females administered 15 or 125 mg/kg/dose (45 or 375 mg/m^2/dose BID), in the ovaries (no corpora lutea) and in the testes (bilateral atrophy/degeneration) of animals given 125 mg/kg/dose (375 mg/m^2/dose BID). There were no compound related microscopic changes in the thymus. A decrease in the absolute and relative thymus weights compared to control observed in high dose animals may be a stress-related secondary effect of axitinib. Thickening of the physeal cartilage (growth plate) in the femur was seen in 2 males and 1 female given 15 mg/kg/dose BID and in 11/16 males and 14/16 females from the 125 mg/kg/dose twice daily. This dose and duration dependent increase in the numbers of hypertrophic chondrocytes in the zone of cell maturation was more severe in females than in males (see Attachment 1 for details).

During a 26–week oral gavage chronic toxicity study with a 13-week interim sacrifice and 4-week recovery period, male and female mice were administered 0 (control), 5, 15, 50, and 125 mg/kg/dose (30, 90, 300, and 750 mg/m^2/day) axitinib twice daily by oral gavage. The interim and terminal sacrifice animals were dosed for 13 and 26 weeks, respectively. Dose-related deceases in body weight and food consumption were noted in 50 and 125 mg/kg/dose animals. The increase in mortality rate at 125 mg/kg/dose resulted in the early sacrifice of the surviving animals of this group during week 22. This high incidence of mortality was associated with poor health, broken and/or missing tooth/teeth and malocclusion. The observations of malocclusion were observed between days 29 and 58. The broken and or missing teeth were noted following day 56 of treatment and after the recovery period. The dental finding correlated with microscopic observation of odontopathy in all treated animals at interim and terminal sacrifices. Hematological changes (~15% decreased erythrocytes) correlated with increased pigment in the spleen in dose- and duration-related manner. Spleen, testes

and uterus weights were reduced in a dose-related manner and correlated with microscopic findings. Other treatment related microscopic changes included mucosal hyperplasia (cecum and colon), hypospermia in the testes and abnormal sperm forms in the epididymis, and decreased number of corpora lutea in the ovaries. These dose and duration dependent changes showed a decrease in severity during the recovery period. Lymphoid depletion seen in the spleen and thymus of high dose animals and mid dose males (50 mg/kg/dose) may be due to treatment-related stress. There were no apparent differences in the toxicokinetics between male and female mice. AUC value did not increase proportionally with the increase in dose levels. This may suggest nonlinear toxicokinetics due to a combination of potential inhibition or saturation of elimination pathways.

Dogs were dosed twice daily for 14 days via oral gavage. Axitinib at 150 mg/kg/dose (3 g/m^2/dose) produced one (1/8) mortality. Clinical effects in high dose animals included thin appearance, hypoactivity, dehydration, and reddening of the gum that was associated with oral mucosal ulcer. Treatment related serum changes included increased cholesterol and triglycerides in the high dose animals suggesting a change in lipid metabolism. There were no treatment-related macroscopic changes. Toxicokinetic and histopathology data were not submitted.

The toxicity of axitinib administered twice daily via oral gavage for 28 days was investigated in dogs. Six of eight (4 males & 2 females) high dose (50 mg /kg/dose) dogs were euthanized. Clinical observations included dehydration, thin appearance, hypoactivity, abnormal stools, and hyperemic oral mucosa and/or oral ulcer. The weight loss in the 50 mg/kg/dose (1 g/m^2/dose) group was associated with decreased food consumption. Serum chemistry results indicated that the compound affected lipid metabolism. Macroscopic findings were observed in the stomach, intestinal tract, and oral cavity. Histomorphologic findings were observed in the stomach (hemorrhage), small and large intestines (congestion/hemorrhage), pancreas (↓ zymogen granules and ↑ acinar apoptosis), thymus (lymphoid depletion), oral mucosa (inflammation/ulcer), bone (thickening of the growth plate), testis (multinucleated cells), and ovaries (absence of corpora lutea). Toxicokinetic data was not submitted for this study (see attachment 1 for details).

In a third repeat dose toxicity study in dogs, axitinib was administered twice daily at 0.5, 1.5, 3, or 5 mg/kg/dose (10, 30, 60, or 100 mg/m^2/dose) by oral gavage for 13 or 26 weeks. Four animals/sex in groups 1 to 4 and 4 males and 3 females in group 5 were sacrificed after 13 weeks of treatment. Two animals/sex in the control group and 3 animals/sex in groups 4 and 5 were sacrificed following at least 13 weeks of treatment and 4 weeks of recovery. Remaining animals (~4/sex/group) were sacrificed after 26 weeks of treatment. Fecal abnormalities were observed in a dose-related manner. Three high dose animals were euthanized due to extreme weigh loss. There were no clear dose-related ophthalmic abnormalities. There were no effects of axitinib on measured cardiographic intervals or heart rate. Two mid dose animals (3 mg/kg/dose) observed with sinus tachycardia may have been due to physiologic excitement. Thymic lymphoid atrophy was the major microscopic finding. The overall exposure (C_{max} and

AUC) after repeated dosing at 0.5, 1.5, 3, or 5 mg/kg/dose twice daily for 26 weeks was variable and did not show significant auto-induction or accumulation.

In a 9-month toxicity study with 8-week recovery, administration of axitinib twice daily (approximately 6 hours apart) at 0.5, 1.5 or 3.0 mg/kg/dose (20, 60, or 120 mg/m^2/day) for 39 weeks did not cause any mortality in male and female dogs. Axitinib-related fecal abnormalities (no feces, liquid, mucoid, nonformed feces) did not correlate with any anatomic pathology findings in the gastrointestinal tract. Administration of axitinib did not affect the mean body weight, food consumption, ophthalmic or electrocardiographic evaluation. Testis weight decreased in a dose-related manner and correlated with histological findings. Histological findings in the testis (testicular degeneration/atrophy and syncytial cells) of mid and high dose animals correlated with decreased testis weight in a dose-related manner. These histological findings were not completely reversed at the end of the recovery phase. Systemic exposure (C_{max} and AUC_{0-24}) at 3 mg/kg/dose for weeks 6, 13, and 39 increased as compared to day 1, indicating potential slight drug accumulation. Gender related differences in mean exposure and extreme interanimal variability within treatment groups were observed. In conclusion, the no-observable-adverse-effect level (NOAEL) for males was 0.5 mg/kg/dose (10 mg/m^2/dose), which corresponded to mean C_{max} and AUC_{0-24} values of approximately 1.72 ng/mL and 5.12 ng*hr/mL, respectively, in week 39 of the dosing phase. The NOAEL for females was 3 mg/kg/dose (60 mg/m^2/dose), which corresponded to mean C_{max} and AUC_{0-24} values of 43.7 ng/mL and 162 ng*hr/mL, respectively.

Pharmacokinetics and Toxicokinetics: Single oral administration of axitinib to Wistar rats gave a dose proportional exposure between the low (50 mg/kg) and mid dose (250 mg/kg). Exposure at high dose (500 mg/kg) was higher between the high dose and lower doses, suggesting potential inhibition or saturation of elimination pathways or increased absorption. The $t_{1/2}$ was 1- 4 hours.

Genotoxicity: Axitinib was not a bacterial mutagen when tested up to the maximum concentration of 5 mg/plate. There was evidence of insolubility in tests at 5 mg/plate with all of the strains and cytotoxicity with one of the Salmonella strains (TA100). Axitinib did not show any clastogenic activity with and without metabolic activation in human lymphocyte cultures. However it induced dose-related increases in polyploid cells. Aneugenic potential of axitinib was assessed by an *in vivo* micronucleus assay. Oral administration of axitinib to mice for three consecutive days induced micronuclei in the polychromatic bone marrow erythrocytes (MNPCE) of males (≥1000 mg/kg) and females (≥500 mg/kg). Kinetochore staining analysis conducted on bone marrow smears from female mice administered axitinib showed that approximately 75% of the evaluated micronuclei were kinetochore positive.

Reproductive and developmental toxicology: A fertility and early embryonic development study was performed in mice to assess the effects of axitinib. Axitinib was administered to male mice twice daily (approximately 6 hours apart) at doses of 0 (vehicle), 5, 15 and 50 mg/kg/dose for at least 70 days before cohabitation and

continued through the day before sacrifice. Female mice received 0 (vehicle), 15, 50 and 125 mg/kg/dose axitinib beginning 15 days before cohabitation and continued through day 7 of presumed gestation (DG 7). Axitinib did not cause any mortality in male and female mice. There were no clinical observations or effects on body weights. Absolute and relative testis weights of 50 mg/kg/dose treated mice were reduced (20 to 22%) as compared to control animals. Sperm density was reduced in a dose-related manner. The cauda epididymal sperm count of high dose males was reduced by 15% as compared to the vehicle control group value. In female mice administered axitinib, the duration of cohabitation increased in the 125 mg/kg/dose dose group as compared to vehicle control mice (4.4 vs. 2.4 days) and fertility was decreased in a dose-dependent manner. Mid (50 mg/kg/dose) and high dose (125 mg/kg/dose) dams had a 57% and 100% litter loss, respectively. Systemic exposure (C_{max} and $AUC_{0\text{-}24h}$) of axitinib (active drug) and PF-03482595 (major circulating metabolite) increased with increasing doses. The male reproductive no-observable-adverse-effect-level (NOAEL) was 5 mg/kg/dose (15 mg/m^2/dose twice daily). A female reproductive and fertility NOAEL for axitinib was not identified in this study.

During a dose-range finding embryo-fetal development toxicity study in mice, axitinib was administered twice daily (approximately six hours apart) at doses of 0 (vehicle), 1.5, 15, 125, and 250 mg/kg/dose (0, 9, 90, 750, and 1500 mg/m^2/day) to pregnant animals from gestation day (GD) 6 through 17. Toxicokinetics animals were dosed at 125 mg/kg/dose (750 mg/m^2/day). Blood samples were collected on DG 6 and DG 17 at 0, 0.5, 1, 2, 6, 6.5, 7, 12, and 24 hours after the first dose of the day. There was no mortality, clinical signs or gross external alterations. The average maternal body weight on GD18 was 98%, 61%, 57%, and 57% of the vehicle control group value in the 1.5, 15, 125 and 250 mg/kg/dose groups, respectively. There were no apparent effects on feed consumption during the dosage period. Postimplantation loss (early resorptions) was increased at ≥1.5 mg/kg/dose (9 mg/m^2/day) as compared to vehicle control group values. Fetal gross external examination revealed rotated hindlimbs, cleft palate, depressed eye bulges, absent digits on both forepaws, whole body edema, and gastroschisis were also observed at ≥ 1.5 mg/kg/dose (9 mg/m^2/day).

In a definitive developmental toxicity study in mice, axitinib was administered orally via gavage twice daily (approximately 6 hours apart) at doses of 0.15, 0.5 and 1.5 mg/kg/dose (0.45, 1.5, and 4.5 mg/m^2/dose) to pregnant animals on gestation days (GD) 6 through 17 to evaluate the effects of the drug on embryo-fetal development. All mice survived to scheduled necropsy. There were no drug-related effects on clinical signs, body weights or feed consumption at any dose level tested. Reductions in female fetal body weights were statistically significant in the 1.5 mg/kg/dose group as compared to control. Axitinib did not significantly effect litter averages for corpora lutea, implantations, litter sizes, live fetuses, or early and late resorptions in a dose-related manner. At 1.5 mg/kg/dose (9 mg/m^2/day), axitinib caused skeletal variations (interfrontal ossification sites, incomplete ossification of the supraoccipitals) and increased fetal and litter incidences of cleft plate. Reversible delays in ossification included a reduction in the average number of ossified hindlimb phalanges and a

reduction in the average number of ossified caudal vertebrae, hindlimb tarsals and hindlimb phalanges at 0.5 and 1.5 mg/kg/dose (3 and 9 mg/m^2/day).

In an embryo-fetal development dose range finding study in rabbits, axitinib was administered to timed-pregnant New Zealand White rabbits by oral gavage during Gestation days (GD) 7-19. Axitinib was administered twice daily, approximately 6 hours apart at doses levels of 5, 15, 50, and 150 mg/kg/dose (60, 180, 600, and 1800 mg/m^2/dose). Cesarean sections were performed on all surviving animals on GD29. Maternal body weight loss occurred in a dose-related manner. Three high dose animals (150 mg/kg/dose) were found dead and the remaining animals were euthanized before scheduled necropsy. Axitinib adversely affected pregnancy outcome. Only 3, 4, and 6 pregnant animals remained in the 15, 5 and 0 mg/kg/dose (360, 120, and 0 mg/m^2/day) groups, respectively, at the time of the scheduled cesarean section. Complete postimplantation loss occurred in all 15 mg/kg/dose animals. Maternal and developmental toxicity was observed at ≥ 5 mg/kg/dose (120 mg/m^2/day).

Local tolerance: Administration of 1 mg axitinib did not cause vascular or perivascular irritation at the injection sites in the ears in the NLW rabbits as compared to vehicle control.

Special toxicology studies: The phototoxic potential of axitinib was evaluated *in vitro* and *in vivo.* Female albino hairless Crl:SKH1 mice were administered axitinib orally (via gavage) as a single dose before exposure to radiation. The phototoxic potential of axitinib was compared with 8-Methoxypsoralen (positive control). A single administration of axitinib up to 100 mg/kg followed 60±10 minutes later by a single exposure to solar-stimulated UVR did not induce any skin reactions indicative of phototoxicity. Skin reactions induced by 8-MOP included grade 1 erythema, grade 1 edema, and scab in 3 of 3 animals. All mice survived to scheduled sacrifice. T_{max} was observed at 1.0 hour and systemic exposure (C_{max} and AUC_{0-24}) increased with increasing doses.

In vitro, the cellular viability of Balb/c 3T3, clone 31 mouse fibroblasts, was significantly decreased (below the threshold of 80%) between 50 and 150 µg/mL of axitinib in the presence of UV light. The photo irritation factor (PIF) and mean photo effect (MPE) value could not be calculated due to software limitations. Axitinib may have phototoxic potential *in vitro*.

Axitinib did not cause hemolysis in human whole blood at lower concentrations (1:10, 1:6 & 1:3). At a higher ratio in PEG400 (1:2 ratio), axitinib showed hemolytic potential.

12 Appendix/Attachments

Attachment 1

Previous Reviews Referenced (IND 63,662)

PHARMACOKINETICS/TOXICOKINETICS

1. Determination of plasma levels in rats following oral administration ((b) (4) study # VFF/526). (b) (4)

Key study findings: Exposure was dose proportional between the low and mid dose, but was higher at high dose.

Conducting laboratory and location: (b) (4)

Date of study initiation: 31 July 2001
GLP compliance: Yes
QA report: yes ( ) no (X)
Drug, lot #, and % purity: AG13736, lot # AC3088, 98% purity
Formulation/vehicle: 0.5% w/v Carboxymethylcellulose

Dosing:

Species/strain: Wistar rats
#/sex/group or time point (main study): 8 males
Satellite groups used for toxicokinetics or recovery: None
Age: 7 weeks
Weight: 155-185 g
Doses in administered units:

Group	Dose (mg/kg)	Number of animals	
		Cohort A	Cohort B
1	Vehicle	4	4
2	50	4	4
3	250	4	4
4	500	4	4

Route, form, volume, and infusion rate: Oral

Observations and times:

Toxicokinetics: Blood samples were collected from cohort A at 15 minutes and 1, 3, 6 and 12 hours post dose. Blood samples from cohort B were collected at 30 minutes and 2, 4, 8 and 24 hours after post dose.

Results:

Toxicokinetics:

Dose (mg/kg)	C_{max} (µg/mL)	T_{max} (hr)	$T_{1/2}$ (hr)	C_{24hr} (µg/mL)	AUC $_{0-\infty}$ (µg.hr/mL)
50	0.03	3.0	1.1	BLQ	0.06
250	0.18	1.0	3.5	BLQ	0.32
500	1.20	2.0	4.1	BLQ	2.20

BLQ = below limit of quantification of 1 ng/mL.

GENERAL TOXICOLOGY

Single-dose toxicity

1. Acute oral toxicity study in mice with AG013736 ((b) (4) study # 22337-0-800). (b) (4) unaudited draft report. Vol. 01, page 238.

Key study findings: The oral NOEL for mice is considered to be 2 g/kg.

Conducting laboratory and location: (b) (4)

Date of study initiation: April 18, 2001
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: AG13736, lot # AC3088, 98% purity
Formulation/vehicle: 0.5% carboxymethylcellulose

Dosing:

Species/strain: Crl:CD-1(ICR)BR
#/sex/group (main study): 5 (total 10 animals)
Satellite groups used for toxicokinetics or recovery: None
Age: 5 weeks
Weight: 21-23 g for % and 19-20 for &
Doses in administered units: 2000 mg/kg, 20 mL/kg
Route, form, volume, and infusion rate: Oral

Observations and times:

Clinical signs:	Twice daily
Body weights:	Pre dose and days 7 and 14 post dose.
Gross pathology:	At necropsy on day 14 (abbreviated cervical, thoracic, and abdominal viscera)

Results:

Mortality:	None
Clinical signs:	No apparent dose-related effects
Body weights:	No effect
Gross pathology:	No drug-related gross findings

Repeat-dose toxicity

1. **14-Day gavage toxicity study with AG013736 in mice ((b) (4) study # 6750-144). (b) (4) audited draft report. Vol. 03, page 001.**

Key study findings: Twice daily gavage administration of AG13736 at 125 mg/kg for 14 days did not cause any toxicity in mice. The test compound at 250 mg/kg/BID caused 4% mortality.

Conducting laboratory and location: (b) (4)

Date of study initiation:	20 February 2001
GLP compliance:	Yes
QA report:	yes ( ) no (X) {to be submitted with finalized report}
Drug, lot #, and % purity:	AG13736, Lot # 2737-C-1P, and 98% purity
Formulation/vehicle:	0.5% carboxymethylcellulose, Lot # PQ0167 from (b) (4)

Dosing:

Species/strain:	Crl:CD-1®(ICR)BR mice
#/sex/group or time point (main study):	15
Satellite groups used for toxicokinetics or recovery:	54/sex/group
Age:	6 weeks
Weight:	males (16-32 g), females (17-18 g)

Doses in administered units:

Group	No. of animals/sex/group	Dose level	
		mg/kg/dose	mg/kg/day
Main Study			
1 (Control)	15	0	0
2 (Low)	15	25	50
3 (Mid)	15	125	250
4 (High)	15	250	500
Toxicokinetic Study			
2 (Low)	54	25	50
3 (Mid)	54	125	250
4 (High)	54	250	500

Route, form, volume, and infusion rate: Oral gavage, 10 mL/kg

Observations and times:

Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Prior to treatment and weekly thereafter
Ophthalmoscopy: Prior to treatment and during week 2.
Hematology: At termination
Clinical chemistry: At termination.
Gross pathology: At termination of the study
Organs weighed: Adrenal, brain, heart, kidneys, liver, lung, ovaries, pituitary, prostate, salivary gland (mandibular), seminal vesicle, testes with epididymis, thymus and thyroids.
Histopathology: Tissues were preserved and retained for possible future analysis.
Toxicokinetics: On days 1 and 14 at 0.25, 0.5, 1, 2, and 6 hours immediately before the second dose, and 6.5, 7, and 8 hours post dose.

Results:

Mortality: Five males and 12 females were found dead or sacrificed in extremis.

Group	Dose (mg/kg/BID)	Main Study		Toxicokinetic	
		Male	Female	Male	Female
1 (Control)	0	0	2	-	-
2 (Low)	25	1	2	0	0
3 (Mid)	125	0	6	0	0
4 (High)	250	1	1	3	1

The deaths of four males and one female in high dose group (4%) were attributed to the test compound.

Remaining deaths were due to perforated esophagus noted at necropsy.

Clinical signs: Hypoactivity, rough hair coat, recumbency and /or hunched posture, labored or audible respiration, cold to touch, red nasal discharge and swelling in the axillary region were observed in moribund mice
Body weights: Unaffected
Food consumption: No effect
Ophthalmoscopy: Within normal limits
Hematology: Decreased red blood cell count, hemoglobin and hematocrit in high dosed females (maximum 20%)
Clinical chemistry: No effect
Organ weights: 125 mg/kg/BID – Absolute thymus wt. (20% decreased in males compared to control)
250 mg/kg/BID – Absolute thymus wt. decreased (20% in males, 29% in females) and decreased absolute testis/epididymis wt. (12%).
Gross pathology: Perforation of the esophagus (1 male and 10 females)
Histopathology: Not performed
Toxicokinetics: TK data are not submitted. The following table is copied from the Investigator's Brochure.

Dose (mg/kg/day)	Day	C_{max} (µg/mL)	T_{max} (h)	AUC (µg.h/mL)
50	1	0.95	6.5	3.73
	14	0.9	7.0	6.23
250	1	2.89	6.5	21.15
	14	8.04	8.0	93.08
500	1	12.38	6.5	138.20
	14	10.2	8.0	122.58

2. 28-Day gavage toxicity study with AG013736 in mice ([redacted] (b) (4) study # 6750-145). [redacted] (b) (4) audited draft report. Vol. 04, page 115.

Key study findings: The oral administration of the compound at 125 mg/kg/BID for 28 days produced 2% mortality in male and female mice. Testicular atrophy/degeneration and absence of corpora lutea were seen in high dose animals. Treatment related thickening of the physeal cartilage was noted in the femur of mid (15 mg/kg/BID) and high (125 mg/kg/BID) dose animals.

Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: 24 April 2001
GLP compliance: yes
QA report: yes (X)
Drug, lot #, and % purity: AG13736, Lot # AC3088, 98% purity
Formulation/vehicle: 0.5% carboxymethylcellulose, Lot # PQ0167 from [redacted] (b) (4)

Dosing:

Species/strain: Crl:CD-1®(ICR)BR mice
#/sex/group or time point (main study): 16
Satellite groups used for toxicokinetics or recovery: 57/sex/group
Age: 6 weeks
Weight: males (25-35 g), females (20-26 g)

Doses in administered units:

Group	No. of animals/sex/group	Dose level	
		mg/kg/dose	mg/kg/day
Main Study			
1 (Control)	16	0	0
2 (Low)	16	5	10
3 (Mid)	16	15	30
4 (High)	16	125	250
Toxicokinetic Study			
2 (Low)	57	5	10
3 (Mid)	57	15	30
4 (High)	57	125	250

6 hours (±15 minutes) between doses

Route, form, volume, and infusion rate: Oral gavage, 10 mL/kg

Observations and times:

Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Prior to treatment and weekly thereafter
Ophthalmoscopy: Prior to treatment and during week 2.
Hematology: At termination
Clinical chemistry: At termination.
Gross pathology: At termination of the study
Organs weighed: See histopathology inventory for this IND
Histopathology: See histopathology inventory
Toxicokinetics: On days 1 and 28 at 0.25, 0.5, 1, 2,and 6 hours immediately before the second dose, and 6.5, 7, and 8 hours post dose.

Results:

Mortality:

Group	Dose level mg/kg/BID	N0. of deaths (day)		Combined deaths (%)	
		Main study	Toxicokinetics	Main study	Toxicokinetics
1 (Control)	0	1M (3)		3	
2 (Low)	5		1M (12)		1
3 (Mid)	15				
4 (High)	125		1M & 2F (11, 18 & 19)		3

The deaths of control male and one high dose female were attributed to a gavage error.

Clinical signs: 125 mg/kg/BID - Yellow discoloration of the fur in the perianal region
Body weights: 125 mg/kg/BID – Decreased (only 6% of control)
Food consumption: No difference
Ophthalmoscopy: No abnormal ophthalmic findings.
Hematology: No effect (small increases in MCV and MCH values)
Clinical chemistry: 125 mg/kg/BID - ↑alkaline phosphatase (17% in male and 35% in female)
Organ weights: 125 mg/kg/BID – absolute testis/epididymis (19%↓ Vs control), absolute thymus weight (↓ 40% in male and 20% in female Vs control)
Gross pathology: No treatment–related changes

Histopathology:

Organ	Sex	Male				Female			
	Group	1	2	3	4	1	2	3	4
Testis, atrophy/degeneration, bilateral		0	0	0	5				
Atrophy/degeneration, unilateral		1	2	0	0				
Mineralization		1	0	0	0				
Unilaterally examined		0	0	1	0				
Epididymis, aspermia, unilateral		0	0	1	0				
Ovary, corpora lutea, absent						2	4	0	11
Bone, femur, thickened, cartilage, Physeal		0	0	2	11	0	0	1	14

The severity of hypertrophic chondrocytes in the zone of cell maturation was greater in females than in males as shown below.

Incidence of severity of thickened physeal cartilage, femur

	Sex	Male		Female	
	Group	3	4	3	4
	Number Examined	16	16	16	16
Minimal		0	3	0	5
Slight		0	3	0	5
Moderate		0	0	0	2
Moderately severe		0	0	0	2
Total		2	11	1	14

Toxicokinetics: Data not submitted. The following table is copied from the Investigator's Brochure.

Dose (mg/kg/day)	Day	C_{max} (µg/mL)	T_{max} (h)	AUC (µg.h/mL)
10	1	0.10	0.5	0.27
	28	0.13	6.5	0.37
30	1	0.42	0.5	1.02
	28	0.46	1.0	1.66
250	1	2.42	2.0	11.89
	28	4.64	8.0	23.72

AUC and C_{max} are proportional to dose.

3. 14-Day gavage toxicity study with AG013736 in dogs ((b) (4) study # 6750-142). (b) (4) unaudited draft report. Vol. 05, page 264.

Key study findings: AG13736 produced one mortality in the 150 mg/kg/BID dogs. The no-observable-effect was observed in the 25 mg/kg/twice daily dogs.

Conducting laboratory and location: (b) (4)

Date of study initiation: 14 February 2001
GLP compliance: Yes
QA report: yes ( ) no (X)
Drug, lot #, and % purity: Not provided

Formulation/vehicle: 0.5% carboxymethylcellulose, Lot # PQ0167 supplied by [redacted] (b) (4)

Dosing:

Species/strain: Beagles
#/sex/group or time point (main study): 4
Satellite groups used for toxicokinetics or recovery: None
Age: 6 months
Weight: 8 to 11 kg for male and 7-9 kg for female
Doses in administered units:

Group	Dosage level (mg/kg/dose)		Dose level (mg/kg/day)	
	Days 1-9	Days 10-14	Days 1-9	Days 10-14
1 (Control)	0	0	0	0
2. (Low)	12.5	25	25	50
3. (Mid)	25	50	50	100
4. (High)	75	150	150	300

There was 6 hours ±30 minutes interval between doses.

Route, form, volume, and infusion rate: Oral gavage

Observations and times:

Clinical signs: Twice daily
Body weights: Weekly
Food consumption: Weekly
Ophthalmoscopy: Prior to treatment and during week 2
EKG: Prior to treatment and near the end of week 2.
Hematology: Day 13
Clinical chemistry: Day 13
Gross pathology: At necropsy
Organs weighed: Adrenal, brain, heart, kidney, liver with drained gallbladder, lung, ovary, pituitary, prostate, salivary gland, testis, thymus, thyroid with parathyroid.
Histopathology: Not submitted (preserved tissues are not evaluated).
Toxicokinetics: Days 1 and 14 at 0, 0.5, 1, 2, 3, 4, 6 hours (5 minutes prior to the second dose), 7, 8, 10, and 24 hours post dose.

Results:

Mortality: 150/300 mg/kg/day – 1 female sacrificed in extremis on day 14.
Clinical signs: 150/300 mg/kg/day – Thin appearance, hypoactivity, dehydration, and reddening of the gums
Body weights:

Sex	Male		Female	
Group	3	4	3	4
% age change Vs control	↓9	↓21	↓7	↓13

Food consumption: 150/300 mg/kg/day – ↓19-29% in male and 24-44% in female compared to control values
Ophthalmoscopy: No treatment related ophthalmoscopic findings
Electrocardiography: According to the sponsor "Electrocardiographic findings were within normal limits and comparable across groups". The data is not submitted.
Hematology: 150/300 mg/kg/day – Absolute reticulocyte (↓89% in male & 98% in female) and % reticulocytes(↓ 91% in male & 75% in female)

Clinical chemistry: Percent increase compared to control value

Sex	Male		Female	
Group	3	4	3	4
Cholesterol	12	121	26	74
Triglycerides		129		137
Total protein		29		15

Organ weights: Absolute organ weights compared to control value

Sex	Male		Female	
Group	3	4	3	4
Thymus	↓ 13%	↓ 50%		↓ 37%
Liver	↑ 5%	↑ 11%	↑ 13%	↑ 23%

Gross pathology: No treatment related findings
Histopathology: Not done
Toxicokinetics: Data not submitted. The following table is copied from the Investigator's Brochure.

Dose (mg/kg/day)	Day	C_{max} (µg/mL)	T_{max} (h)	AUC (µg.h/mL)
50	1	1.63±1.39	7.0±2.3	6.64±5.31
	14	0.60±0.35	5.4±3.8	3.33±3.41
100	1	3.60±1.69	6.1±2.4	13.9±8.3
	14	1.17±0.96	6.4±2.4	6.83±4.96
300	1	8.41±5.54	6.4±2.3	41.1±32.7
	14	2.57±2.05	6.4±3.1	24.9±24.0

C_{max} and AUC are proportional to dose.

4. 28-Day gavage toxicity study with AG013736 in dogs ((b) (4) study # 6750-143). (b) (4) Vol. 06, page 107.

Key study findings: Twice daily oral administration of AG13736 at 100 mg/kg/day produced 75% mortality. The compound produced toxicity at all doses tested.

Conducting laboratory and location: (b) (4)

Date of study initiation: 20 April 2001
GLP compliance: Yes
QA report: yes ( ) no (X) to be submitted
Drug, lot #, and % purity: AG13736, Lot # AC3088, 98% purity
Formulation/vehicle: 0.5% carboxymethylcellulose, Lot # PQ0167 from (b) (4)

Dosing:

Species/strain: Beagles
#/sex/group (main study): 4
Satellite groups used for toxicokinetics or recovery: None
Age: 9 months
Weight: % 8-10 kg, & 7-9 kg
Doses in administered units:

Group	Dosage level	
	mg/kg/dose	mg/kg/day
1 (Control)	0	0
2 (Low)	5	10
3 (Mid)	15	30
4 (High)	50	100

6 hours between doses.

Route, form, volume, and infusion rate: Oral gavage, 5 mL/kg/dose

Observations and times:

Clinical signs:	Twice daily
Body weights:	Weekly
Food consumption:	Weekly
Ophthalmoscopy:	Prior to treatment and during week 4
EKG:	Prior to treatment and near the end of week 4
Hematology:	Near the end of week 4 and prior to unscheduled sacrifices
Gross pathology:	At necropsy
Organs weighed:	See histopathology inventory for list of organs
Histopathology:	See histopathology inventory
Toxicokinetics:	On days 1 and 28 at 0, 0.25, 0.5, 1, 2, 3, 6, 6.5, 7, 8, 9, 12, and 24 hours after the first dose

Results:

Mortality: 100 mg/kg/day – Six of eight animals were euthanized in extremis on days 15 (2 males & 1 female), 17 (1 female), or 18 (2 males).

Clinical signs: 10 & 30 mg/kg/day – Abnormal stools and hyperemia of the oral mucosa.

100 mg/kg/day – Dehydration, thin appearance, hypoactivity, abnormal stools, and hyperemic oral mucosa and/or ulcers. One female had tachycardia (heart rate =180 bpm) on day 13 and a male had bradycardia (heart rate = 56) on day 17.

Body weights:

Changes in body weight (% initial)

Group	Sex	
	Males	Females
3 (30 mg/kg/day)	↓ 11	↓ 15
4 (100 mg/kg/day)	↓ 21	↓ 17

Food consumption:

Changes in food consumption (% control)

Group	Sex	
	Males	Females
3 (30 mg/kg/day)	85	65
4 (100 mg/kg/day)	31	44

Ophthalmoscopy: No treatment-related ophthalmoscopic findings

Electrocardiography: According to the sponsor "Electrocardiographic findings were within normal limits and comparable across groups". The data is not submitted.

Hematology: Changes in hematology parameters (% initial)

	Sex	Male		Female	
	Group	3	4	3	4
Reticulocytes (%)		↓ 75	-		
Absolute reticulocytes		↓ 63	-		↓ 47

- Samples not available

Clinical chemistry: Changes in clinical chemistry parameters (% initial)

	Sex	Male		Female	
	Group	3	4	3	4
Cholesterol		↑ 41	-	↑ 20	↑ 23
Triglycerides		↑ 27	-	↑ 53	↑ 64

- Samples not available

Urinalysis: Comparable between groups

Organ weights: 100 mg/kg/day – Thymus (↓ 67% in females Vs control)

Gross pathology: 100 mg/kg/day - Dark mucosa/dark areas in the stomach, jejunum, cecum, and colon.

Histopathology: Treatment-related histomorphologic findings are shown below.

Incidence and severity of microscopic observations in pyloric stomach

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Number Examined	4	4	4	4	4	4	4	4
Hemorrhage								
Unremarkable	4	4	3	1	4	4	4	2
Minimal	0	0	1	0	0	0	0	0
Slight	0	0	0	0	0	0	0	1
Moderate	0	0	0	2	0	0	0	0
Moderately Severe	0	0	0	1	0	0	0	1
Mean Severity Grade	0	0	0.3	2.5	0	0	0	1.5
Chronic Active Inflammation								
Unremarkable	4	4	3	3	4	4	4	2
Minimal	0	0	1	1	0	0	0	1
Slight	0	0	0	0	0	0	0	1
Mean Severity Grade	0	0	0.3	0.3	0	0	0	0.8
Necrosis								
Unremarkable	4	4	3	4	4	4	4	3
Minimal	0	0	1	0	0	0	0	0
Moderate	0	0	0	0	0	0	0	1
Mean Severity Grade	0	0	0.3	0	0	0	0	0.8
Ulcer								
Present	0	0	0	0	0	0	0	1

Incidence and severity of fibrinoid necrosis/inflammation in the submucosal vessels of stomach

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Number Examined	4	4	4	4	4	4	4	4
Cardiac Stomach								
Unremarkable	4	3	3	4	4	4	4	3
Minimal	0	1	1	0	0	0	0	1[b]
Mean Severity Grade	0	0.3	0.3	0	0	0	0	0.3
Fundic Stomach								
Unremarkable	3	4	4	3	4	4	4	4
Minimal	1	0	0	0	0	0	0	0
Slight	0	0	0	1[a]	0	0	0	0
Mean Severity Grade	0.3	0	0	0.5	0	0	0	0
Pyloric Stomach								
Unremarkable	4	4	3	1	4	4	4	2
Minimal	0	0	0	2	0	0	0	1
Slight	0	0	1	1[a]	0	0	0	1[b]
Mean Severity Grade	0	0	0.5	1.0	0	0	0	0.8
(Cardiac + Fundic + Pyloric) Stomach								
Unremarkable	3	3	3	1	4	4	4	2
Minimal	1	1	1	2	0	0	0	1
Slight	0	0	1	1	0	0	0	1
Mean Severity Grade	0.3	0.3	0.5	1.0	0	0	0	1.0

a Animal number H38580 had lesions in both fundic and pyloric stomach.
b Animal number H38597 had lesions in both cardiac and pyloric stomach.

Incidence and severity of congestion/hemorrhage in small and large intestine

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Number Examined	4	4	4	4	4	4	4	4
Duodenum								
Minimal	0	0	0	2	0	0	0	0
Slight	0	0	0	0	0	0	0	1
Mean Severity Grade	0	0	0	0.5	0	0	0	0.5
Jejunum								
Minimal	1	0	0	0	0	0	0	0
Slight	0	0	0	0	0	0	0	1
Mean Severity Grade	0.3	0	0	0	0	0	0	0.5
Ileum								
Minimal	0	0	0	1	0	0	0	0
Slight	0	0	0	2	0	0	1	0
Moderate	0	0	0	0	0	0	0	1
Mean Severity Grade	0	0	0	1.3	0	0	0.5	0.8
Cecum								
Minimal	0	0	0	2	0	0	0	1
Slight	0	0	0	1	0	0	0	0
Mean Severity Grade	0	0	0	1.0	0	0	0	0.3
Colon								
Minimal	0	0	0	2	0	0	0	0
Slight	0	0	0	2	0	0	0	0
Moderate	0	0	0	0	0	0	0	1
Mean Severity Grade	0	0	0	1.5	0	0	0	0.8
Rectum								
Minimal	0	0	0	1	0	0	0	0
Slight	0	0	0	2	0	0	0	1
Mean Severity Grade	0	0	0	1.3	0	0	0	0.5

Incidence and severity of microscopic observations in pancreas

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Number Examined	4	4	4	4	4	4	4	4
Decreased Zymogen Granules								
Unremarkable	4	4	0	0	4	3	1	0
Minimal	0	0	2	0	0	1	2	0
Slight	0	0	1	0	0	0	1	1
Moderate	0	0	1	1	0	0	0	1
Moderately Severe	0	0	0	3	0	0	0	2
Mean Severity Grade	0	0	1.8	3.8	0	0.3	1.0	3.3
Acinar Cell Proliferation								
Unremarkable	4	4	0	0	4	4	1	0
Minimal	0	0	3	0	0	0	2	1
Slight	0	0	0	0	0	0	1	1
Moderate	0	0	1	1	0	0	0	2
Moderately Severe	0	0	0	3	0	0	0	0
Mean Severity Grade	0	0	1.5	3.8	0	0	1.0	2.3
Increased Acinar Cell Apoptosis								
Unremarkable	4	4	4	2	4	4	3	2
Minimal	0	0	0	2	0	0	1	2
Mean Severity Grade	0	0	0	0.5	0	0	0.3	0.5

Incidence and severity of lymphoid depletion in thymus

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Number Examined	4	4	4	4	4	4	4	4
Lymphoid Depletion								
Unremarkable	3	3	2	0	4	4	1	0
Minimal	1	1	1	0	0	0	1	1
Slight	0	0	1	2	0	0	2	2
Moderate	0	0	0	2	0	0	0	1
Mean Severity Grade	0.3	0.3	0.8	2.5	0	0	1.3	2.0

Incidence and severity of microscopic observations in tongue

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Number Examined	4	4	4	4	4	4	4	4
Inflammation, Chronic Active								
Unremarkable	4	4	3	1	4	4	4	0
Slight	0	0	0	2	0	0	0	1
Moderate	0	0	1	1	0	0	0	3
Mean Severity Grade	0	0	0.8	1.8	0	0	0	2.8
Ulcer								
Unremarkable	4	4	3	1	4	4	4	1
Present	0	0	1	3	0	0	0	3
Necrosis								
Unremarkable	4	4	4	3	4	4	4	1
Minimal	0	0	0	0	0	0	0	1
Slight	0	0	0	1	0	0	0	1
Moderate	0	0	0	0	0	0	0	1
Mean Severity Grade	0	0	0	0.5	0	0	0	1.5

Incidence and severity of microscopic observations in oral cavity

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Number Examined	0	0	2	4	0	1	2	4
Chronic Active Inflammation								
Minimal	0	0	1	0	0	0	0	0
Slight	0	0	0	1	0	0	1	0
Moderate	0	0	1	3	0	1	1	3
Severe	0	0	0	0	0	0	0	1
Mean Severity Grade	0	0	2.0	2.8	0	3.0	2.5	3.5
Ulcer								
Present	0	0	0	3	0	0	1	4

Incidence and severity of thickening of growth plate in rib

Sex	Male				Female			
Group	1	2	3	4	1	2	3	4
Number Examined	4	3	4	4	3	4	4	2
Growth Plate, Thickened								
Not Examined	0	1	0	0	1	0	0	2
Minimal	0	0	2	0	0	0	0	0
Slight	0	0	0	3	0	0	0	0
Mean Severity Grade	0.0	0.0	0.5	1.5	0.0	0.0	0.0	0.0

(excerpted from the Applicant's submission)

- 100 mg/kg/day – Hypocellularity in the bone marrow (femur) of males and multinucleated cells in the seminiferous tubules

- ≥10 mg/kg/day – delayed sexual maturity in females (2 at 10 mg/kg/day, 3 at 30 mg/kg/day and 2 at 100 mg/kg/day) as evidenced by presence of small follicles and absence of corpora lutea in the ovary, and presence of inactive uterus and mammary gland.

Toxicokinetics: Data not submitted. The following table is copied from the Investigator's Brochure.

Dose (mg/kg/day)	Day	C_{max} (µg/mL)	T_{max} (h)	AUC (µg.h/mL)
10	1	0.41±0.24	5.6±3.3	1.68±1.21
	28	0.04±0.03	4.9±3.7	0.07±0.07
30	1	1.00±0.98	3.4±2.3	5.25±6.45
	28	0.97±1.56	5.9±3.3	4.15±6.09
100	1	0.72±0.78	7.0±7.8	3.71±3.56
	28	0.82	5.5	4.40

GENETIC TOXICOLOGY

1. Microbial Reverse Mutation Assays. (Pfizer Study # 01-2191-02). Vol. 07, Page 001.

Key findings: AG13736 did not induce mutation in Ames test up to 5 mg/plate with or without metabolic activation (S9).

Conducting laboratory and location: Drug Safety Evaluation Department, Pfizer Global Research and Development, Pfizer Inc., Groton, Connecticut 06340.
Date of study initiation: April 17, 2001
GLP compliance: Yes
QA reports: yes (X) no ()
Drug, lot #, and % purity: AG13736, lot # 2737-C-1P (also referred to as AC3088), 98% purity
Formulation/vehicle: DMSO

Methods:

Strains: Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA100. Escherichia coli strain WP2 uvrA pKM101.
Concentration selection criteria: Insolubility (precipitate) and cytotoxicity
Basis of concentration selection: Preliminary plate incorporation assay
Range finding studies: Yes

Test agent stability: Stable
Analysis: 90-110 %
Metabolic activation system: S9 from Aroclor 1254-induced rats
Exposure conditions: 37^0C
Incubation and sampling times: ~48 hours for E. coli and ~72 hours for Salmonella
No. of replicates: 3
Counting method: Electronically or manually
Criteria for positive results: A reproducible dose-related 2-fold increase over the control

Results:

Bacterial mutation assay

Compound	Concentration (mg/plate)	S9	TA98	TA100	TA1535	TA1537	WP2 uvrA pKM101
DMSO	0.1 mL	-	31±5	132±11	20±3	10±5	159±33
		+	28±12	135±5	20±5	14±8	203±13
AG13736	1.5	-	22±3	125±9	20±5	9±3	170±14
		+	22±6	144±14	23±5	8±3	184±25
	5.0	-	20±2	125±6	22±11	8±4	157±15
		+	24±3	129±8	15±3	9±4	145±9
2-Nitrofluorene	0.005	-	1134±262				
9-Aminoacridine	0.04	-				72±9	
ENNG	0.005	-					1881±28
Nitrofurantoin	0.002	-		1193±127			
Sodium nitrite	2	-			361±35		
2-Anthramine	0.005	+		1496±96	302±36		

Conclusions: Concentration-related cytotoxicity was observed at 5 mg/plate in the absence of S9 metabolic activation. No evidence of significant concentration-related increases in the number of revertant colonies compared to negative control were observed.

2. *In Vitro* Cytogenetic Assays. (Pfizer Study # 01-2191-03). Vol. 07, Page 053.

Key findings: AG13736 did not induce structural chromosome aberration in human lymphocyte cultures *in vitro* either with or without metabolic activation.

Conducting laboratory and location: Drug Safety Evaluation Department, Pfizer Global Research and Development, Pfizer Inc., Groton, Connecticut 06340.
Date of study initiation: April 20, 2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AG13736, lot # 2737-C-IP (AC3088), 98% purity
Formulation/vehicle: DMSO

Methods:

Cell line: Human peripheral lymphocytes
Concentration selection criteria: Test article-induced cytotoxicity and chromosome damage (50% suppression of the mitotic index compared to negative control),
Basis of concentration selection: Preliminary cytotoxicity assays
Range finding studies: 7.8 to 500 μg/mL
Test agent stability: data not submitted
Metabolic activation system: S9
Vehicle: DMSO
Positive controls: Mitomycin-C (without S9) and cyclophosphamide (+ S9)
Exposure conditions: $37^{O}C$
Incubation and sampling times: 3 hours in the presence of S9 or 24 hours (3 hours followed by a 21 hours recovery in fresh complete medium) without S9.
No. of replicates: Duplicate
Criteria for positive results: Dose-related significant increase in aberrant cells as compared to negative control.

Results:

HUMAN LYMPHOCYTE ABERRATION ASSAY: SUMMARY OF TEST

3 HOUR TREATMENT
WITHOUT METABOLIC ACTIVATION

Treatment[a]	Mean (%) Mitotic Suppression[b]	Total Cells Analyzed	Mean (%) Abnormal Cells[c]	p-Value[d]	Mean (%) Polyploid Cells[e]
Negative Control: DMSO					
1.00%	0	200	1.0	--	0.4
Test Article: AG13736 (µg/ml)					
0.220*	--	--	--	--	--
0.370	5	200	2.0	0.343	2.7
1.04	32	200	1.0	>0.500	5.0
1.73	37	--	--	--	--
2.88	56	200	1.5	0.500	9.8
4.80**	--	--	--	--	--
Positive Control: Mitomycin-C					
0.400	37	100	30.0	<0.001	--

a: Two replicate cultures evaluated for each treatment group; when possible 100 cells per culture are analyzed for chromosome damage.
b: Mean (%) Mitotic Suppression = [One minus the quotient (Mean Test Article Mitotic Index / Mean Negative Control Mitotic Index)] (x) 100.
c: Mean (%) Abnormal Cells = Sum of % Abnormal Cells for duplicate cultures / 2.
d: Data analyzed by 1-tailed Fisher's Exact test for increases in abnormal cells compared to the negative control values.
e: Mean (%) Polyploid = Sum of % Polyploidy for duplicate cultures / 2.

Abbreviations:
--: Dashes indicate data not available or determined.
*:Cultures treated with test article concentrations ≤0.220 µg/ml were not evaluated.
**: Cultures treated with test article concentrations ≥4.80 µg/ml were not evaluated since sufficient mitotic suppression was achieved at a lower concentration.

HUMAN LYMPHOCYTE ABERRATION ASSAY: SUMMARY OF TEST

3 HOUR TREATMENT
WITH METABOLIC ACTIVATION

Treatment[a]	Mean (%) Mitotic Suppression[b]	Total Cells Analyzed	Mean (%) Abnormal Cells[c]	p-Value[d]	Mean (%) Polyploid Cells[e]
Negative Control: DMSO					
1.00%	0	200	1.0	--	0.6
Test Article: AG13736 (µg/ml)					
0.050*	--	--	--	--	--
0.080	11	200	1.5	0.500	0.8
2.88	15	200	1.0	>0.500	7.0
4.80	57	200	0.5	>0.500	19.5
8.00**	70	--	--	--	--
Positive Control: Cyclophosphamide					
10.0	11	100	24.0	<0.001	--

HUMAN LYMPHOCYTE ABERRATION ASSAY: SUMMARY OF TEST

24 HOUR TREATMENT
WITHOUT METABOLIC ACTIVATION

Treatment[a]	Mean (%) Mitotic Suppression[b]	Total Cells Analyzed	Mean (%) Abnormal Cells[c]	p-Value[d]	Mean (%) Polyploid Cells[e]
Negative Control: DMSO					
1.00%	0	200	1.0	--	0.6
Test Article: AG13736 (µg/ml)					
0.130*	--	--	--	--	--
0.220	16	200	0.0	>0.500	0.8
0.370	31	200	0.0	>0.500	1.4
0.620	47	200	0.5	>0.500	12.7
1.04**	93	--	--	--	--
Positive Control: Mitomycin-C					
0.050	33	100	27.0	<0.001	--

(Excerpted from the Applicant's submission)

Conclusions: There was no significant increase in the number of abnormal cells aberrations compared to concurrent negative control at any concentration evaluated. However, the no effect level (NOEL) for AG13736 induced polyploidy in this system was less than 0.220 µg/mL.

3. Mouse Micronucleus Assay. (Pfizer Study # 01-2191-01). Vol. 07, Page 085.

Key findings: AG13736 induced micronuclei in bone marrow PCE of male and female mice at doses ≥ 1000 and 500 mg/kg, respectively.

Conducting laboratory and location: Drug Safety Evaluation Department, Pfizer Global Research and Development, Pfizer Inc., Groton, Connecticut 06340.
Date of study initiation: April 23, 2001
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: AG13736, lot # AC3088, 99% purity

Methods:

Strains/species: Crl: CD-1 (ICR)BR VAF/Plus® mice
#/sex/group 5 (Test) and 3 (PK)
Basis of dose selection: Single dose mouse gavage toxicity study
Test agent stability: Stable (±10 % range)
Negative controls: 0.5% methylcellulose

Positive controls: Mitomycin C (0.5 mg/kg by intraperitoneal injection)
Exposure conditions: Once per day for three days
Doses used in definitive study: 500, 1000, and 2000 mg/kg/day

Results:

Test 1: Mouse micronucleus assay in treated animals compared to negative control animals

Compound	Dose (mg/kg)	Wt gain (%)		PCE (%)		MNPCE (%)	
		Male	Female	Male	Female	Male	Female
Methylcellulose	0.5 %	13.2 ± 5.1	0.4 ± 3.3	67.4 ± 3.8	73.8 ± 7.3	0.16 ± 0.14	0.12 ±. 010
Mitomycin C	0.5	8.1 ± 1.8	7.7 ± 3.7	52.9 ± 7.1	66.8 ± 7.6	2.33 ± 0.79	1.98 ± 0.07
AG13736	500	8.7 ± 6.3	4.5 ± 3.3	45.8 ± 10.5	69.6 ± 8.1	0.13 ± 0.09	0.66 ± 0.29
	1000	12.9 ± 5.1	4.2 ± 5.9	24.7 ± 13.6	42.2 ± 15.0	1.57 ± 0.7	1.46 ± 1.30
	2000	0.3 ± 5.5	5.3 ± 3.7	2.1 ± 1.2	3.3 ± 4.4	*	1.65

PCE: Polychromatic erythrocyte % MNPCE: Percentage of micronucleated PCE
*: No data due to toxicity at 2000 mg/kg.

AG13736 at 2000 mg/kg produced severe cytotoxicity in the bone marrow in both males (2.1% PCE) and females (3.3% PCE) compared to negative control. The compound produced an elevation in MNPCE at 1000 and 500 mg/kg in males and females, respectively, when compared to the concurrent vehicle control values.

Test 2: Mouse micronucleus assay in treated females compared to negative control females

Compound	Dose (mg/kg)	Wt. gain (%)	PCE (%)	MNPCE (%)
methylcellulose	0.5%	-1.4±4.6	62.0±6.5	0.08±0.08
Mitomycin C	0.5	0.9±5.5	48.7±6.9	1.60±0.20
AG13736	60	2.1±3.5	38.4±5.5	0.15±0.05
	125	5.1±5.4	42.7±11.2	0.10±0.05
	250	5.4±6.3	52.5±7.0	0.09±0.07
	500	3.7±3.2	29.9±18.0	0.47±0.36

AG13736 produced ~6 fold elevation in MNPCE at 500 mg/kg compared to vehicle control.

Mean pharmacokinetic parameters of AG13736 in mice

Test #	Dose (mg/kg)	Gender	C_{max} (µg/mL)	T_{max} (h)	$AUC_{0\text{-last}}$ (µg.h/mL)
`2	60	F	1.32	1	2.25
2	125	F	2.22	1	5.24
2	250	F	3.54	4	18.35
2	500	F	8.52	2	40.81
		M	11.57	4	58.55
1	2000	F	51.83	4	361.49
		M	28.70	2	307.76

The increase in exposure (C_{max} and AUC) was approximately dose proportional. C_{max} and AUC values were higher for males than females at 500 mg/kg/day, but were lower in males than females at 2000 mg/kg/day.

Kinetochore staining analysis was conducted on the bone marrow smears from two females treated with 500 mg/kg of AG13736. The results showed that ~75% of the evaluated micronuclei were kinetochore positive. This limited data suggest an aneuploidy mechanism for the induction of micronuclei seen in this study.

Conclusions: Oral administration of AG13736 for three consecutive days induced micronuclei in the polychromatic bone marrow erythrocytes of male (≥ 1000 mg/kg) and female (≥ 500 mg/kg) mice.

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/s/

M A GOHEER
01/06/2012

TODD R PALMBY
01/06/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 202-324 **Applicant:** Pfizer **Stamp Date:** April 13, 2011

Drug Name: INLYTA tablets (Axitinib) **NDA Type:** NME

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		CTD format
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		Electronic submission
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
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)?	√		Carcinogenicity – not done/not required Mutagenicity – done Teratogenicity – done Fertility – not required Juvenile studies – not done/not required Acute and repeat dose toxicity – done (26-week in mice & dogs and 9-month in dogs) ADME – done Safety pharmacology – done(cardio, local tolerance)
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).	√		Similar formulations were used in pivotal preclinical and clinical studies
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?	√		Same route of administration

	Content Parameter	Yes	No	Comment
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?	√		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		
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?	√		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			Appears sufficient
11	Has the applicant addressed any abuse potential issues in the submission?		√	
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable. This NDA is NME

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: N/A

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter: None

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

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/s/

M A GOHEER
05/13/2011

WHITNEY S HELMS
05/13/2011