CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-465

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22, 465**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/19/2008**
PRODUCT:	**Pazopanib**
INTENDED CLINICAL POPULATION:	**Renal Cell Carcinoma**
SPONSOR:	**GlaxoSmithKline**
DOCUMENTS REVIEWED:	**Electronic Submission**
REVIEW DIVISION:	**Oncology Drug Products**
PHARM/TOX REVIEWER:	**Robeena Aziz, MPH, PhD/ Whitney Helms, Ph.D**
PHARM/TOX SUPERVISOR:	**Leigh Verbois, PhD**
DIVISION DIRECTOR:	**Robert Justice, MD**
PROJECT MANAGER:	**Kim Robertson**

Date of review submission to Division File System (DFS): **9/17/2009**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
Approvable. The non-clinical studies with pazaponib support the safety of its use in renal cell carcinoma.

B. Recommendation for nonclinical studies
No additional non-clinical studies are required for pazopanib

C. Recommendations on labeling
The recommendations to the sponsor's proposed labeling are given, with a detailed report regarding the rationale for the recommended changes, in a subsequent review.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The nonclinical findings have shown the target sites of toxicity with pazopanib to be the teeth, bone marrow, gastrointestinal, and reproductive systems. Many of these toxicities are seen clinically and are thought to be direct effects of the pharmacology of pazopanib.

Pazopanib was not mutagenic or clastogenic in the *in vitro* assays. Pazopanib was not clastogenic (induction of micronuclei) in the *in vivo* rat micronucleus assay (highest dose = 2000 mg/kg; 12,000 mg/m^2).

Pazopanib did not impair fertility when administered to male rats. Female rats showed reduced fertility at doses ≥ 30 mg/kg (180 mg/m^2). Pazopanib was found to be teratogenic in the rat and induced embryo-fetal toxicity in both rats and rabbits at doses significantly below those that caused maternal toxicity.

B. Pharmacologic activity

The major pharmacological activity of pazopanib is inhibition of angiogenic signaling pathways through a panel of growth factors and receptors, primarily the VEGF receptor family. Inhibition of these growth factors and/or their receptors results in the inhibition of neovascularization, disruption of existing tumor vascularization, and inhibition of proangiogenic growth factor release.

In nonclinical studies, pazopanib inhibited vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, VEGFR-3, platelet-derived growth factor receptor (PDGFR)-α and -β, fibroblast growth factor receptor (FGFR) -1 and -3, cytokine receptor (Kit), interleukin-2 receptor inducible T-cell kinase (Itk), leukocyte-specific protein tyrosine kinase (Lck), transmembrane glycoprotein receptor tyrosine kinase (c-Fms), and mitogen-activated protein kinase (P38).

Pazopanib inhibited autophosphorylation of VEGFR-2, c-Kit and PDGFR-β receptors in human umbilical vein endothelial cells (HUVEC), NCI-H526 (human small cell lung carcinoma), human foreskin fibroblast (HFF) and RS4;11 cells (human B-cell acute lymphoblastic leukemia). Pazopanib showed modest activity (IC_{50} values $\leq$ 1 nM) in 282 human cell lines obtained from AML, myeloma, colon carcinoma, kidney leiomyoblastoma, thyroid carcinoma, rhabdomyosarcoma, CML, lymphoma cutaneous T-cell, and stomach carcinoma. When evaluated in imatinib-resistant c-Kit mutant cell lines (V654A, T670I, Y816V, Y823D, K642E), and in CHO-K1 cells transiently-transfected with wild type c-Kit, pazopanib inhibited wild-type c-Kit activation (IC_{50} values of 3 nM).

In vivo studies in mouse lung stimulated with VEGF showed that pazopanib inhibited VEGF-induced phosphorylation at 8, 16, and 24 hours post-dosing and at doses of 10, 30, and 100 mg/kg (30, 90, and 300 mg/m^2). Pazopanib showed an improved dose-dependent inhibition of tumors in CB-17 SCID mice compared to Swiss mice at doses $\geq$10 mg/kg/day (30 mg/m^2) when both mouse models were injected with renal cell carcinoma tumor xenografts (CAKI-2 and A498). Pazopanib also showed modest inhibition of tumor growth at $\geq$10 mg/kg/day (30 mg/m^2) when the human renal cell cancer xenograft (ACHN) was injected in Swiss nude mice. Compared to the dihydrchloride form, the monohydrochloride form of the drug was not as effective at inhibiting tumors in a mouse model of angiogenesis; however, there was significant tumor inhibition when the monohydrochloride was administered at $\geq$30 mg/kg (90 mg/m^2). The drug does not appear to disrupt delivery of other anti-angiogenic agents to tumor tissues when given in combination. .

The pharmacological classification of pazopanib is a kinase inhibitor. This is consistent with other drugs of this class that act by competitively inhibiting tyrosine kinase function.

C. Nonclinical safety issues relevant to clinical use

The nonclinical safety issues observed in the toxicology program with pazopanib included toxicities in the bone marrow, gastrointestinal and reproductive system, hepatic system and teeth (mice and rats only) which are consistent with other tyrosine kinase inhibitors.

There were no remarkable findings in studies investigating the effect of pazopanib on pulmonary or CNS function. Cardiovascular safety studies conducted in cynomolgus monkeys showed that there were transient increases in mean arterial pressure and decreases in heart rate following administration of pazopanib. Decreased heart rate lasted up to 13 hours following dosing at the doses $\geq$ 50 mg/kg/day (600 mg/m^2) and changes were evident at earlier time points even at the low dose of 5 mg/kg/day (60 mg/m^2.) Investigators reported similar findings when pazopanib was administered by IV infusion at 3.75 mg/kg (45 mg/m^2) with animals displaying decreased heart rate for up to 24.5 hours post dose. ECG waveforms obtained for monkeys were, however, within normal limits for the species and there was no significant change in QTc. These studies suggest that pazopanib does not have an acute adverse effect on cardiovascular function.

The profile of metabolites of pazopanib were similiar across species, including mouse, rat, dog and human. Following oral administration of radio-labeled pazopanib in mouse, rat, monkey and human plasma, the principal radio-labeled component was unchanged pazopanib. *In vitro* studies with radiolabelled pazopanib in with hepatocytes and hepatic microsomes from mice, rat, rabbit, dog, monkey and human showed that mono-oxygenation, di-oxygenation, and potential oxidation of methyl to carboxylic acid were routes of metabolism. No human specific Phase 1 metabolites were detected in human microsomal or hepatocyte incubations. In studies with human liver microsomes, pazopanib showed moderate to marked inhibition of cytochrome P450 enzymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4. Pazopanib metabolism is mediated mainly by CYP3A4 with minor contributions from CYP1A2 and CYP2C8. In addition, pazopanib was shown to be a direct inhibitor of human UGT1A1 (IC_{50} = 1.2 μM) *in vitro*. Pazopanib was also found to be an inhibitor of the xenobiotic liver transport protein OATP1B1 at clinically relevant concentrations.

In vivo studies showed that pazopanib was widely distributed through the bodies of male and female rats and had strong association with melanin producing cells, particularly in the uveal tract. Other organs with high concentrations of pazopanib included the meninges, skin, and liver. The primary route of excretion of pazopanib and its metabolites was fecal.

In the 26-week rat study, dosing with pazopanib resulted in growth plate hypertrophy and hypocellular bone marrow in addition to trabecular atrophy of the femur and periosteal chondroid change in the sternum at ≥30 mg/kg/day (180 mg/m^2). In mice, similar changes in the sternum and femur/stifle joints were noted after 13 weeks at ≥100 mg/kg/day (300 mg/m^2). Bone and bone marrow effects in mice and rats are consistent with the pharmacological inhibition of VEGF-2. Crystalline pigment in the small intestine and mesenteric lymph node were noted in mice (1000 mg/kg/day, 3000 mg/m^2) and rats (≥ 30 mg/kg/day, 180 mg/m^2) and attributed to clinical signs of pale, few and non-formed feces. Atrophy/degeneration of testes with aspermia, hypospermia and cribiform change in the epididymis was observed at ≥30 mg/kg/day (180 mg/m^2). This correlated with decreases in absolute and relative epididymis and testes weights. A dose-response in sparse corpora lutea and epithelial cysts was observed in mice (≥ 100 mg/kg/day 300 mg/m^2) while atrophy in rats was observed only at 300 mg/kg/day (1800 mg/m^2).

In addition, the hepatic system is an additional site of toxicity that was observed in rodents. The hepatic effects consisted of eosinophilic foci and adenoma in female mice at 1000 mg/kg/day (3000 mg/m^2). Male mice had elevated ALT and AST levels. In a 26-week rat study, increases in ALT were observed at 300 mg/kg/day (1800 mg/m^2) after 4 weeks of dosing. For both mice and rats, there was no clear association of liver enzyme elevations with proliferative changes.

In rodents, mortality was attributed to decreases in body weight and food consumption parameters resulting from animal's inability to eat food due to severe teeth toxicities. In rats, clinical signs of excessively long, brittle, loose, broken and missing teeth correlating to dentine and enamel degeneration and thinning were observed at ≥30 mg/kg/day (180

mg/m^2). These findings were present in 4, 13, and 26 week studies and occurred approximately at Week 6 with the onset of mortality at Week 10. In mice, overgrown and broken teeth were also present, however, no mortality occurred. No teeth findings were seen in non-rodents, however, these findings are relevant if future development of the drug includes administration to pediatric populations.

In the 52-week monkey study, severe gastrointestinal effects (colored feces and excretion) required early termination on Weeks 26 and 34. Crystalline pigment in the duodenum, jejunum and mesenteric lymph node and were secondary to the reduced body weight observed at 500 mg/kg/day (6000 mg/m^2). Active/mature corpora lutea and presence of increased endometrial stoma/glands were observed at 5 mg/kg/day (60 mg/m^2). However, these findings had to no correlation to organ weights.

Pazopanib was not mutagenic or clastogenic in the *in vitro* assays. Pazopanib was not clastogenic (induction of micronuclei) in the *in vivo* rat micronucleus assay (highest dose = 2000 mg/kg; 12,000 mg/m^2). (b) (4) a starting material for the drug substance (pazopanib monohydrochloride), has a structural alert for genotoxicity. Additionally, (b) (4) was found to be positive in both the Ames Assay and the mouse lymphoma assay. However the level of (b) (4) in the drug substance is significantly lower (b) (4) than the acceptable daily intake of genotoxic impurities defined in the draft Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches for products administered for ≥12 months (1.5 μg/day) and is therefore acceptable.

Reproductive toxicology studies were performed in rats and rabbits to assess the effects of the drug on fertility and embryofetal development. Male fertility was not affected, though there were dose-dependent decreases in reproductive organ weights and sperm production, concentration, and motility. Decreased motility occurred only at the highest administered dose of 100 mg/kg/day (600 mg/m^2, AUC<877 h*μg/mL). Female fertility was affected. At 300 mg/kg/day (1800 mg/m^2, ~1.3 times the human clinical exposure based on AUC) the fertility index of these animals dropped to 32% with a 100% resorption rate. At 3 mg/kg/day (180 mg/m^2,~0.5 times the human clinical exposure based on AUC) females had a 25.6% rate of resorption and there was decreased fetal body weight.

Embryofetal development studies conducted in the rat showed that pazopanib is teratogenic and embryotoxic. In the rat study malformations of the great vessels, including retroesophogeal subclavian arteries and missing innominate vessels were evident, increasing in a dose-dependent manner. Incomplete or absent ossification, particularly in the thoracic vertebrae, also increased with increasing dose. The fetal NOAEL in this study was 1 mg/kg (6 mg/m^2). Beginning at 10 mg/kg (60 mg/m^2) there was an increase in post-implantation loss which increased to 100 % at ≥ 30 mg/kg (180 mg/m^2, ~0.5 times the human clinical exposure based on AUC). Similarly, in rabbits there was increasing post-implantation loss at ≥ 10 mg/kg (118 mg/m^2), with decreased fetal body weight at doses ≥ 3 mg/kg (35.4 mg/m^2). Among the rabbit litters there were single incidences of gastroschisis (3 mg/kg, 35.4 mg/m2) and microtia (30 mg/kg, 354 mg/m^2). A fetal NOAEL was not determined in the rabbit and the AUC for this species at the frankly toxic dose of 30 mg/kg (354 mg/m^2) was ~0.07 times the expected human

clinical exposure. The conclusion of these studies is that administration of pazopanib during pregnancy may pose a risk for fetal toxicity. Pregnancy category D is recommended.

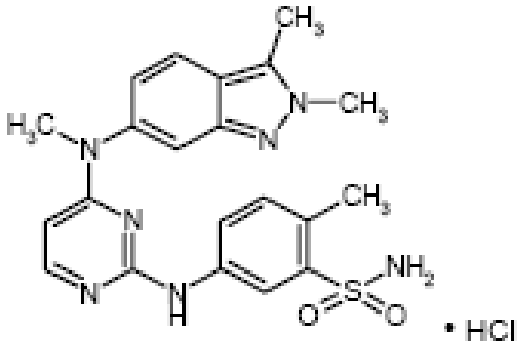
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22, 465
Review number: 1
Sequence number/date/type of submission: 000/December 19, 2008/Electronic
Information to sponsor: Yes () No (X)
Sponsor and/or agent: Glaxo Smith Kline
King of Prussia, PA 19406
Manufacturer for drug substance: Glaxo Operations UK Limited
Ware, Hertfordshire SG12 0DJ
United Kingdom
Reviewer name: Robeena M. Aziz, MPH, PhD
Division name: Division of Drug Oncology Products
Review completion date: July 30, 2009

Drug:

Trade name: Votrient
Generic name: Pazopanib (r-INN), pazopanib hydrochloride (USAN)
Code name: GW786034B denotes the monohydrochloride salt of the free base, GW786034X
CAS name: Benzenesulfonamide, 5-[4-[(2,3-dimethyl-2*H*-indazol-6 yl)methylamino]-2-pyrimidinyl]amino]-2-methyl-monohydrochloride
CAS name: 635702-64-6
Molecular formula/molecular weight: $C_{21}H_{23}N_7O_2S$ HCl/474 g/mol
Structure:



Relevant INDs/NDAs/DMFs: IND 65, 747

Drug class: kinase inhibitor

Intended clinical population: advanced stage renal cell carcinoma (RCC)

Clinical Formulation: 200 and 400 mg tablets

Table 1 Composition of Pazopanib Tablets, 200 mg and 400 mg[1]

(b) (4)

Route of administration: oral

Starting dose: 800 mg/day

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:
Pharmacology
Primary Pharmacodynamics:

Title	Study No.	Module
GW786034 is a potent and selective inhibitor of vascular endothelial growth factor	RR2002/00010/01	4.2.1.1
IC50 profiling of 4 compounds (GW771806A, GW786034B, GW654652X, and GW695612A) using 16 protein kinases	RH2003/00076/00	4.2.1.1
GW786034B (pazopanib) biochemical kinase inhibitory activity and differentiation studies	UH2008/00035/00	4.2.1.1
Effects of GW786034 on cellular proliferation and receptor phosphorylatiion assays	RH2002/00042/00	4.2.1.1
Effects of pazopanib on the proliferation of human tumor cell lines	UH2008/00110/00	4.2.1.1
Cellular activities of pazopanib, sunitinib, and sorafenib against various receptor tyrosine kinases	UH2008/00016/00	4.2.1.1
Cellular activity of pazopanib against imatinib-resistant c-kit mutants	UH2008/00015/00	4.2.1.1
Inhibition of VEGFR2 phosphorylation by GW786034B *in vivo*	RH2003/00005/00	4.2.1.1
Anti-tumor activity of GW786034B in various human tumor xenografts	RH2002/00043/00	4.2.1.1
Effects of pazopanib on the growth of human renal cell carcinoma xenografts in mice	UH2008/00109/00	4.2.1.1
Head to head comparison of the anti-tumor activity of three small molecule angiogenesis inhibitors: pazopanib, sunitinib and sorafenib	UH2008/00114/00	4.2.1.1
Effect of GW786034B on the growth of prostate tumors in CR2-T-Ag transgenic mice	RH2003/00006/00	4.2.1.1
The effects of VEGFR2 antagonist GW786034A di-salt in the bFGF/Matrigel® model of angiogenesis	RH2002/00048/00	4.2.1.1
The effects of VEGFR2 antagonist GW786034B mono-HCl salt in the bFGF/Matrigel® model of angiogenesis	RH2002/00049/00	4.2.1.1

Pharmacodynamic drug interactions:

Title	Study No.	Module
Effect of pazopanib and bevacizumab on the delivery of chemotherapeutic agents in the human tumor grown in mice	UH2008/00107/00	4.2.1.4

Safety Pharmacology

Title	Study No.	Module
GW786034B: Safety Pharmacology Study of Overt Central and Peripheral Pharmacodynamic Effects Following Oral Administration in Conscious Sprague Dawley® Rats	RD2002/00100/00 R41033	4.2.1.3
GW786034B: A Single Oral Dose Respiratory Study in Rats	RD2001/01691/00 R41018	4.2.1.3
GW786034B: Effect on hERG Tail Current Recorded from Stably Transfected HEK-293 Cells	FD2008/00125/00 V28197	4.2.1.3
GW786034B: Effect on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibres	FD2002/00060/00 D23802	4.2.1.3
Hemodynamic evaluation of GW-786034A in the anesthetized rat	CH2002/00002/00	4.2.1.3

Title	Study No.	Module
GW786034B: Acute Effects on Cardiovascular Function Following Intravenous Administration in the Conscious Cynomolgus Monkey (Safety Pharmacology Study)	CD2006/00750/00 G05397	4.2.1.3
Single Oral Dose Cardiovascular Study in Cynomolgus Monkeys	CD2002/00099/00 G02047	4.2.1.3

Pharmacokinetics

Absorption:

Title	Study No.	Module
Exposure of GW786034X following oral administration of GW786034A at 10, 30, and 100 mg/kg to female CD-1 mice	RD2001/01451/00	4.2.2.2
Preliminary investigation of the intravenous pharmacokinetics and oral bioavailability of GW786034B in the conscious rat	CD2002/00094/00	4.2.2.2
Preliminary Investigation to evaluate the oral PK for GW786034B HCl salt ((b) (4) in the male Sprague-Dawley rats	CD2003/00102/00	4.2.2.2
Pharmacokinetics and oral bioavailability of GW786034X following intravenous and oral administration of GW786034A to male Han Wistar rats	RD2001/01452/00	4.2.2.2
Pharmacokinetics and oral bioavailability of GW786034X following intravenous and oral administration of GW786034A to male beagle dogs	RD2001/01514/00	4.2.2.2
Preliminary PK evaluation of GW786034B following iv an oral administration in the male cynomolgus monkey	CD2002/00093/00	4.2.2.2
Pharmacokinetics and oral bioavailability of GW786034X following intravenous and oral administration of GW786034A to non-naïve male cynomolgus monkeys	RD2001/01169/00	4.2.2.2
Exposure of GW786034X following oral administration of GW786034B to non-naïve male monkeys	RD2002/00061/00	4.2.2.2
Preliminary investigation to evaluate the oral PK on GW786034 HCl salt ((b) (4) in the male cynomolgus monkeys	CD2003/00103/00	4.2.2.2
Pharmacokinetic of GW786034 and its metabolites following daily oral administration of GW786034B for 7 days in male CD-1 mice	CD2007/00494/01	4.2.2.2
Pharmacokinetic of GW786034 and its metabolites following daily oral administration of GW786034B for 7 days in male Sprague Dawley rats	CD2007/00493/01	4.2.2.2
Plasma exposure to GW786034 and metabolites following 7-day oral administration to male and female cynomolgus monkeys (pharmacokinetic support for in-life contract study no. CMS83603A)	CD2006/01538/01	4.2.2.2

Distribution:

Title	Study No.	Module
Quantitative Tissue Distribution of Drug-Related Material Using Whole Body Autoradiography Following a Single Oral Dose of [14C]GW786034B (10 mg/kg) to Male Long-Evans Rats	CD2004/00153/01	4.2.2.3
Quantitative Tissue Distribution of Drug-Related Material Using Whole-Body Autoradiography Following a Single Oral Dose of [14C]GW786034 (10 mg/kg) to Female Long-Evans Rats	CD2007/00356/00	4.2.2.3
Interaction of GW786034B and GW771806A with human serum albumin	RH2002/00074/01	4.2.2.3
Preliminary *in vitro* protein binding of GW786034B in mouse, rat, dog, monkey and human plasma	RD2002/00877/00	4.2.2.3
Assessment of GW786034B protein binding in mouse, rat, dog, monkey and human plasma by equilibrium dialysis *in vitro*	CD2004/00451/00	4.2.2.3
An *in vitro* investigation into the inhibition by GW786034B of xenobiotic transport via human OATPIBI heterologously expressed in CHO cells	CD2006/00629/00	4.2.2.3

Metabolism:

Title	Study No.	Module
A preliminary *in vitro* investigation into the oxidative metabolism of GW786034	CD2004/00901/00	4.2.2.4
Preliminary identification of *in vitro* and *in vivo* metabolites of GW786034	RD2002/000874/00	4.2.2.4
An *in vitro* investigation into the inhibition of human UDP-glucurononsyltransferase enzyme UGT1A1 by GW786034	CD2007/00811/00	4.2.2.4
An *in vitro* investigation into the inhibition of cytrochrome P450 enzymes by GW786034	CD2003/00864/00	4.2.2.4
An *in vitro* investigation of the hepatic metabolism of [^{14}C]GW786034 in the mouse, rat, female rabbit, dog, monkey and man	CD2003/00965/00	4.2.2.4
Investigation of GW786034 metabolites following a preliminary toxicity study by oral gavage administration to CD-1 mice for 13 weeks	CD2005/00865/00	4.2.2.4
Quantification of the metabolites of GW786034 in the Sprague-Dawley rat following a single oral administration of [^{14}C]GW786034 at 10 mg/kg	CD2003/00860/00	4.2.2.4
Metabolism of GW786034 following a single oral administration of [^{14}C]GW786034 to male and female intact and bile duct-cannulated cynomolgus monkeys	CD2004/00028/00	4.2.2.4
Preliminary characterization of metabolites of GW786034 in human plasma and urine following oral administration in adults with solid tumors	CD2005/01355/00	4.2.2.4

Excretion:

Title	Study No.	Module
Elimination of radioactivity following a single oral (10 mg free base/kg) administration of [^{14}C]GW786034 (solution dose) to male and female Intact and Bile Duct-Cannulated Rats	CD2002/00088/00	4.2.2.5
Elimination of radioactivity following a single oral (5 mg/kg) administration of [^{14}C]GW786034 to male and female Intact and Bile Duct-Cannulated Cynomolgus Monkeys	CD2002/00108/00	4.2.2.5

Toxicology:

Single dose:

Title	Study No.	Module
GW786034B: Single dose intravenous injection toxicity study in rats	RD2006/00221/00	4.2.3.1
GW786034B: Single dose investigative toxicity study in beagle dogs	RD2001/01637/00	4.2.3.1

Repeat dose:

Title	Study No.	Module
GW786034B toxicity study by oral gavage administration to CD-1 mice for 13 weeks	WD2005/00481/00	4.2.3.2
GW786034B: 28 Day Oral Investigative Toxicity Study in Rats	RD2002/00721/01	4.2.3.2
GW786034B: 13-Week Oral Investigative Study in the Male Sprague Dawley Rat	VD2006/00544/00	4.2.3.2
GW786034B: 26-Week Oral Gavage Dose Toxicity Study in Rats	RD2002/01337/01	4.2.3.2
GW786034B: 52-Week Oral Gavage Dose Toxicity Study in Cynomolgus Monkeys	RD2002/01338/02	4.2.3.2

Genotoxicity

Title	Study No.	Module
Bacteria mutagenicity report (12 to 800 µg/plate)	RD2001/01168/00	4.2.3.3
Bacteria mutagenicity report (100 to 5000 µg/plate)	RD2002/00279/00	4.2.3.3
Bacteria mutagenicity report (100 to 5000 µg/plate)	RD2002/00280/00	4.2.3.3
Bacteria mutagenicity report (33.3 to 5000 µg/plate)	RD2002/00887/00	4.2.3.3
GW786034B: *in vitro* assay for chromosomal aberrations in cultured human peripheral blood lymphocytes (5.05 to 200 µg/mL)	RD2002/00238/00	4.2.3.3
GW786034B: micronucleus frequencies in bone marrow polychromatic erythrocytes form male Sprague Dawley rats following oral administration	RD2002/00227/00	4.2.3.3

Reproductive toxicology

Fetal embryo development:

Title	Study No.	Module
GW786034B: oral female fertility and early embryonic development study in rats	CD2004/00559/00 G03407	4.2.3.5.1
GW786034B: oral male fertility study in rats	CD2004/00940/00 G03372	4.2.3.5.1

Embryo fetal development:

Title	Study No.	Module
GW786034B: oral dose range study in non-pregnant rats	CD2004/00029/00 D03311	4.2.3.5.2
GW786034B: oral dose range finding embryo/fetal development study in rats	CD2004/00030/01 (b) (4) -472019	4.2.3.5.2
GW786034B: oral embryo-fetal development dose range study in rabbits	CD2004/00268/00 D030409	4.2.3.5.2
GW786034B: oral embryo-fetal development study in rabbits	CD2004/00399/00 G04040	4.2.3.5.2

Studies <u>previously</u> reviewed within IND 65, 747:

Toxicology

Repeat dose:

GW786034A: Nonaudited 4-day oral toxicity study in female Sprague Dawley rats	RD2001/01061/00 R40961	4.2.3.2
GW786034B: 1 month oral toxicity and reversibility study in rats	CD2002/00063/00 G01200	4.2.3.2
GW786034B: 1 month oral toxicity in cynomolgus monkeys	CD2002/00103/00 G02013	4.2.3.2

Studies <u>not</u> reviewed within this submission:

Pharmacology

Primary Pharmacodynamics:

Title	Study No.	Module

(b) (4)

Title	Study No.	Module

(b) (4)

Secondary Pharmacodynamics:

Title	Study No.	Module

(b) (4)

Pharmacokinetics

Analytical method validation:

Title	Study No.	Module
(b) (4)		

Absorption:

Title	Study No.	Module
(b) (4)		

Distribution:

Title	Study No.	Module

(b) (4)

Metabolism:

Title	Study No.	Module
(b) (4)		

Other PK studies:

Title	Study No.	Module
(b) (4)		

Toxicology

Repeat dose:

Title	Study No.	Module
(b) (4)		

Local tolerance studies:

Title	Study No.	Module
(b) (4)		

Impurity studies:

Title	Study No.	Module
(b) (4) Reverse mutation assay Ames test using *Samonella Typhimurium* single experiment	CD2005/01104/00	4.2.3.7.6
(b) (4): Reverse mutation assay Ames test using *Samonella Typhimurium* single experiment	CD2005/01159/00	4.2.3.7.6
(b) (4) Screening L5178Y TK+/- mutation assay	CD2005/01105/00	4.2.3.7.6
(b) (4): Screening L5178Y TK+/- mutation assay re-issue report	CD2005/01160/01	4.2.3.7.6
(b) (4) Micronucleus test in the mouse	CD2006/00012/00	4.2.3.7.6
(b) (4): Micronucleus test in the mouse	CD2006/01475/00	4.2.3.7.6
GW786034B: Investigative effect of (b) (4) (GW786034B synthesis intermediates) on L5178Y mouse lymphoma cell cycle	WD2007/00312/00	4.2.3.7.6
In vitro micronucleus test to assess the aneugenic potential of (b) (4) using L5178Y mouse lymphoma cells (screening study)	WD2008/00798/00	4.2.3.7.6

Other toxicology studies:

Title	Study No.	Module
GW786034B: Evaluation of *in vitro* phototoxicity on Balb/c 3T3 fibroblasts using the Neutral Red uptake assay	WD2005/00242/00	4.2.3.7.7
GW786034B: *In vitro* hemolysis testing in rat and monkey blood	CD2006/00192/00	4.2.3.7.7
GW786034B: Study of the potential for *in vitro* hemolysis and plasma protein flocculation in human blood	RD2006/00375/00	4.2.3.7.7

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Pazopanib (also known as GW786034) is an orally bioavailable, adenosine triphosphate-competitive multi-tyrosine kinase inhibitor of vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, VEGFR-3, platelet-derived growth factor receptor (PDGFR)-α and -β, fibroblast growth factor receptor (FGFR) -1 and -3, cytokine receptor (Kit), interleukin-2 receptor inducible T-cell kinase (Itk), leukocyte-specific protein tyrosine kinase (Lck), transmembrane glycoprotein receptor tyrosine kinase (c-Fms), and mitogen-activated protein kinase (P38). A range of *in vitro* and *in vivo* studies have been conducted to investigate the primary pharmacology and drug-drug interactions of pazopanib.

The pharmacology studies discussed below were conducted using the monohydrochloride salt of pazopanib or referred to as GW786034B throughout this section. (b) (4) . Since the monohydrochloride salt (GW786034B) is the one intended for clinical use, studies using this formulation were primarily reviewed with a few exceptions. Study No. RR2002/00010/01 used a synthetic intermediate of the GW786034 or GW786034X while Study No's RH2002/00043/00 and RH2002/00048/00 used dihydrochloride salt of pazopanib (GW786034A) alone and/or in combination with the mono-hydrochloride salt of pazopanib.

The table below is excerpted from the sponsor and includes the exposure of patients given a daily dose of pazopanib (the monohydrochloride salt or GW786034B was used in pharmacology studies) at the proposed treatment dose. For *in vitro* studies, IC_{50} for enzymes or targets were calculated using the AUC value after repeated doses (AUC value = 1040 μg*h/mL).

Summary of Derived Pharmacokinetic Parameters for Pazopanib and Metabolites After a Single Dose in Study VEG10005 and After 16 Days of 800 mg Pazopanib Administered Once Daily in Study VEG10007

Analyte	**AUC(0-t)**		**Cmax**	
	(μg*h/mL)1		**(μg/mL)1**	
	Single Dose	Repeated Dose**,2**	Single Dose	Repeated Dose
	(VEG10005)	(VEG10007)	(VEG10005)	(VEG10007)
Pazopanib	669 (526.3, 851.4)	1,040 (879, 1,230)	20.4 (16.0, 25.9)	58.1 (49.5, 68.3)

*Exceprted from sponsor

In vitro, GW786034B was shown to be a selective inhibitor of vascular endothelial growth factor receptors (VEGFR). GW786034B inhibited substrate phosphorylation catalysed by human VEGFR-1, VEGFR-2 and VEGFR-3 (IC_{50} values of 10, 30 and 47 nM, respectively) and mouse, rat and dog VEGFR-2 (IC_{50} values of 42, 17 and 17 nM, respectively). When evaluated against a wide range of kinases which included receptor tyrosine kinases, cytoplasmic tyrosine kinases, proline-directed kinases, and serine/threonine directed kinases, GW786034B was sensitive to receptor tyrosine kinase

(c-Fms), cytoplasmic tyrosine kinases (ITK and Lck), and serine/threonine directed kinases (P38) with IC_{50} values of 146, 430, 411, and 105.6 nM, respectively.

In a study to examine the inhibitory effects of GW786034B against a number of kinases *in vitro*, GW786034B inhibited PDGFR-α, PDGFR-β and Kit (IC_{50} values of 71, 84 and 74 nM, respectively). GW786034B also inhibited FGFR-1 and 3, however, to a lesser extent with IC_{50} values of 140 and 130 nM, respectively.

In a comparison study with sunitinib and sorafenib using apparent inhibition constant (Kiapp) values, GW786034B inhibited the activity of VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β and c-Kit (Kiapp values for GW786034B = 15, 8, 10, 30, 14, and 2.4 nM, respectively) compared to sunitinib but not sorafenib. GW786034B has weaker affinity for Flt-3 compared to both sunitinib and sorafenib (Kiapp values for GW786034B = 230, 0.6, and 22 nM, respectively). GW786034B and sunitinib tended to have less activity for Raf enzymes which included: B-Raf wildtype, B-Raf V600E mutant, and C-Raf (Kiapp values for GW786034B = 160, 68, and 109 nM, respectively). Sorafenib, however, had a greater affinity for Raf enzymes with Kiapp value of 1.9 nM.

In a cell proliferation assay with a variety of cell lines including the human umbilical vein endothelial cells (HUVEC) alone and also stimulated with basic fibroblast growth factor (bFGF), normal human foreskin fibroblasts (HFF), human tumor cells lines HT29 (colon), MDA-MB-468 (breast), PC3 (prostate) and A375P (melanoma), GW786034B inhibited the proliferation of HUVEC stimulated with VEGF (IC_{50} = 21 nM) compared to HUVEC stimulated with bFGF (IC_{50} = 721 nM). In addition, GW786034B was 48-fold selective for VEGF-induced HUVEC proliferation compared to normal human foreskin fibroblasts (HFF) proliferation (IC_{50} = 1012 nM).

In a panel of 282 human cell lines, GW786034B showed modest activity against proliferation with a small number of cells having IC_{50} values $\leq$ 1 nM. These included cells lines obtained from AML, myeloma, colon carcinoma, kidney leiomyoblastoma, thyroid carcinoma, rhabdomyosarcoma, CML, lymphoma cutaneous T-cell, and stomach carcinoma. From these results, GW786034B was a weak or inactive inhibitor of proliferation in a majority of human cell lines tested.

The cellular activity of GW786034B against VEGFR-2, c-Kit, PDGFR-β, and Flt-3 receptors was evaluated in ligand-induced receptor autophosphorylation assay using human umbilical vein endothelial cells (HUVEC), NCI-H526 (human small cell lung carcinoma), human foreskin fibroblast (HFF) and RS4;11 cells (human B-cell acute lymphoblastic leukemia). Sunitinib and sorafenib were also evaluated in these assays to compare their activity with that of pazopanib. GW786034B inhibited autophosphorylation of VEGFR-2, c-Kit and PDGFR-β receptors (estimated IC_{50} values of 8, 3, and 3 nM, respectively). GW786034B was less active at blocking Flt-3 receptor activation (IC_{50} = $\geq$1000 nM) compared to sunitinib and sorafenib (IC_{50}= 1nM for both compounds). All three drugs (sunitinib, sorafenib, and pazopanib) inhibited VEGFR-2, PDGFR-β and c-Kit with no significant difference in terms of IC_{50} values (IC_{50} each drug: VEGFR-2 = 8-10 nM, IC50 values for PDGFR-β = 2-7 nM, and IC_{50} values for c-Kit = 3 nM, respectively).

When evaluated in imatinib-resistant c-Kit mutants which included V654A, T670I, Y816V, Y823D, K642E (imatinib-sensitive mutant), and wild type c-Kit in transiently-transfected CHO-K1 cells, GW786034B and imatinib inhibited wild-type c-Kit activation (IC_{50} values of 3 nM and 230 nM, respectively). GW786034B was moderately active against T670I, the gatekeeper mutation (IC_{50} of 310 nM) while imatinib was inactive (IC_{50} = ≥10000 nM). Both GW786034B and imatinib had weak activity against the V654A mutant (IC_{50} = 1300 to 1450 nM) and were inactive against the D816V and Y823D mutations.

In vivo, pre-treatment of GW786034B in mice lungs stimulated with exogenous VEGF inhibited VEGF-induced phosphorylation in a time and dose-dependent manner. An oral dose of 30 mg/kg of GW786034B inhibited phosphorylation at 8, 16, and 24 hours post-dosing. GW786034B also inhibited VEGFR2 phosphorylation at doses of 10, 30, and 100 mg/kg with no significant inhibition the low-dose of 3 mg/kg.

Inhibition of tumor growth was slightly lower with GW786034B (the monhydrochloride salt of pazopanib) compared to GW786034A (the dihydrochloride salt of pazopanib) when given at orally at doses 10, 30, and 100 mg/kg either once (qd) or twice a day (bid) in colon, head and neck, prostate and melanoma tumor xengraft models.

When renal cell carcinoma tumor xenografts (CAKI-2 and A498) were injected in different mouse models, GW786034B showed a dose-dependent inhibition of tumors in CB-17 SCID mice (90%, 77%, and 99% at 10, 30, and 100 mg/kg, respectively) compared to Swiss nude mice. However, a modest inhibition of tumor growth (38%, 28%, and 46% at 10, 30, and 100 mg/kg, respectively) was observed when Swiss nude mice were treated with the ACHN renal cell xenograft.

GW786034B inhibited tumors to a lesser extent in colon carcinoma tumor xenografts Colo-205 (33% and 70% at 30 and 100 mg/kg bid, respectively) and HT-29 (45% and 79% at 30 and 100 mg/kg bid, respectively) compared to sorafenib (Colo-205: 40%, >100%, and >100% at 20, 40, and 80 mg/kg qd, respectively and HT-29: 78%, 81%, and 94% at 20, 40, and 80 mg/kg qd, respectively) when given orally for 21 days in female CD-1 nude mice.

GW786034B had no effect at inhibiting prostate tumors in the transgenic prostate cancer mouse model, CR2-T-Ag, when given orally at doses of 10, 30, or 100 mg/kg/day.

GW786034A, the dihydrochloride of pazapanib, showed a greater inhibition of tumors (qd/bid: 57/58% at 30 mg/kg and 83/86% at 100 mg/kg) compared to GW786034B, the monohydrochloride salt of pazaopanib (qd/bid: 24/9% at 3 mg/kg, 26/31% at 10 mg/kg, 45/42% at 30 mg/kg and 56/59% at 100 mg/kg) in the basic fibroblast growth factor (bFGF)/Matrigel model of angiogenesis at doses of ≥30 mg/kg. Although the inhibitory activity of GW786034B was lower, significant tumor inhibition was observed whether the drug was administered qd or bid at doses ≥30 mg/kg.

A pharmacodynamic drug interaction study in tumor bearing mice (CD-1 nude) with GW786034B and bevacizumab showed that clinical administration of anti-angiogenic

agents in combination with GW786034B is unlikely to affect the delivery of chemotherapeutic agents to the tumor tissue.

2.6.2.4 Primary pharmacodynamics

Mechanism of Action:
RR2002/00010/01: GW786034 is a potent and selective inhibitor of vascular endothelial growth factor

This study examined the effects of both the mono-hydrochloride salt (GW786034B) and free-base (GW786034X) of pazapanib to inhibit vascular endothelial growth factor (VEGF) 1, 2, and 3 as well as a variety of other protein kinases in cell free assays. Human VEGFR1, VEGFR2, and VEGFR 3, and VEGFR2 from mouse, rat and dog, and twenty-three enzymes that represent a variety of kinase enzyme classes were used in this study. The kinase enzymes included the following: receptor tyrosine kinases (rbB-4, ErbB-2, EGFR, PDGFR1B, c-Fms, and Tie-2); cytoplasmic tyrosine kinases (Src, ITK, and Lck); proline-directed kinases (CDK-1/Cyclin A, CDK-2/Cyclin A, JNK1, JNK2 and TrJNK3); and serine/threonine directed kinases (GSK3, P38, AKT3, zPKC, PLK1 and PLK3).

GW786034X was tested at a single concentration of 12 μM and the percent inhibition relative to the uninhibited reaction was determined for AKT3. GW786034X did not inhibit AKT3 when compared to controls. The average inhibition was 11.6% (Table 1). Based on this study, it appears that the IC_{50} of GW786034X for AKT3 is >10 μM.

Table 1 Single concentration analysis of the effect of GW786034X on peptide substrate phosphorylation catalyzed by AKT3

Enzyme	GW786034X Concentration (μM)	Average % Inhibition	Notebook References
AKT3	12	11 ± 6 (n = 2)	U19100/121

[Table excerpted from sponsor]

Table 2 shows a comparison of GW786034B and GW786034X against human VEGFR1, 2, and 3. GW786034B was shown to be an inhibitor of human VEGFR1, VEGFR2 and VEGFR3 with IC_{50} values of 10 nM, 30 nM and 47 nM, respectively.

Table 2 Dose response analysis of the effect of GW786034 salt forms on peptide substrate phosphorylation catalyzed by human VEGFR2, VEGFR1 and VEGFR3

Enzyme	Ave. IC_{50} (µM) GW786034B	Ave. IC_{50} (µM) GW786034X	Notebook References
VEGFR2	0.030 ± 0.013 (n = 3)	0.0275 (n = 1)	U19079/97;U19195/40; U20515/1; U19195/26
VEGFR1	0.010 ± 0.004 (n = 3)	ND [1]	U19932/101
VEGFR3	0.0470 ± 0.0061 (n = 2)	0.0490 (n = 1)	U19001/151; U19195/26; U19195/40;

1. ND means Not Determined

[Table excerpted from sponsor]

Similar to what was seen in human VEGFRs, GW786034B was shown to be an inhibitor of mouse, rat and dog VEGFR2 with IC_{50} values of 10 nM, 30 nM and 47 nM, respectively (Table 3).

Table 3 Dose response analysis of the effect of GW786034B on peptide substrate phosphorylation catalyzed by dog, mouse and rat VEGFR2

Enzyme	Species	Ave. IC_{50} (µM) GW786034B	Notebook References
VEGFR2	Dog	0.017 ± 0.006 (n = 2)	U19932/120
VEGFR2	Mouse	0.042 ± 0.009 (n = 2)	U19932/120
VEGFR2	Rat	0.017 ± 0.003 (n = 2)	U19932/120

[Table excerpted from sponsor]

In addition to the above study, twenty-three additional enzymes were evaluated. Theses enzymes represent a wide variety of kinase enzyme classes. These enzyme classes consisted of the following: receptor tyrosine kinases, non-receptor tyrosine kinases, and serine/threonine directed kinases. From the enzyme classes tested, c-Fms, PDGFR1B, murine Lck and ITK were shown to be the most sensitive to GW786034B with an IC_{50} value of 146 nM, 195 nM, 411 nM and 430 nM, respectively. ALK6, JNK1, P38, Tie-2, Src, and TrJNK3 have IC_{50} values in the range of 1 to 5 µM. Finally, IC_{50} values for CDK1/CyclinA, CDK2/CyclinA, c-RAF/MEK/ERK, ErbB-2, ErbB-4, JNK2, PLK1, PLK3, and zPKC are >10 µM (Table 4).

The selectivity profile GW786034X, parallels that of GW786034B but the data is not as complete. GW786034X inhibits the tyrosine kinase activity of human VEGFR2 and VEGFR3 with IC_{50} values of 47 nM and 49 nM, respectively. GW786034X IC_{50} values for c-Fms, ITK, murine Lck, and Src range from 0.230 to 0.800 µM. GSK3, JNK1, P38, Tie-2 and TrJNK3 have IC_{50} values ranging from 1 to 6.3 µM. Finally, the GW786034X IC_{50} values for CDK2/CyclinA, EGFR, EPHB4, ErbB-2, ErbB-4, and PLK1 are >20 µM. GW786034X therefore has 4 to 400-fold selectivity for the VEGF receptor kinases when compared to the other kinases tested (Table 4).

Table 4 Dose response analysis of the effect of GW786034 salts on peptide substrate phosphorylation catalyzed by various protein kinases or on fluorescent ligand binding to various protein kinases

Enzyme	Ave. GW786034B IC_{50} (µM)	Ave. GW786034X IC_{50} (µM)	Notebook References
ALK6	4.266 (n = 1)	ND [1]	U20484/99
CDK-1/Cyclin A	> 20 (n = 1)	ND [1]	U19971/160
CDK-2/Cyclin A	> 20 (n = 2)	> 20 (n = 3)	U18154/167; U18437/9
c-Fms	0.146 ± 0.113 (n = 3)	0.239 ± 0.088 (n = 3)	U17966/100; U17966/136; U20186/137; U17966/134
c-RAF/MEK/ERK	14.454 (n = 1)	ND [1]	U20206/188
EGFR	ND [1]	> 20 (n = 3)	U17965/106; U18104/22; U18104/25
EPHB4	ND [1]	> 20 (n = 2)	U19349/125
ErbB-2	> 20 (n = 2)	> 20 (n = 3)	U17965/106; U18104/22; U18104/25; U18104/27
ErbB-4	> 20 (n = 2)	> 20 (n = 3)	U17965/106; U18104/22; U18104/25, U18104/27
GSK3	ND [1]	3.462 ± 1.147 (n = 3)	U18606/41; U18607/30
ITK	0.430 ± 0.061 (n = 2)	0.699 ± 0.233 (n = 2)	R7097/68
JNK1	2.466 + 0.078 (n = 2)	4.110 + 2.059 (n = 3)	R7097/75
JNK2	10.233 (n = 1)	ND [1]	U20055/21
Lck (murine)	0.411 ± 0.020 (n = 2)	0.594 ± 0.187 (n = 2)	R7097/71

[Table excerpted from sponsor]

Enzyme	Ave. GW786034B IC_{50} (µM)	Ave. GW786034X IC_{50} (µM)	Notebook References
P38	1.056 ± 0.069 (n = 2)	1.240 ± 0.250 (n = 2)	R7097/81
PDGFR1B	0.195 (n = 1)	ND [1]	U20319/76
PLK1	> 20 (n = 2)	> 20 (n = 3)	U17400/185; U19005/10; U19005/8
PLK3	> 20 (n = 1)	ND [1]	U20929/30
Src	3.09 (n = 1)	0.795 ± 0.127 (n = 3)	U18606/44; U18607/32; U20484/123
Tie-2	4.52 + 4.841 (n = 2)	1.10 (1)	U19387/151; U20929/38; U19387/1
TrJNK3	4.065 ± 1.556 (n = 2)	6.250 ± 1.278 (n = 3)	R7097/78
Z-PKC	> 20 (n = 1)	ND [1]	U20236/22

1. ND means Not Determined

[Table excerpted from sponsor]

Results of this study indicate that of all the kinases tested, c-Fms, PDGFR1B, murine Lck and ITK show the most sensitivity to GW786034B. These kinases are 3 to 9-fold less sensitive to GW786034B inhibition compared to other VEGF receptors. The remaining enzymes are even less sensitive to inhibition by GW786034B with IC_{50} values ranging from 1 µM to greater than 20 µM. Therefore, GW786034B has more activity (3 to 400-fold) at VEGFR2 than for the protein kinases discussed above.

RH2003/00076/00: IC_{50} profiling of 4 compounds (GW771806A, GW786034B, GW654652X, and GW695612A) using 16 protein kinases

This study examined the inhibitory effects in terms of IC_{50} of 4 different compounds of pazopanib including the mono-hydrochloride salt or GW786034B and intermediates against 16 different protein kinases. The compounds that were tested included the following: GW771806A, GW786034B, GW654652X, and GW695612A. The kinases used were as follows: ABL1, MET, PDGFR-α, PDGFR-β, IGF2-R, KIT, INS-R, WEE1, PKC- β1, PKC- β2, FAK, FGF-R4, FGF-R1, and FGF-R3. VEGFR-1 and VEGFR-2 were included for comparison purposes.

The table below shows the IC_{50} of 4 compounds using a proprietary protein kinase assay. The inhibitory profile of the compounds tested ranged from $1.2x10^{-8}$ M to > $1x10^{-8}$ M.

The table also shows that GW786034B inhibited PDGFR-α, PDGFR-β, and c-Kit. GW786034B partially precipitated in DMSO at the 3 highest concentrations tested. Therefore, the IC_{50} values could not be calculated and were estimated for kinases: ABL1, MET, IGF1-R, INS-R, FAD and FGF-R4.

	GW 771806A	GW 786034B	GW 654652X	GW 695612A
	Compound 1	Compound 2	Compound 3	Compound 4
VEGF-R1	0.022	0.013	0.17	0.1
VEGF-R2	0.021	0.012	0.02	0.022
PDGFR-α	0.16	0.071	0.56	0.074
PDGFR-β	0.47	0.084	0.61	0.057
KIT	0.68	0.08	0.44	0.018
ABL1	9.3	2*	0.48	0.28
FGF-R1	0.28	0.14	0.3	0.17
FGF-R3	0.49	0.13	0.32	0.15
FGF-R4	3.6	0.8*	4.8	0.75
IGF1-R	9.5	8*	>100	22
INS-R	24	20*	>100	15
MET	8.9	6*	>100	10
FAK	2.9	0.8*	7.5	7.6
WEE1	61	100	>100	31
PKC-β1	100	>100	>100	>100

*Estimated IC_{50}

Table A: Enzyme IC_{50} (μM) of 4 compounds against various kinases

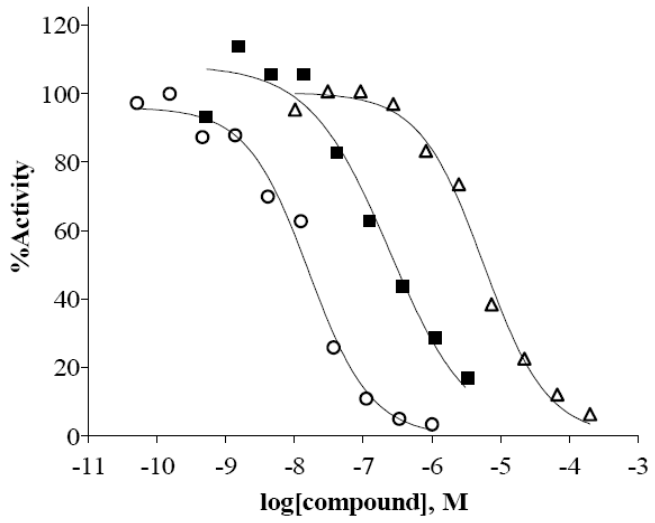
[Table excerpted from sponsor]

UH2008/00035/00: GW786034B (pazopanib) biochemical kinase inhibitory activity and differentiation studies

This purpose of this study was to further characterize the inhibitory activity and selectivity of GW786034B against a number of human protein kinases (VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α/β, Flt-3, c-Kit, B-Raf V600E mutant, B-Raf wild type and C-Raf) by using the apparent inhibition constant (K_{iapp}) value. Two competitor molecules, sunitinib (GSK280667A) and sorafenib (SB-706991) were included for comparison purposes. The *in vitro* Kiapp values were determined for pazopanib (GW786034B), sunitinib (GSK280667A) and sorafenib (SB-706911) against purified VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β, Flt-3 and c-Kit receptor tyrosine kinases using either ^{33}P ATP filter binding or HTRF assays. The *in vitro* Kiapp against purified serine/threonine kinase Raf enzymes was determined using the B-Raf/C-Raf Activated MEK ATPase (BRAMA/CRAMA) biochemical activity assays.

Figure 1 and Table 1 show that sorafenib is the most active at inhibiting B-Raf V600 kinase with a B-Raf V600 Kiapp value of 6.1 nM. GW786034B is less active with a B-Raf V600 Kiapp value of 160 nM. Sutitinib is the least active with a B-Raf V600 Kiapp value of 3000 nM. All three compounds do not show significant selectivity between Raf enzymes.

Figure 1 **Inhibition of Purified B-Raf V600E Kinase Activity by Pazopanib (Closed Squares), Sunitinib (Open Triangles), and Sorafenib (Open Circles).**



[Figure excerpted from sponsor]

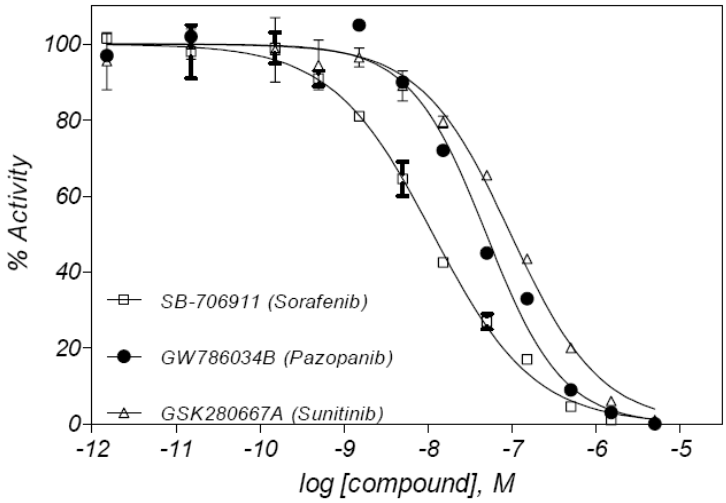
Table 1 **Summary of Inhibition (K_i^{app}) of Purified Raf Enzymes by Pazopanib, Sunitinib, and Sorafenib.**

Compound	K_i^{app} (nM)			Number of replicates
	B-Raf V600E	B-Raf WT	C-Raf	
Pazopanib	160 ± 30	68 ± 6	109 ± 9	3
Sunitinib	3000 ± 300	470 ± 50	2000 ± 200	3
Sorafenib	6.1 ± 0.8	1.97 ± 0.08	1.9 ± 0.1	2

[Table excerpted from sponsor]

As shown in Figure 2 and Table 3, GW786034B has a high inhibitory activity for VEGFR-2 with a K_{iapp} value of 8 nM. GW786034B also showed similar affinity at VEGFR-1, VEGFR-3, PDGFR-α, PDGFR-β and c-Kit (Figure 3) with K_{iapp} values of 15, 10, 30, 14 and 2.4 nM, respectively. GW786034B, however, possessed a weak affinity for Flt-3 with a mean K_i value of 230 nM which is 25-fold weaker compared to VEGFR-2. In contrast, sunitinib possessed 85-fold and 100-fold greater affinity for Flt-3 and c-Kit compared to VEGFR-2 with $K_{i\ app}$ values of 0.6, 0.45 and 51 nM respectively. Sorafenib showed the least selectivity among these tyrosine kinase receptors with affinity values ranging from 2-22 nM.

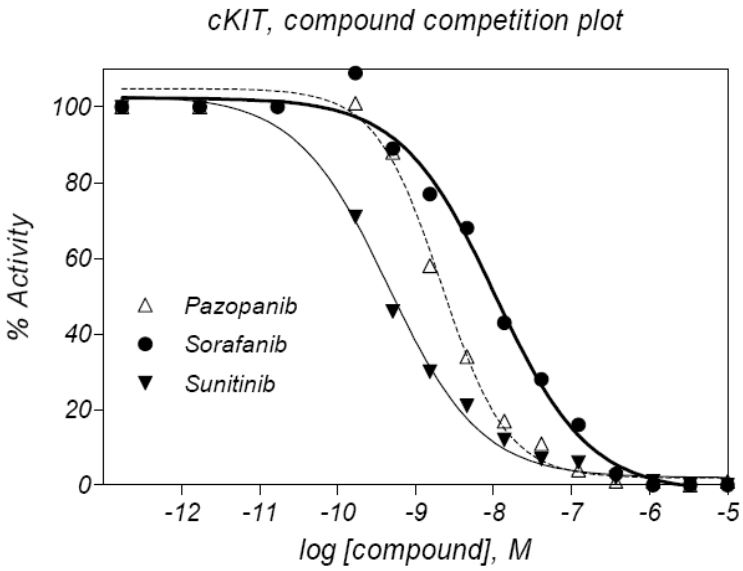
Figure 2 **Inhibition of VEGFR-2 Kinase Activity by Pazopanib, Sunitinib and Sorafenib.**



These VEGFR-2 kinase data are representative of data obtained in subsequent independent experiments (n=2-7), as well as of data obtained with VEGFR-1/3, PDGFR α/β, and Flt-3 truncate enzymes.

[Figure excerpted from sponsor]

Figure 3 **Inhibition of c-Kit Kinase Activity by Pazopanib, Sunitinib and Sorafenib (HTRF).**



These c-Kit truncate data are representative of data obtained in subsequent independent experiments (n=2-4).

[Figure excerpted from sponsor]

Table 3 Summary of Inhibition (K_i^{app}) of Purified c-Kit Kinase Activity by Pazopanib, Sunitinib and Sorafenib.

	Ki^{app} (nM) ± STDEV		
ENZYME	Pazopanib (GW786034B)	Sunitinib (GSK280667A)	Sorafenib (SB-706991)
VEGFR1	15 ± 6	229 ± 76	10 ± 5
VEGFR2	8 ± 3	51 ± 13	4 ± 2
VEGFR3	10 ± 0.6	30 ± 14	6 ± 2
PDGFRα	30 ± 9	28 ± 9	2 ± 1
PDGFRβ	14 ± 8	7 ± 5	5 ± 2
FLT3	230 ± 113	0.6 ± 0.2	22 ± 12
c-KIT	2.4 ± 1†	0.45 ± 0.07†	15 ± 5†

n=2-7

† - HTRF c-Kit assay

[Table excerpted from sponsor]

In summary, GW786034B, sunitinib and sorafenib inhibit VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α, PDGFR-β and c-Kit with sunitinib also inhibiting Flt-3 and sorafenib inhibiting Raf kinases.

Effects on Cellular Proliferation:

RH2002/00042/00: Effects of GW786034 on cellular proliferation and receptor phosphorylatiion assays

The effects of GW786034 (pazaponib) on cellular growth were examined in cultured human umbilical vein endothelial cells or HUVEC (alone and also stimulated with basic fibroblast growth factor or bFGF), normal human foreskin fibroblasts (HFF), and human tumor cells lines HT29 (colon), MDA-MB-468 (breast), PC3 (prostate) and A375P (melanoma). Cell proliferation was assessed by bromodeoxyuridine (BrdU) incorporation using enzyme-linked immunosorbent assay. To confirm the biochemical activity of GW786034, Western blot analysis was performed with HUVEC cells treated with several concentrations of GW786034 followed by VEGF (10 ng/ml) stimulation for 10 minutes.

Table 1 indicates that GW786034 inhibited proliferation in human umbilical vein endothelial cells (HUVEC) stimulated with VEGF and bFGF. The IC_{50} were 0.021 and 0.721 μM, respectively. GW786034 was 48-fold selective for HUVEC cells stimulated with VEGF compared to HFF proliferation. GW786034 did not inhibit proliferation of the four other human tumor cells lines tested (HT-29, PC3, MDA468, A375P).

Table1. Inhibition of Human Cell Lines by GW786034

Concentrations that inhibit growth by 50% (IC_{50}) and 90% (IC_{90}) in the proliferation assay (BrdU incorporation) were interpolated from multiple experiments using Levenberg-Marquardt non-linear regression and the equation, $y = V_{max}*(1-(x/(K+x)))$. Values are presented as mean ± sem from multiple experiments.

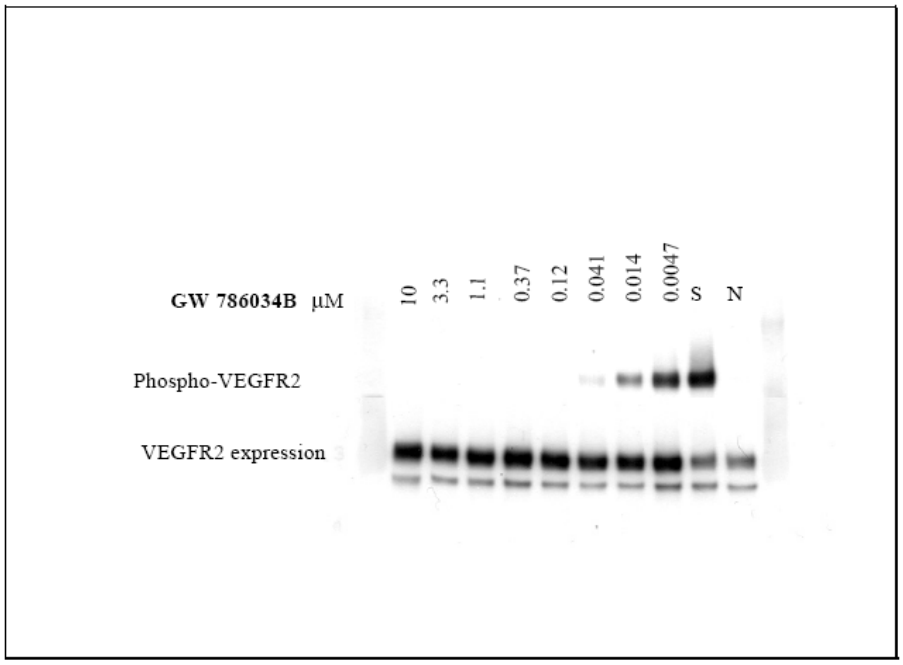
	IC_{50} (µM)	IC_{90} (µM)	Fold Selectivity[$]
HUVEC-v[a]	0.0213 ± 0.0045 (n=15)	0.1559 ± 0.0325 (n=15)	-
HUVEC-b[b]	0.7209 ± 0.2395 (n=11)	5.9812 ± 1.7313 (n=11)	33.84
HFF	1.0123 ± 0.1533 (n=5)	8.6202 ± 1.1564 (n=5)	47.53
HT29	>30 (n=5)	-	>1400
MDA468	>30 (n=5)	-	>1400
PC3	>30 (n=5)	-	>1400
A375P	>30 (n=5)	-	>1400

[a]Human umbilical vein endothelial cells (HUVEC) stimulated with VEGF.
[b]HUVEC stimulated with bFGF.
[$]The selectivity index was estimated by dividing the mean IC_{50} against cell line of interest with the mean IC_{50} against HUVEC-v.

[Table excerpted from sponsor]

Figure 3 shows the results of the Western blot analysis. GW786034 inhibited tyrosine phosphorylation in human umbilical vein endothelial cells (HUVEC) stimulated with VEGFR2.

Figure 3. Effect of GW786034 on Tyrosine Autophosphorylation of VEGFR2 in HUVEC Stimulated with VEGF



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[Figure excerpted from sponsor]

To summarize, GW786034B inhibited proliferation of various cell types was evaluated in an *in vitro* assay for 3 days. Consistent with kinase activity, GW786034B selectively inhibited the proliferation of human umbilical vein endothelial cells (HUVEC) stimulated with VEGF (IC_{50} = 21 nM) compared to bFGF-stimulated proliferation (IC_{50} = 721 nM). Pazopanib was >1400-fold selective for VEGF-induced HUVEC proliferation relative to HT-29, MDA468, PC3, and A375P and 48-fold selective relative to human foreskin fibroblast (HFF) cell line.

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The effect of mono-HCl salt of pazopanib (or GW786034B) was further evaluated in a cell proliferation assay using a panel of 282 human cell lines. Of these cell lines, 281 were tumor cell lines derived from various tissue types while MCF10A came from a non-transformed breast cell line. Cells were incubated in the presence of GW786034B at concentrations ranging from 0.003 to 10 uM for 72 hours. The cell nuclei were stained with 4, 6-diamindino-2-phenylindole which is a fluorescent stain that binds to DNA. Cell proliferation was measured by fluorometric analysis using the fluorescent intensity of cells.

Table 2 shows the IC_{50} from the cells tested. Values ranged from 0.01 to >10 μM. Few cell lines showed IC_{50} <1 μM. These cell lines included the following (values list the IC_{50} high density to low density range): GDM1 AML (IC_{50} 0.094-0.010 μM), ARH-77 myeloma (IC_{50} 0.037-0.13 μM, NCI-H716 colon carcinoma (IC_{50} 0.20-0.18 μM), G402 kidney leiomyoblastoma (IC_{50} 0.19-0.19 μM), CGTH-W-1 thyroid carcinoma (IC_{50} 0.21-0.24 μM), A204 rhabdomyosarcoma, (IC_{50} 0.26-0.27 μM), CML-T1 CML blast phase (IC_{50} 0.61-0.79 μM), HUT78 lymphoma cutaneous T cell (IC_{50} 1.7-0.5 μM), and KATOIII stomach carcinoma (IC_{50} 1.8-0.6 μM).

The IC_{50} values of GW786034B in the cells listed above were 2.4-33 fold higher than the IC_{50} for human umbilical vein endothelial cells (HUVEC) stimulated with VEGF which had an IC_{50}=21 nM (Reference - Study No. RH2002/00042/00).

Based on the data obtained in this study, it appears that GW786034B is a weak or inactive inhibitor of proliferation in the majority of human cell lines tested. .

Table 2 Activity of Pazopanib in Human Cell Panel

			IC_{50} (μM)	
Cell Line	**Tissue**	**Histology**	**High Cell Density**	**Low Cell Density**
NCI-H295R	Adrenal gland	adrenocortical carcinoma	>10	>10
5637	Bladder	carcinoma	>10	>10
639-V	Bladder	carcinoma	>10	>10
647-V	Bladder	carcinoma	>10	>10
BFTC-905	Bladder	carcinoma	6.2	6.4
HT1197	Bladder	carcinoma	>10	9.2
HT1376	Bladder	carcinoma	>10	>10
J82	Bladder	carcinoma	>10	>10
SCaBER	Bladder	squamous cell carcinoma	>10	>10
SW780	Bladder	carcinoma	>10	>10
UM-UC-3	Bladder	carcinoma	>10	>10
BT-20	Breast	carcinoma	>10	>10
BT-474	Breast	carcinoma	>10	>10
DU4475	Breast	carcinoma	>10	6.5
EFM-19	Breast	carcinoma	>10	>10
HCC1143	Breast	carcinoma	>10	>10
HCC1395	Breast	carcinoma	3.7	9.5
HCC1937	Breast	carcinoma	>10	
HCC2218	Breast	carcinoma	>10	
HCC38	Breast	carcinoma	>10	>10
HCC70	Breast	carcinoma	>10	>10
KPL-1	Breast	carcinoma	3.5	4.6
MCF10A	Breast	normal	>10	10
MCF7	Breast	carcinoma	>10	9.8
MDA-MB-175-VII	Breast	carcinoma	>10	
MDA-MB-231	Breast	carcinoma	>10	>10
MDA-MB-435	Breast		9.7	>10
MDA-MB-453	Breast	carcinoma	6.4	4.3
MDA-MB-468	Breast	carcinoma	>10	>10
MT-3	Breast	carcinoma	>10	9.7
MX-1	Breast	carcinoma	>10	>10
NCI/ADR-RES	Breast	carcinoma	>10	>10
SK-BR-3	Breast	carcinoma	>10	>10
T-47D	Breast	carcinoma	7.8	6.3
UACC-812	Breast	carcinoma	>10	>10
ZR-75-1	Breast	unavailable	>10	
C-33A	Cervix	carcinoma	>10	7.6
C-4I	Cervix	carcinoma	>10	>10
C-4II	Cervix	carcinoma	5.6	5.4
DoTc2-4510	Cervix	carcinoma	>10	>10
HeLa	Cervix	carcinoma	8.1	>10
HT-3	Cervix	carcinoma	>10	>10
SiHa	Cervix	carcinoma	>10	>10
SW756	Cervix	carcinoma	>10	>10
A172	CNS	glioma	3.2	3.4
BE2-C	CNS	neuroblastoma	>10	8.0
CCF-STTG1	CNS	glioma	3.0	>10

			IC_{50} (μM)	
Cell Line	**Tissue**	**Histology**	**High Cell Density**	**Low Cell Density**
CHP-212	CNS	neuroblastoma	4.9	5.3
D283Med	CNS	medulloblastoma	>10	>10
DBTRG-05MG	CNS	glioma	>10	>10
DK-MG	CNS	glioma	>10	>10
H4	CNS	glioma	4.1	4.1
MC-IXC	CNS	neuroblastoma	8.3	8.4
SF-268	CNS	glioma	>10	>10
SF-295	CNS	glioma	10	10
SF-539	CNS	glioma	0.89	1.5
SK-N-AS	CNS	neuroblastoma	>10	8.6
SK-N-DZ	CNS	neuroblastoma	>10	>10
SK-N-F1	CNS	neuroblastoma	>10	>10
SNB-19	CNS	glioma	>10	>10
SNB-75	CNS	glioma	>10	>10
SW1088	CNS	glioma	>10	>10
SW1783	CNS	glioma	>10	>10
U-138MG	CNS	glioma	>10	>10
U251	CNS	glioma	>10	9.2
U-87MG	CNS	glioma	>10	>10
COLO201	Colon	carcinoma	>10	>10
COLO205	Colon	carcinoma	6.7	8.0
COLO-320 HSR	Colon	carcinoma	7.7	8.5
COLO-320DM	Colon	carcinoma	>10	>10
DLD-1	Colon	carcinoma	5.7	6.2
HCC2998	Colon	carcinoma	9.3	>10
HCT116	Colon	carcinoma	8.8	6.7
HCT-15	Colon	carcinoma	6.4	4.6
HCT-8	Colon	carcinoma	9.5	7.3
HT-29	Colon	carcinoma	3.6	4.5
KM12	Colon	carcinoma	>10	>10
LS1034	Colon	carcinoma	>10	>10
LS174T	Colon	carcinoma	>10	5.4
NCI-H508	Colon	carcinoma	>10	9.7
NCI-H630	Colon	colorectal carcinoma	>10	>10
NCI-H716	Colon	carcinoma	0.20	0.18
NCI-H747	Colon	carcinoma	>10	>10
RKO	Colon	carcinoma	7.7	5.6
SW1116	Colon	carcinoma	>10	
SW1417	Colon	carcinoma	>10	>10
SW1463	Colon	colorectal carcinoma	5.3	
SW403	Colon	carcinoma	>10	>10
SW48	Colon	carcinoma	>10	>10
SW480	Colon	carcinoma	>10	>10
SW620	Colon	carcinoma	>10	>10
SW837	Colon	carcinoma	>10	>10
SW948	Colon	carcinoma	>10	8.4
T84	Colon	carcinoma	8.1	>10
WIDR	Colon	carcinoma	3.1	3.6
Y79	Eye	retinoblastoma	>10	>10
CAL27	Head and Neck	carcinoma	>10	>10

			IC_{50} (μM)	
Cell Line	**Tissue**	**Histology**	**High Cell Density**	**Low Cell Density**
FaDu	Head and Neck	carcinoma	>10	>10
HN5	Head and Neck	squamous cell carcinoma	>10	6.9
RPMI2650	Head and Neck	carcinoma	7.3	6.3
SCC-12	Head and Neck	squamous cell carcinoma	>10	>10
SCC-13	Head and Neck	squamous cell carcinoma	>10	>10
SCC-15	Head and Neck	carcinoma	>10	>10
SCC-25	Head and Neck	carcinoma	>10	>10
SCC-4	Head and Neck	carcinoma	8.2	>10
SCC-9	Head and Neck	carcinoma	>10	>10
ARH-77	Hematopoietic and lymphoid	myeloma - plasma cell	0.037	0.13
BC-1	Hematopoietic and lymphoid	lymphoma - B cell unspecified	7.7	8.8
BC-2	Hematopoietic and lymphoid	lymphoma - B cell unspecified	7.8	5.3
BC-3	Hematopoietic and lymphoid	lymphoma - B cell unspecified	4.4	4.1
BV-173	Hematopoietic and lymphoid	CML blast phase	5.5	9.8
CEM-C1	Hematopoietic and lymphoid	acute lymphoblastic leukaemia	2.1	3.2
CESS	Hematopoietic and lymphoid	AML	6.2	9.0
CML-T1	Hematopoietic and lymphoid	CML blast phase	0.61	0.79
CRO-AP2	Hematopoietic and lymphoid	lymphoma - B cell unspecified	8.1	3.5
CRO-AP5	Hematopoietic and lymphoid	lymphoma - B cell unspecified	4.5	
DB	Hematopoietic and lymphoid	lymphoma - diffuse large B cell	10	>10
EM-2	Hematopoietic and lymphoid	CML blast phase	8.4	7.3
GDM-1	Hematopoietic and lymphoid	AML	0.094	0.010
HEL-92-1-7	Hematopoietic and lymphoid	erythroleukemia	5.3	6.4
HuT78	Hematopoietic and lymphoid	lymphoma - cutaneous T cell	1.7	0.5
J-RT3-T3-5	Hematopoietic and lymphoid	acute lymphoblastic leukaemia	7.3	4.0
K-562	Hematopoietic and lymphoid	CML	>10	7.2
MC/CAR	Hematopoietic and lymphoid	Myeloma - plasma cell	1.6	3.8
MJ	Hematopoietic and lymphoid	lymphoma - cutaneous T cell	>10	8.1
ML-2	Hematopoietic and lymphoid	AML	4.9	4.5
MOLT-16	Hematopoietic	acute lymphoblastic	2.6	4.2

			IC_{50} (μM)	
Cell Line	**Tissue**	**Histology**	**High Cell Density**	**Low Cell Density**
	and lymphoid	leukaemia		
MV-4-11	Hematopoietic and lymphoid	acute leukaemia of ambiguous lineage	1.3	
Raji	Hematopoietic and lymphoid	lymphoma - Burkitt	7.3	6.2
RL	Hematopoietic and lymphoid	lymphoma - non-Hodgkin	8.3	>10
RPMI6666	Hematopoietic and lymphoid	lymphoma - Hodgkin	4.4	2.8
RPMI8226	Hematopoietic and lymphoid	Myeloma	5.1	5.6
SKO-007	Hematopoietic and lymphoid	Myeloma	>10	
SR	Hematopoietic and lymphoid	lymphoma - large cell immunoblastic	8.4	9.4
ST486	Hematopoietic and lymphoid	lymphoma - Burkitt	>10	8.8
THP-1	Hematopoietic and lymphoid	AML	>10	8.8
TO175.T	Hematopoietic and lymphoid	haematopoietic	>10	
U266B1	Hematopoietic and lymphoid	Myeloma	8.9	
769-P	Kidney	carcinoma	>10	>10
786-0	Kidney	carcinoma	1.9	2.6
A-498	Kidney	carcinoma	>10	>10
ACHN	Kidney	carcinoma	3.3	4.0
Caki-1	Kidney	carcinoma	3.3	3.4
Caki-2	Kidney	choriocarcinoma	>10	>10
CAL-54	Kidney	carcinoma	>10	9.9
G-401	Kidney	Wilms tumour	3.4	2.6
G-402	Kidney	leiomyoblastoma	0.19	0.19
RXF393	Kidney	carcinoma	9.2	5.5
SK-NEP-1	Kidney	Wilms tumour	7.0	
SN12C	Kidney	carcinoma	9.7	9.0
TK-10	Kidney	carcinoma	7.2	6.2
UO-31	Kidney	unavailable	>10	7.6
C3A	Liver	carcinoma	7.8	9.8
Hep3B	Liver	hepatoma	4.0	4.9
HepG2	Liver	carcinoma	8.2	8.1
SNU-182	Liver	carcinoma		>10
SNU-387	Liver	carcinoma	>10	>10
SNU-398	Liver	carcinoma	8.2	7.7
SNU-423	Liver	carcinoma	>10	>10
SNU-449	Liver	carcinoma	>10	>10
SNU-475	Liver	carcinoma	7.3	9.4
A427	Lung	adenocarcinoma	3.2	4.3
A549	Lung	alveoloar basal epithelial-squamous	7.5	6.5
Calu-3	Lung	airway epithelial	>10	>10
EKVX	Lung	adenocarcina	>10	>10

			IC_{50} (μM)	
Cell Line	**Tissue**	**Histology**	**High Cell Density**	**Low Cell Density**
HOP-62	Lung	adenocarcinoma	>10	>10
HOP-92	Lung	NSCLC	>10	>10
MV522	Lung	adenocarcinoma	>10	9.2
NCI-H1299	Lung	NSCLC	>10	>10
NCI-H1395	Lung	carcinoma	>10	>10
NCI-H146	Lung	SCLC	>10	>10
NCI-H157	Lung	NSCLC	>10	>10
NCI-H187	Lung	SCLC	>10	>10
NCI-H2052	Lung	mesothelioma	>10	9.2
NCI-H2087	Lung	carcinoma	>10	7.6
NCI-H209	Lung	SCLC	>10	5.7
NCI-H2122	Lung	adenocarcinoma	4.4	8.3
NCI-H2170	Lung	squamous	9.2	>10
NCI-H2171	Lung	SCLC	>10	>10
NCI-H2195	Lung	SCLC	>10	>10
NCI-H226	Lung	squamous cell carcinoma	>10	>10
NCI-H23	Lung	adenocarcinoma	>10	>10
NCI-H292	Lung	mucoepidermoid	>10	7.8
NCI-H322	Lung	branchio-alveolar	>10	>10
NCI-H358	Lung	branchio-alveolar	7.2	6.0
NCI-H460	Lung	NSCLC	8.1	7.8
NCI-H520	Lung	squamous	4.0	4.5
NCI-H522	Lung	adenocarcinoma	>10	>10
NCI-H526	Lung	SCLC	>10	>10
NCI-H596	Lung	adeonsquamous	>10	>10
NCI-H661	Lung	NSCLC	7.0	5.1
NCI-H69	Lung	SCLC	>10	>10
NCI-H727	Lung	pulmonary carcinoid	>10	>10
NCI-H82	Lung	SCLC	4.1	1.1
SCLC-3	Lung	SCLC	>10	8.2
SHP-77	Lung	SCLC	7.4	>10
A2780	Ovary	carcinoma	>10	>10
CaOv-3	Ovary	carcinoma	>10	>10
COLO-704	Ovary	adenocarcinoma	>10	>10
ES-2	Ovary	carcinoma	8.3	8.5
IGROV1	Ovary	carcinoma	9.8	9.5
OV-90	Ovary	carcinoma	>10	>10
OVCAR-3	Ovary	carcinoma	>10	>10
OVCAR-4	Ovary	carcinoma	6.9	>10
OVCAR-5	Ovary	carcinoma	>10	>10
OVCAR-8	Ovary	carcinoma	>10	>10
SK-OV-3	Ovary	carcinoma	>10	>10
AsPC-1	Pancreas	carcinoma	>10	>10
BxPC-3	Pancreas	carcinoma	>10	
Capan-1	Pancreas	carcinoma	>10	>10
Capan-2	Pancreas	adenocarcinoma	>10	>10
HPAC	Pancreas	adenocarcinoma	>10	
HPAFII	Pancreas	carcinoma	>10	>10
HuP-T4	Pancreas	carcinoma	>10	>10
MIA PaCa-2	Pancreas	carcinoma	>10	>10

			IC_{50} (μM)	
Cell Line	**Tissue**	**Histology**	**High Cell Density**	**Low Cell Density**
SW1990	Pancreas	carcinoma	>10	>10
YAPC	Pancreas	carcinoma	>10	>10
Detroit562	Pharynx	carcinoma		7.4
BeWo	Placenta	choriocarcinoma	>10	>10
JAR	Placenta	choriocarcinoma	>10	8.3
JEG-3	Placenta	choriocarcinoma	>10	>10
22Rv1	Prostate	carcinoma	8.4	8.1
BM1604	Prostate	carcinoma	>10	9.5
BPH1	Prostate	hyperplasia	>10	>10
DU145	Prostate	carcinoma	9.2	7.9
LNCaP	Prostate	carcinoma	2.6	7.1
NCI-H660	Prostate	unavailable	>10	
PC-3	Prostate	carcinoma	>10	>10
RWPE-1	Prostate	carcinoma	>10	
A204	Sarcoma	rhabdomyosarcoma	0.26	0.27
A673	Sarcoma	rhabdomyosarcoma	9.1	>10
GCT	Sarcoma	sarcoma	>10	6.4
HOS	Sarcoma	osteosarcoma	6.2	6.2
HT-1080	Sarcoma	fibrosarcoma	>10	5.1
KHOS-240S	Sarcoma	osteosarcoma	4.8	4.6
MES-SA	Sarcoma	sarcoma	>10	7.0
RD	Sarcoma	osteosarcoma	>10	>10
RD-ES	Sarcoma	sarcoma	>10	8.2
Saos-2	Sarcoma	osteosarcoma	>10	>10
SJRH30	Sarcoma	rhabdomyosarcoma	6.4	9.5
SK-LMS-1	Sarcoma	sarcoma	>10	>10
SK-UT-1	Sarcoma	leiomyosarcoma	>10	>10
SW1353	Sarcoma	sarcoma	>10	>10
SW684	Sarcoma	fibrosarcoma	>10	>10
SW872	Sarcoma	liposarcoma	>10	>10
TE381.T	Sarcoma	rhabdomyosarcoma	5.3	5.2
U-2OS	Sarcoma	osteosarcoma	5.2	>10
A101D	Skin	malignant melanoma	>10	>10
A375	Skin	malignant melanoma	>10	>10
A-431	Skin	epithelial carcinoma	>10	8.0
A7	Skin	unavailable	9.6	9.7
C32TG	Skin	malignant melanoma	>10	>10
CHL-1	Skin	malignant melanoma	6.2	5.9
COLO-829	Skin	malignant melanoma	>10	>10
HMCB	Skin	malignant melanoma	3.5	4.6
LOX IMVI	Skin	melanoma	1.1	6.6
M14	Skin	malignant melanoma	6.9	10
Malme-3M	Skin	malignant melanoma	>10	>10
SH-4	Skin	malignant	5.9	6.2

			IC_{50} (μM)	
Cell Line	**Tissue**	**Histology**	**High Cell Density**	**Low Cell Density**
		melanoma		
SK-MEL-1	Skin	malignant melanoma	8.2	8.0
SK-MEL-28	Skin	malignant melanoma	>10	>10
SK-MEL-3	Skin	malignant melanoma	8.0	6.7
UACC-257	Skin	malignant melanoma	>10	>10
UACC-62	Skin	malignant melanoma	>10	>10
WM115	Skin	malignant melanoma	>10	>10
AGS	Stomach	carcinoma	>10	>10
HS746T	Stomach	carcinoma	>10	>10
KATOIII	Stomach	carcinoma	1.8	0.6
NCI-N87	Stomach	carcinoma	7.8	6.2
SNU-1	Stomach	carcinoma	>10	8.5
SNU-5	Stomach	carcinoma	>10	>10
BHT-101	Thyroid	carcinoma	>10	7.4
CAL-62	Thyroid	carcinoma	>10	>10
CGTH-W-1	Thyroid	carcinoma	0.21	0.24
SW579	Thyroid	carcinoma	1.2	1.0
AN3CA	Uterus	carcinoma	2.7	6.6
HEC-1-A	Uterus	carcinoma	>10	9.0
HEC-1-B	Uterus	carcinoma	>10	>10
KLE	Uterus	carcinoma	>10	>10
RL95-2	Uterus	carcinoma	>10	>10
SW954	Vulva	carcinoma	>10	>10
SW962	Vulva	carcinoma	>10	>10

[Table excerpted from sponsor]

Effects on Receptor Phosphorylation in Cells:

UH2008/00016/00: Cellular activities of pazopanib, sunitinib, and sorafenib against various receptor tyrosine kinases

This study was conducted in three parts.

In the first part of the study, the cellular activity of GW786034B against VEGF-2, c-Kit, PDGFR-β, and Flt-3 receptors were evaluated in a ligand-induced receptor phosphorylation assay using the following cell lines: human umbilical vein endothelial (HUVEC); human small cell lung cancer carcinoma (NCI-H526); human foreskin fibroblast (HFF); human B-cell acute lymphoblastic leukemia (RS4-11) and human actue myelogenous leukemia (MV4-11).

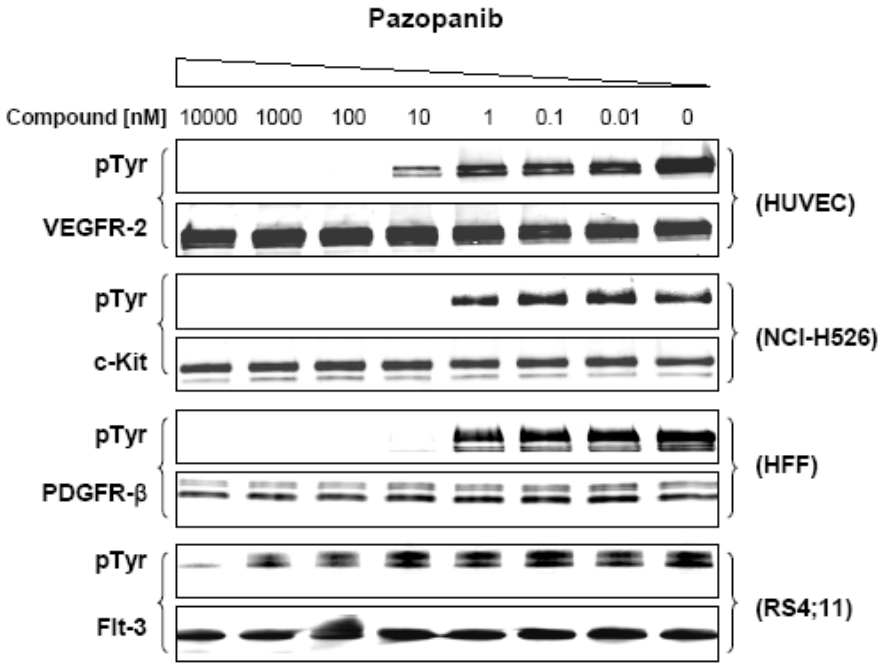
As a comparator, the second part of the study evaluated the cellular activities of competitor compounds, sunitinib (GSK280667A) and sorafenib (SB-706991) using the same cell lines as above.

The study also examined the effects of GW786034B on receptor autophosphorylation, downstream signaling as well as cellular proliferation using RS4-11(human B-cell acute lymphoblastic leukemia) and MV4-11 (human acute myelogenous leukemia) cell lines. This last part of the study further elucidated the impact of Flt-3 inhibition by GW786034B, sunitinib and sorafenib.

Part 1: *Pazopanib Inhibits Cellular Auto-phosphorylation of VEGFR-2, c-Kit and PDGFR- β, but not Flt-3 Receptor*

Western blot analyses showed at physiologically relevant concentrations, GW786034B dose-dependently suppressed ligand-induced activation of VEGFR-2, c-Kit, and PDGFR-β with an estimated IC_{50} of 8, 3 and 3 nM, respectively in HUVEC, NCI-H526 and HFF cells. GW786034B was significantly less active against Flt-3 receptor activation with an $IC_{50} \geq 1$ μM when tested in RS4-11 cells (Figure 1 and Table 1).

Figure 1 Pazopanib Inhibits Receptor Phosphorylation of VEGFR-2, c-Kit, PDGFR-β, and Flt-3



[Figure excerpted from sponsor]

Table 1 Cellular IC_{50} for Inhibition of Ligand-induced Receptor Autophosphorylation

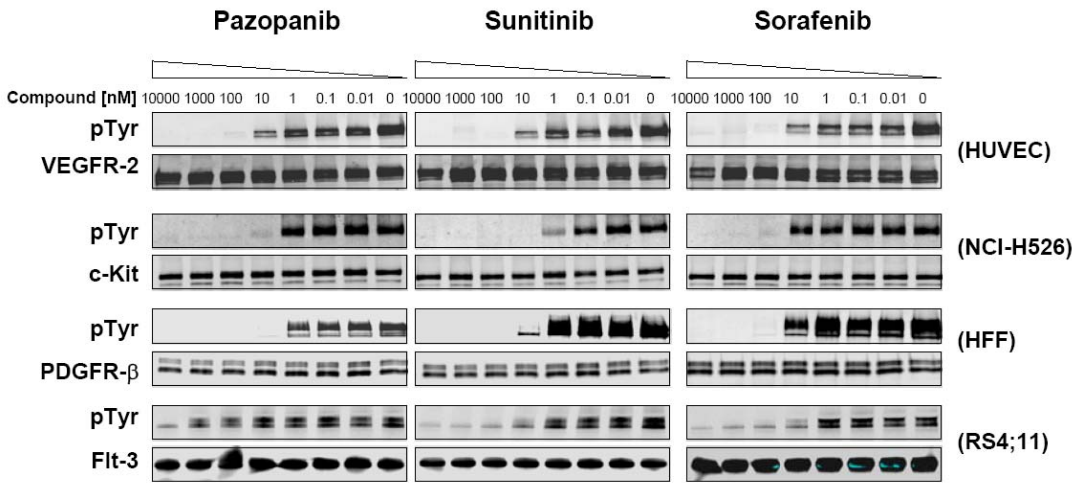
Compound	IC_{50} (μM)			
	c-Kit	PDGFR-ß	VEGFR-2	Flt-3
Pazopanib	0.0026	0.003	0.008	≥1
Sunitinib	0.0003	0.002	0.005	0.001
Sorafenib	0.0290	0.007	0.010	0.001

[Table excerpted from sponsor]

Part 2: *Activity of Pazopanib, Sunitinib, and Sorafenib against Ligand-induced Autophosphorylation of Various Receptor Tyrosine Kinases*

Western blot analyses showed that cells treated with GW786034B, sunitinib, and sorafenib inhibited ligand-induced activation of VEGFR-2, c-Kit, PDGFRβ, and Flt-3 receptors in dose-dependent manner while total receptor levels remain constant (Figure 2). All three inhibitors exhibited similar activity in suppressing activation of VEGFR-2 and PDGFR-β (Table 1). However, sunitinib had a 10-fold greater activity compared to GW786034B and 100-fold greater activity than sorafenib against c-Kit activation. Both sunitinib and sorafenib inhibited wild-type Flt-3 receptor activation with IC_{50} of 1nM. As mentioned earlier, GW786034B was 1000-fold less active against Flt-3 with IC_{50} ≥1μM (Table 1).

Figure 2 Inhibition of Receptor Phosphorylation by Pazopanib, Sunitinib and Sorafenib



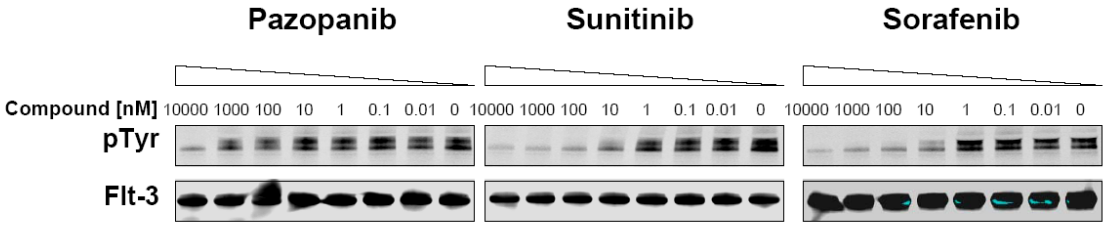
[Figure excerpted from sponsor]

Part 3: *Effect of Pazopanib, Sunitinib and Sorafenib on Flt-3 Receptor Phosphorylation, Downstream Signaling, and Cell Proliferation in Tumor Cells with Wild-type and Mutant Flt-3*

As shown in Figure 3A, GW786034B was less active compared to sunitinib and sorafenib at inhibiting Flt-3 mediated signaling in RS4-11 cells. Figure 3B shows that all 3 compounds inhibited the proliferation of RS4-11 cells with similar activity suggesting that Flt-3 inhibition does not involve anti-proliferative activity. Phospho-AKT levels were not inhibited by any of the 3 compounds suggesting that AKT activation is independent of Flt-3 activation (Figure 3C). Treatment with sunitinib and sorafenib induced a dose-dependent inhibition of MV4-11 cell growth with IC_{50} values of 5 and 4 nM, respectively, whereas GW786034B was much less active with an IC_{50} of 1.4 µM (Figure 4 and Table 2).

Figure 3 Effect of Pazopanib, Sunitinib and Sorafenib on Flt-3 Signaling and Cell Proliferation in RS4;11 Cells

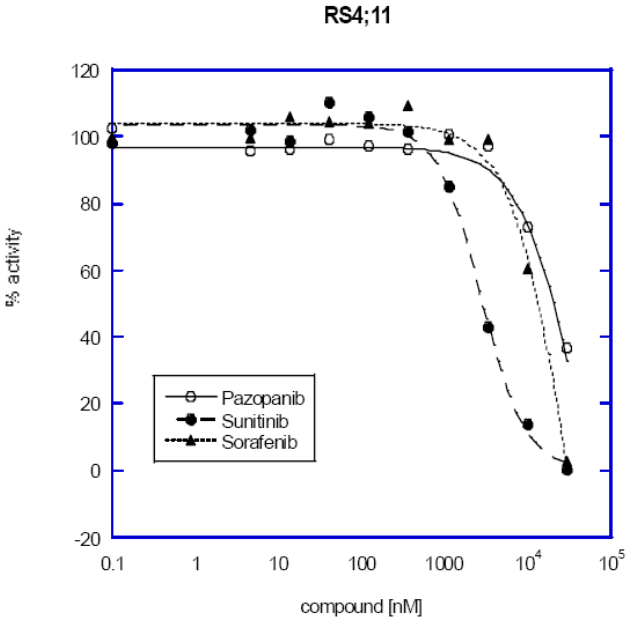
A. IP with anti-Flt-3 antibody, followed by western blot:



[Figure excerpted from sponsor]

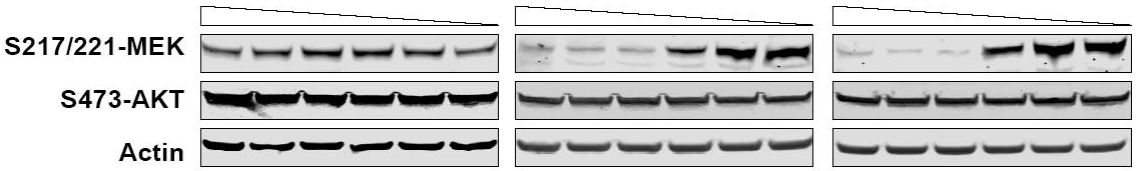
Figure 3 continued

Figure 3B. Proliferation



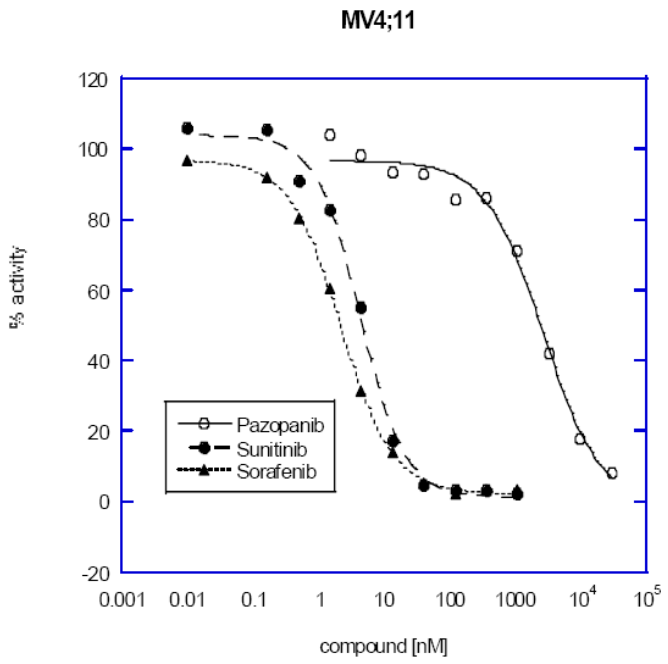
[Figure excerpted from sponsor]

Figure 3C. Western Blot



[Figure excerpted from sponsor]

Figure 4



[Figure excerpted from sponsor]

Table 2 IC50 of Pazopanib, Sunitinib, and Sorafenib in Cell Proliferation Assay

	Cellular IC_{50} [μM]	
Cell line	RS4;11	MV4-11
Flt-3 genotype	Wild-type	ITD mutant
Pazopanib	8.56±2.9	1.44±0.57
Sunitinib	2.24±0.88	0.005±0.002
Sorafenib	9.3±1.37	0.004±0.003

[Table excerpted from sponsor]

UH2008/00015/00: Cellular activity of pazopanib against imatinib-resistant c-kit mutants

The cellular activity of GW786034B and imatinib (a marketed kinase inhibitor) against variants of c-kit receptors were evaluated using imatinib-resistant c-kit mutants (V654A, T670I, Y816V, Y823D) and one imatinib-sensitive mutant, K642E, along with wild-type c-kit in transiently transfected CHO-K1 cells.

In CHO-K1 cells expressing wild type c-kit, GW786034B reduced c-kit activation with an IC_{50} of <0.01 μM. Imatinib, however, showed a >23-fold lower activity with an IC_{50} of 0.23 μM (Table 2).

V654A and T670I are mutations found in exon 13. Imatinib was active against both mutants with IC_{50} values of 1.3 and >10 μM, respectively. GW786034B, however, showed moderate activity with IC_{50} of 1.45 and 0.31 μM, respectively (Table 2).

The D816V mutation in exon 17 is commonly associated with mastocytosis and is resistant to imatinib. Both GW786034B and imatinib appeared to be inactive against this mutation with an IC_{50} of >10 μM (Table 2).

Y823D is an activating mutation similar to the D816V mutation. Similar to the D816V mutation, GW786034B and imatinib showed little or no activity against this mutation with IC_{50} values of >5 μM for both inhibitors (Table 2).

As shown in Table 2, imatinib retained its ability to inhibit K642E activation with an IC_{50} of 0.26 μM, which is similar in activity to wildtype c-Kit (IC_{50} 0.23 μM). Although GW786034B was similar in activity to imatinib with IC_{50} of 0.48 μM in the K642E mutant, this is much higher than the IC_{50} of GW786034B against wild-type c-Kit (<0.01 μM).

In conclusion, GW786034B and imatinib inhibited wild-type c-Kit activation with IC_{50} values of 0.003 μM to 0.230 μM, respectively. GW786034B and imatinib inhibited the K642E mutant with IC_{50} values of 0.480 and 0.260 μM, respectively. GW786034B was moderately active against T670I, the gatekeeper mutation, with an IC_{50} of 0.310 μM whereas imatinib was inactive (IC_{50} > 10 μM). Both GW786034B and imatinib had weak activity against the V654A mutant (IC_{50} = 1.3 to 1.45 μM) and were inactive against D816V and Y823D mutations. The latter two mutations are not expressed in RCC, the indication that GW786034B will be used for.

Table 2 Inhibitory activity of pazopanib and imatinib in wild-type and c-kit mutants in CHO-K1 cells

Compound	IC50 (μM)					
	Wild-type	V654A	T670I	D816V	Y823D	K642E
Pazopanib	<0.01	1.45	0.31	>10	>5	0.48
Imatinib	0.230	1.30	>10	>10	>5	0.26

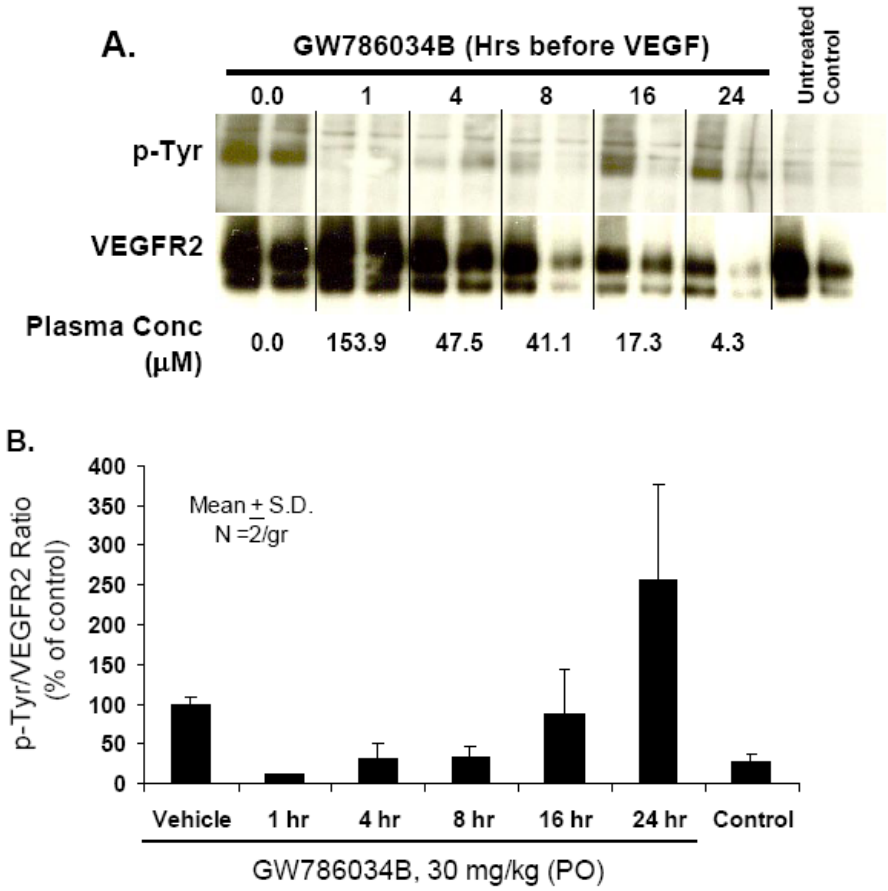
[Table excerpted from sponsor]

***In Vivo* Studies – Inhibition of VEGFR2 phosphorylation:**

RH2003/00005/00: Inhibition of VEGFR2 phosphorylation by GW786034B *in vivo*

The effects of GW786034B on VEGF-2 phosphorylation were evaluated in the lung tissue of female Swiss nude mice stimulated with exogenous VEGF ($VEGF_{121}$). Mice (n=2 to 3/dose) were administered a single dose of GW786034B at 0 or 30 mg/kg via oral gavage. At different times post-dosing, mice were then given 15 ug of $VEGF_{121}$ intravenously before being immediately sacrificed. Lungs were harvested after 5 minutes to measure total and phosphorylated VEGFR2 levels. Blood was collected for plasma analysis. Figure 1A shows that mice treated with VEGF alone showed increased phosphorylation of VEGFR2 compared to untreated controls. Figure 1B shows that pre-treating mice with GW786034B at 30 mg/kg inhibited VEGFR2 phosphorylation in a time-dependent manner. An oral dose of 30 mg/kg inhibited phosphorylation for ≥8 hours. There was no significant inhibition of phosphorylation at 16 and 24 hours post dosing, compared to animals treated with vehicle alone.

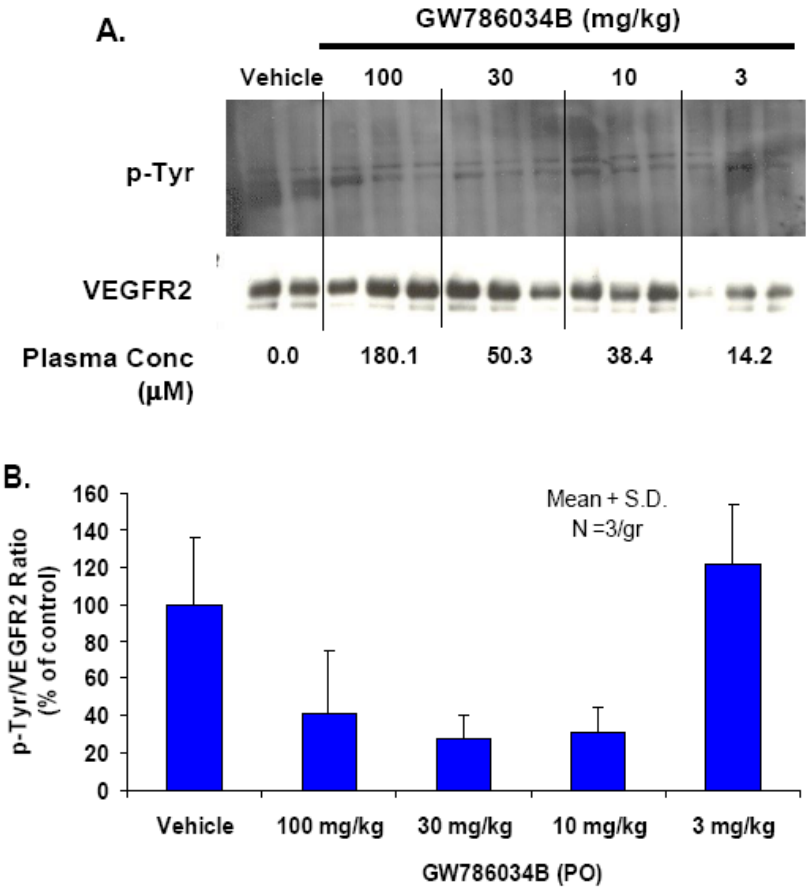
Figure 1. Time-course inhibition of VEGF-R2 phosphorylation by GW786034B in murine lungs stimulated with VEGF



[Figure excerpted from sponsor]

In addition, a dose-response study was conducted where mice received a single dose of GW786034B at 3, 10, 30 and 100 mg/kg. Two hours after the administration. VEGF was given intravenously and the lungs were collected after 5 minutes. Figure 2B shows that GW786034B inhibited VEGFR2 phosphorylation at doses ≥ 10 mg/kg. No significant inhibition was observed in mice treated with 3 mg/kg GW786034B compared controls. Mean plasma concentrations of 14.2, 38.4, 50.3, and 180.1 uM were achieved in mice dosed at 3, 10, 30, and 100 mg/kg. Therefore, data from this study suggest that plasma concentrations of approximately 40 uM or higher are required for optimal inhibition of VEGFR-2 phosphorylation in mice.

Figure 2. Dose-dependent inhibition of VEGF-R2 phosphorylation by GW786034B in murine lungs stimulated with VEGF



[Figure excerpted from sponsor]

***In Vivo* studies – Anti-Tumor Activity in Human Tumor Xenografts in Mice:**

RH2002/00043/00: Anti-tumor activity of GW786034B in various human tumor xenografts

This study was conducted to investigate the anti-tumor activity of GW786034B and GW786034A (pazopanib dihydrochloride) in four tumor xenografts models in immuno-compromised mice. Human tumor xenografts consisted of the following: colon carcinoma (HT29), head and neck (HN5), prostate (PC3), and melanoma (A375P). When tumor volume reached to 100 to 200 mm^3 in size, mice (N=8/dose/group) were administered 0, 10, 30, and 100 mg/kg of GW786034B or GW786034A once daily (qd) or twice daily (bid) via oral gavage for 21 days.

As shown in Table 1, HN5 tumors showed the greatest inhibition of tumor growth when given the highest dose of 100 mg/kg either twice daily (bid) or once daily (qd).

Following 21 days at 100 mg/kg once daily (qd), HT29 and HN5 tumor growth was inhibited by 69% and 110%, respectively, with GW786034A, and by 65% to 90%, respectively, with GW786034B (Table 1).

Following 21 days at 100 mg/kg twice daily (bid), HT29 and HN5 tumor growth was inhibited by 82% and 101%, respectively, with GW786034A, and by 66% to 90%, respectively, with GW786034B (Table 1).

Inhibition of tumor growth in A375P and PC3 xenografts was more modest with GW786034A and little to no inhibition was observed with GW786034B.

In conclusion, inhibition of tumor growth was slightly lower with GW786034B compared to GW786034A in all 4 tumor models. This may have been attributed to the 2-fold reduced plasma levels of GW786034B in mice dosed orally (Table 2). Table 2 lists the average plasma concentrations of mice given GW786034B and GW786034A.

Table 1. Percent Inhibition of tumor growth in mice treated with GW786034

	HT29		A375P		PC3		HN5	
Experiment #	I	II	I	II*	I	II	I	II
GW786034 - 100mg/kg,bid	82	66	64	44	53	0	101	90
GW786034 - 30mg/kg, bid	64	54	8	32	38	0	99	33
GW786034 - 10mg/kg, bid	32	15	16	37	50	5	104	40
GW786034 - 100mg/kg, qd	69	65	55	47	58	26	110	90
GW786034 - 30mg/kg, qd	44	20	35	13	21	24	102	13
GW786034 - 10mg/kg, qd	32	39	16	15	23	17	99	53

Percent inhibition of tumor growth in mice treated with different doses of GW786034A (experiment I) or GW786034B (experiment II) compared to vehicle treated mice after 21 days of treatment.

*Percent inhibition after 15 days of treatment.

[Table excerpted from sponsor]

Table 2. Plasma concentration of two salt forms of GW786034 in mice after single oral administration

	Average Plasma Concentration (μM)					
	GW 786034A			GW 786034B		
Time	10 mg/kg	30 mg/kg	100 mg/kg	10 mg/kg	30 mg/kg	100 mg/kg
0.25h	39.8	68.7	201.8			
0.5h	35.2	106.0	269.5			
1h	33.1	111.5	230.1	23.0	29.5	125.7
2h	40.3	122.4	227.7			
4h	38.8	106.0	198.7	15.2	47.7	91.4
6h	41.2	77.6	135.3			
8h	21.4	70.5	121.2			
12h	13.3	52.4	81.3	5.4	22.6	28.4
24h	2.1	5.1	5.2			

Plasma concentrations of GW786034 were determined in female CD-1 mice following a single oral dose of GW786034A (RD2001/01451/00) or GW786034B at 10, 30 and 100 mg/kg.

[Table excerpted from sponsor]

UH2008/00109/00: Effects of pazopanib on the growth of human renal cell carcinoma xenografts in mice

The anti-tumor activity of GW786034B was evaluated in 5 different human renal cell carcinoma (RCC) tumor xenografts (CAKI-1, CAKI-2, ACHN, A498, and 786-O) in female CD-17 SCID and Swiss nude mice. GW786034B was administered to mice either once/day (QD) or twice/day (BID) when tumor volume reached 100-250 mm^3 in size.

CB-17 SCID mice (N=8/dose/group) bearing CAKI-2 or A498 tumor xenografts were administered GW786034B orally once daily at 0, 10, 30, and 100 mg/kg for 24 days. Swiss nude mice (N=8/dose/group) bearing A498 or ACHN tumor xenografts were administered GW786034B orally twice daily at 0, 10, 30, and 100 mg/kg for 46 days. Swiss nude mice (N=8/dose/group) bearing 786-O or CAKI-1 tumor xenografts were administered GW786034B orally twice daily at 0, 10, 30, and 100 mg/kg for 31 or 21 days, respectively.

As shown in Table 1, CAKI-2 tumors showed the most inhibition of tumor growth (90, 77 and 99%) when GW786034B was given at 10, 30 and 100 mg/kg once daily for 24 days. Doses of 100 mg/kg/day inhibited A498 tumor growth by 64% in SCID mice whereas the same dose resulted in only a 45% inhibition in nude mice. The lower doses of 10 and 30 mg/kg/day in both SCID and nude mice had no effect on tumor growth rate.

In the ACHN and 786-O tumor xenografts, the mid and high doses of GW786034B induced moderate inhibition of tumor growth. No tumor growth inhibition was observed in CAKI-1 xenografts in mice at all doses.

In conclusion, GW786034B inhibited growth of 4 out of 5 human RCC xenografts growth in mice to varying degree with CAKI-2 model being the most sensitive to GW786034B treatment.

Table 1 Percent inhibition of RCC tumor xenograft growth in mice treated with pazopanib relative to vehicle treated mice at the end of dosing period

RCC Model	Mouse Strain	Pazopanib Schedule	GW786034B Dose (mg/kg)		
			10 mg/kg	30 mg/kg	100 mg/kg
CAKI-2	CB-17 SCID	QD x24 days	90%	77%	99%
A498	CB-17 SCID	QD x 24 days	0	0	64%
A498	Swiss Nude	BID x 46 days	0	0	45%
ACHN	Swiss Nude	BID x 46 days	38%	28%	46%
786-O	Swiss Nude	BID x 31 days	0	37%	30%
CAKI-1	Swiss Nude	BID x21 days	0	0	0

Data represent percent inhibition of tumor growth at the end of dosing period for each group relative to vehicle treated mice.

[Table excerpted from sponsor]

UH2008/00114/00: Head to head comparison of the anti-tumor activity of three small molecule angiogenesis inhibitors: pazopanib, sunitinib and sorafenib

The anti-tumor activity of GW786034B against HT29 and Colo-205 (colon carcinoma) tumor xenografts in female CD-1 nude mice was compared to that of sunitinib and sorafenib. Female mice (N=8/dose/group) bearing Colo-205 and HT-29 tumors were administered either GW786034B (at 30 or 100 mg/kg/day twice a day), sutitinib (at 20, 40, or 80 mg/kg/day once a day), or sorafenib (at 15, 30, or 60 mg/kg/day once a day). All doses were given orally for a total of 21 days.

Tables 1 and 2 show that all 3 compounds inhibited tumor growth in mice. In Colo-205 tumors, GW786034B inhibited tumor growth by 45 to 90% at the low dose of 30 mg/kg/day and 51 to 79% at the high dose of 100 mg/kg/day. In HT29 tumors, GW786034B inhibited tumor growth by 33 to 55% at 30 mg/kg/day and 69 to 70%, at 100 mg/kg/day. Sunitinib, at the low-dose of 20 mg/kg, inhibited HT29 tumor growth by

40%. At higher doses, 100% inhibition was observed. Sunitinib doses of 20, 40 or 80 mg/kg inhibited Colo-205 tumor growth by 78, 81 and 94%, respectively.

Table 1 The Effect of Pazopanib and Sunitinib on the Growth of Colo-205 and HT-29 (Colon) Xenografts Tumors

	Tumor Growth Inhibition (%)	
Study Groups:	**Colo-205**	**HT-29**
1. Vehicle I*, BIDx21, PO	--	--
2. 30 mg/kg Pazopanib, BIDx21, PO	45%	33%
3. 100 mg/kg Pazopanib, BIDx21, PO	79%	70%
4. Vehicle II*, QDx21, PO	--	--
5. Sunitinib, 20 mg/kg, QDx21, PO	40%	78%
6. Sunitinib, 40 mg/kg, QDx21, PO	>100%	81%
7. Sunitinib, 80 mg/kg, QDx21, PO	>100%	94%

*Vehicle I = 0.5% HPMC/0.1% Tween; Vehicle II = 20%SBE-CD pH3.0

Tumor growth inhibition (%) relative to vehicle treated group, represented as a mean of n=8 mice animals per group.

[Table excerpted from sponsor]

Sorafenib at doses of 15, 30 and 60 mg/kg inhibited HT29 tumor growth by 5, 10 and 22%, respectively and Colo-205 tumor growth by 59, 42 and 58%, respectively (Table 2).

Table 2 The Effect of Pazopanib and Sorafenib on the Growth of Colo-205 and HT-29 (Colon) Xenografts Tumors

	Tumor Growth Inhibition (%)	
Study Groups:	**Colo-205**	**HT-29**
1. Vehicle I*, BIDx21, PO	--	--
2. 30 mg/kg Pazopanib, BIDx21, PO	90%	55%
3. 100 mg/kg Pazopanib, BIDx21, PO	51%	69%
4. Vehicle II*, QDx21, PO	--	--
5. Sorafenib, 15 mg/kg, QDx21, PO	59%	5%
6. Sorafenib, 30 mg/kg, QDx21, PO	42%	10%
7. Sorafenib, 60 mg/kg, QDx21, PO	58%	22%

*Vehicle I = 0.5% HPMC/0.1% Tween; Vehicle II = 12.5% CREM/12.5% Ethanol

Tumor growth inhibition (%) relative to vehicle treated group, represented as a mean of n=8 mice animals per group

[Table excerpted from sponsor]

In conclusion, all three agents have some activity in the two models of human colon carcinoma (Colo-205 and HT-29) with sunitinib having the most effect on tumor growth compared to GW786034B (~100% tumor growth inhibition vs ~70%, respectively). GW786034B, however, was more effective at inhibiting tumors compared to sorafenib (~70% tumor growth inhibition vs ~40%, respectively).

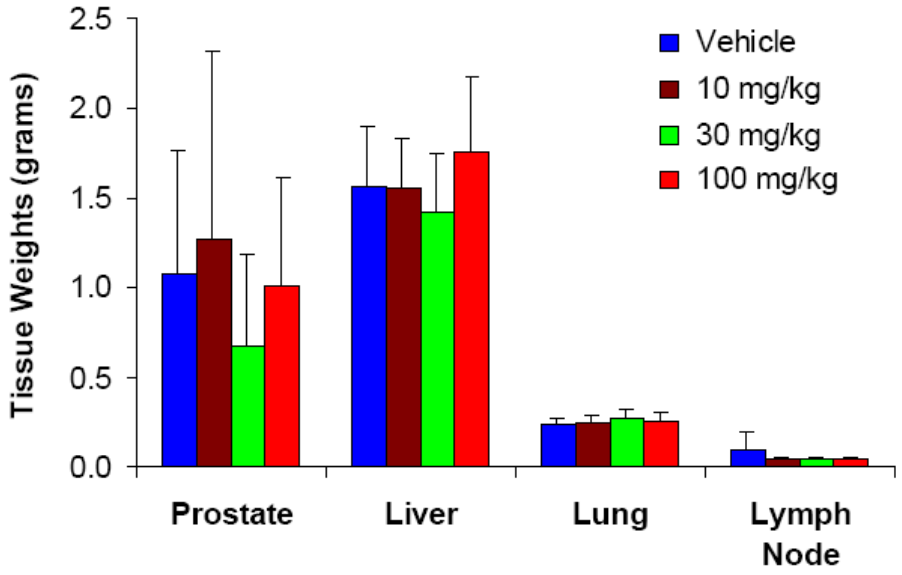
Anti-tumor activity in transgenic mice:
RH2003/00006/00: Effect of GW786034B on the growth of prostate tumors in CR2-T-Ag transgenic mice

This study was conducted to examine the anti-tumor activity of GW786034B in the transgenic mouse model of metastatic prostate cancer. This study is a better representation of human tumor growth compared to the xenograft tumor model in mice.

Mice were treated with vehicle or GW786034B at 10, 30 or 100 mg/kg/day once a day (qd) by oral gavage. At 24 weeks of treatment, animals were euthanized and the prostates and other associated tumors were excised and weighed. Liver, lung, and abdominal lymph nodes were also collected and weighed.

Figure 1 shows that GW786034B had no significant effect on prostate tumor growth. In addition, there was no difference in the weights of the areas where tumor growth occur (the liver, lung and abdominal lymph nodes- Table 1).

Figure 1 **Effects of GW786034B on Prostate, Liver, Lung and Lymph Node Weights in Male CR2-T-Ag mice**



Tissue weights represented as mean±SD in male CR2-T-Ag mice treated with different doses of GW786034B or vehicle for 8 weeks.

[Figure excerpted from sponsor]

Table 1 **Tissue weights in CR2-T-Ag mice treated with GW786034B**

	Tissue Wet Weight (g)			
	Prostate	Liver	Lung	Lymph Node
Vehicle	1.076 (1.177)	1.565 (1.601)	0.241 (0.238)	0.096 (0.043)
GW786034B - 10 mg/kg, SID	1.271 (0.896)	1.548 (1.536)	0.250 (0.250)	0.041 (0.043)
GW786034B - 30 mg/kg, SID	0.681 (0.601)	1.422 (1.416)	0.264(0.281)	0.039 (0.041)
GW786034B - 100 mg/kg, SID	1.012 (0.852)	1.755 (1.713)	0.258 (0.260)	0.042 (0.037)

Tissue weights represented as mean (median) in male CR2-T-Ag mice treated with different doses of GW786034B or vehicle for 8 weeks.

[Table excerpted from sponsor]

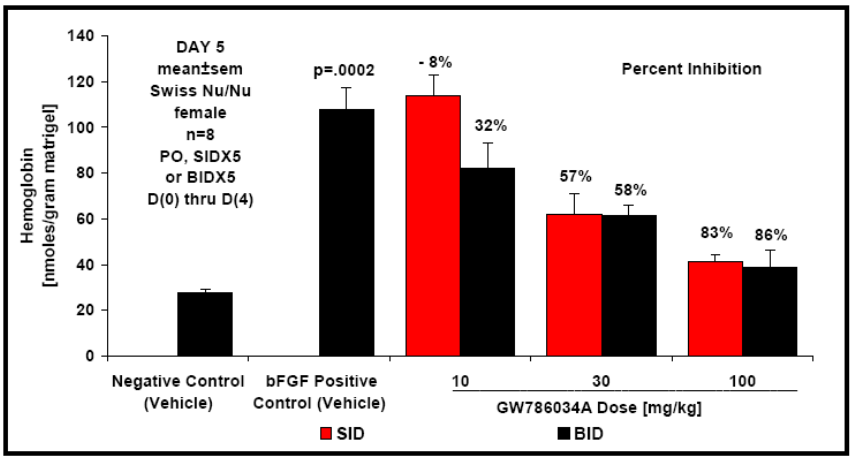
Effects on angiogenesis:

RH2002/00048/00: The effects of VEGFR2 antagonist GW786034A di-salt in the bFGF/Matrigel® model of angiogenesis

This study examined the effects of di-HCl salt of pazopanib (or pazopanib dihydrochloride or GW786034A) on basic fibroblast growth factor (bFGF)/Matrigel® model of angiogenesis. Basic-FGF (bFGF) is a growth factor with pro-angiogenic activities. Matrigel® is a murine extracellular matrix preparation that provides a substrate for blood vessel growth. In mice, subcutaneous implantation of bFGF containing 1 µg/mL of Matrigel results in new blood vessel growth in the plug area which is highly dependent on VEGFR-2 signaling. In the current study, female swiss nu/nu mice (N=8/group/dose) were given doses of pazopanib dihydrochloride (di-HCl salt) at 0, 10, 30, and 100 mg/kg/day twice a day (bid) and once a day (qd) by oral gavage for 5 days. On day 5, mice were killed and the Matrigel removed, weighed and homogenized.

As shown in Figure 1, tumor inhibition was equal, regardless of schedule (bid vs qd). Dosing at 100 mg/kg bid resulted in 86% inhibition of tumors when given twice a day and 83% inhibition of tumors when given once a day. Dosing at 30 mg/kg exhibited a similar pattern, dosing twice a day resulted in 58% inhibition vs. 57% inhibition when given once a day. The only difference between the twice a day and once a day dosing regiment was observed at the 10 mg/kg dose (bid was a 32% inhibition and qd was a -8% inhibition with a p value of 0.058).

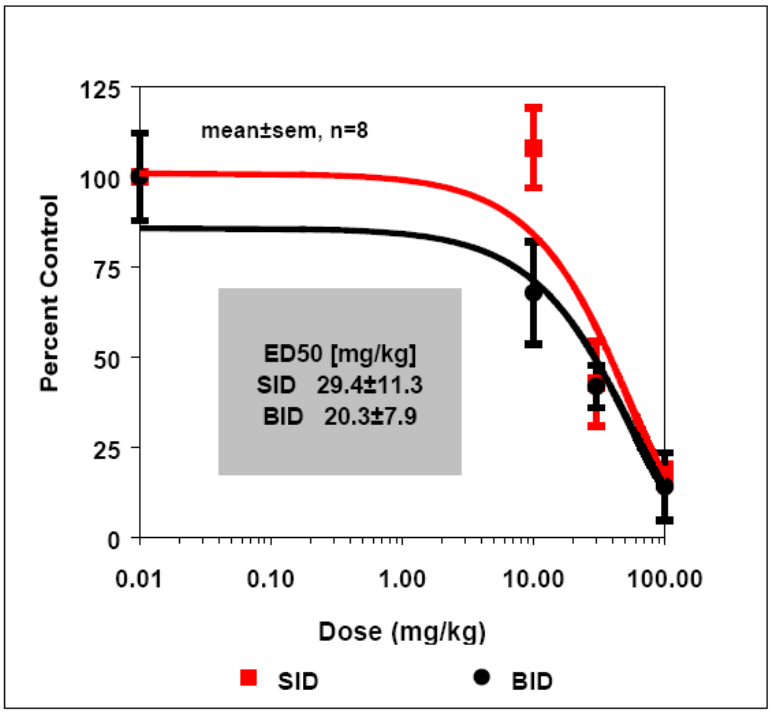
Figure 1. Anti-angiogenic dose response of GW786034A (di-HCl salt) in the mouse bFGF/Matrigel® angiogenesis assay.



[Figure excerpted from sponsor]

Comparing ED_{50} values for once a day and twice a day dosing regimens suggests similar total amounts of drug are required on a daily basis to achieve an ED_{50} and reinforces the fact that bid and qd regimens appear equivalent for GW786034A in this study (Figure 2).

Figure 2. **A comparison of bid and sid dosing regimens for GW786034A (di-HCl salt) in the mouse bFGF/Matrigel® angiogenesis assay.**



[Figure excerpted from sponsor]

RH2002/00049/00: The effects of VEGFR2 antagonist GW786034B mono-HCl salt in the bFGF/Matrigel® model of angiogenesis

This study was similar to the above study (Study No. RH2002/00048/00). The current study, however, examined the effects of the mono-HCl salt of pazopanib (GW786034B) instead of the di-HCl salt of pazopanib (GW786034A) on angiogenesis in the Matrigel® model. Swiss nu/nu mice were given doses of pazopanib mono-hydrochloride at 0, 3, 10, 30, and 100 mg/kg/day twice a day (bid) and once a day (qd) by oral gavage for 5 days. On day 5, mice were killed and the Matrigel removed, weighed and homogenized.

Similar to the results obtained from Study No. RH2002/00048/00, administration of GW786034B in a twice a day (bid) regimen showed no advantage of over the once a day (qd) dosing.

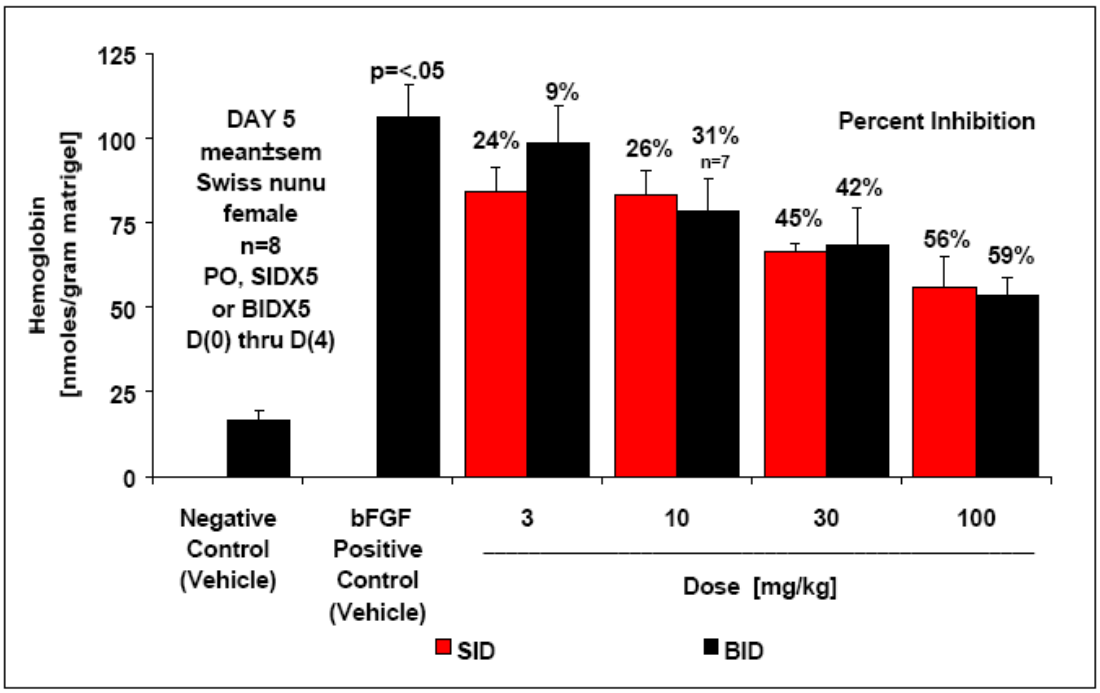
At the high dose of 100 mg/kg, twice a day dosing resulted in 59% inhibition of angiogenesis. This did not differ significantly from 56% inhibition produced when 100 mg/kg was given once a day.

Dosing at 30 mg/kg exhibited a similar pattern. When mice were given 30 mg/kg twice a day, a 42% inhibition was observed compared to a 45% inhibition when given once a day.

Similarly at 10 mg/kg twice a day, a 31% inhibition was observed compared to a 26% inhibition when given once a day.

As seen in the previous study (Study No. RH2002/00048/00), the similar levels of activity demonstrated by the mono-HCl salt of pazopanib when given either once or twice a day could be explained by the extended plasma half-life in mice (data discussed in Study No. RH2002/00043/00).

Figure 1 **Anti-angiogenic dose response of GW786034B (mono-HCl salt) in the mouse bFGF/Matrigel® angiogenesis assay.**



[Figure excerpted from sponsor]

2.6.2.3 **Secondary pharmacodynamics –** No studies reviewed

2.6.2.4 Safety Pharmacology

Safety pharmacology studies were performed to investigate the effect of pazopanib (GW786034) on cardiovascular, overt central and peripheral neurological, and pulmonary function. No overt effects of single dose treatment with the drug were seen on pulmonary or neurologic function. The sponsor included cardiovascular studies in rats and cynomolgus monkeys as well as *in vitro* studies including the hERG assay and a purkinje cell fiber assay. There were no remarkable findings from either of the *in vitro* studies. In the *in vivo* oral dose monkey study, the sponsor reported decreased heart rates and increased mean arterial pressure compared to vehicle control in animals at doses ≥ 600

mg/m^2. These changes, though mild, were evident, respectively, from 2-13 and 2-8 hours after dosing. At the 4-5 hour time point the same effects were evident at even the lowest dose of 60 mg/m^2 in a dose-dependent manner. The veterinary cardiologist certified that the ECG waveforms in this study were within normal limits for the species. No other significant changes were noted. A second study done in monkeys using an IV dose of 45 mg/m^2 of pazopanib revealed similar cardiovascular effects. There was a brief increase in mean arterial pressure between 0.3 and 0.5 hours after dosing. In addition animals displayed a decreased heart rate from 75 minutes to 24.5 hours post dose. While this decrease was associated with prolonged QT and PR intervals, these intervals are inversely correlated with heart rate and the QTc showed no significant change. Finally, the cardiovascular study performed in rats also showed a very mild decrease in heart rate compared to baseline at all doses tested (≥6 mg/m^2). Overall, these studies suggest that pazopanib does have an effect on cardiovascular function.

Study #/Organ System	Method of Administration/ GLP?	Species	Doses	Gender/N	Findings
R41033 CNS-FOB	Oral/ Yes	Rat	0, 18, 60, 600, 1800 mg/m^2	6F/group	No remarkable observations
G02047 Cardiovascular	Oral/ Yes	Monkey	0, 60, 600, 6000 mg/m^2	4M	60 mg/m^2 : mild ↑ in mean arterial pressure and ↓ in heart rate 2-5 hrs. 600 mg/m^2 : ↑in MAP between 2-8 hrs., ↓ heart rate (compared to baseline) 2-13 hrs. 6000 mg/m^2 : ↑in MAP between 2-8 hrs., ↓ heart rate (compared to baseline) 2-13 hrs.; maximum change from baseline was an 11% increase in MAP at 5 hrs. post dosing
G05397 Cardiovascular	IV/ Yes	Monkey	0, 45 mg/m^2	4M/group	Tmax 1 minute AUC 41098 ng*hr/mL Cmax 55240 ng/mL ↓ heart rate btwn. 1.25 hrs. And 24.5 hrs post dose associated with prolonged QT and PR intervals. QTc showed no significant change. QT did not exceed 250 msec. Transient ↑ in MAP 0.3-0.5 hrs.
CH2002/00002/00 No number assigned Cardiovascular	IV/ No	Rat	6, 18, 60 mg/m^2	3M	Slightly ↓ heart rate at all doses 60 mg/m^2 Cmax 57578 ng/mL
D23802 Canine Purkinje Fibers	In vitro	Dog tissue	40, 80 nM		No remarkable observations
V28197	In vitro/ Yes (in	NA	0, 1.241,		IC_{50} = not determined,

Study #/Organ System	Method of Administration/ GLP?	Species	Doses	Gender/N	Findings
hERG current	accordance with UK GLP regs.)		and 4.137 µM		Insufficient inhibition at max. soluble concentration to allow estimation 4.137 µM ↓19.5% 1.241 µM ↓ 18.5%
R41018 Respiratory	Oral/ Yes	6M			No remarkable findings

Neurological effects:

RD2002/001/00 GW786034B: Safety Pharmacology Study of Overt Central and Peripheral Pharmacodynamic Effects Following Oral Administration in Conscious Sprague Dawley Rats

Key Study Findings: No remarkable observations

Report #:	R41033
Conducting Laboratory and Location:	GlaxoSmithKline Safety Assessment Research Triangle Park, NC USA
Date of Study Initiation:	February 25, 2002
GLP Compliance:	Yes
QA Report:	Yes (X), No ()
Drug, lot #, and % purity:	GW786034B, 786034-A2-01M, 90.1%

Doses:	0, 3, 10, 100, and 300 mg/kg
Species/strain:	Sprague Dawley Rat/ Crl:CD (SD)IGS BR
Number/sex/group or time point:	6 females/group
Route; formulation; volume; and infusion rate:	Oral gavage once on Day 1; 0.5% hydroxypropyl methylcellulose in reverse osmosis water with 0.1% Tween 80; pH adjusted to 1.38 with 1N HCl; 10 mL/kg/day
Age:	Approximately 13 weeks
Weight:	234.4-280.5 g
Parameters:	• Behavior, • Skeletal muscle tone, • Reflexes, • Body temperatures • Overt autonomic, gastrointestinal and neurological effects
Methods	Functional Observational Battery measurements taken predosing, and at

	approximately 3 and 24 hours after dosing

Cardiovascular effects:

CD2002/00099/00 Single Oral Dose Cardiovascular Study in Cynomolgus Monkeys

Report #: G02047 (GSK) MN102441 (b) (4)
Conducting Laboratory and Location: (b) (4)

Date of Study Initiation April 12, 2002
GLP Compliance Yes
QA Report Yes (X), No ()
Drug, lot #, and % purity GW786034B, 786034-A3-01P, 99.1%

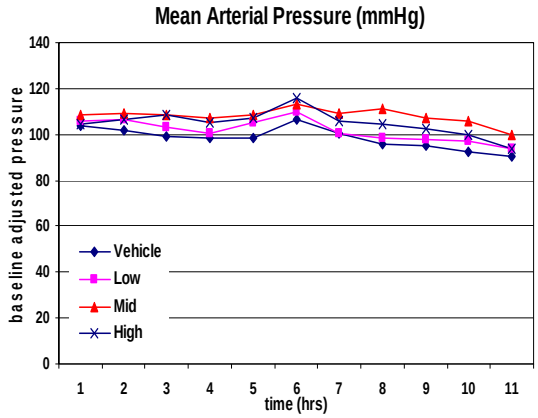
Doses:	0, 5, 50, and 500 mg/kg
Species/strain:	Cynomolgus monkeys/ *Macaca fascicularis*
Number/sex/group or time point:	1 male/group/ time-point (4 males/dose)
Route; formulation; volume; and infusion rate:	Oral gavage once weekly for 4 weeks; 0.5% Hydroxypropylmethylcellulose/0.1% Tween® 80 (pH-adjusted to 1.3 using 1N HCl); 5 mL/kg
Age:	3-5 years
Weight:	4.5-6.0 kg
Parameters:	Systemic arterial blood pressures (systolic, diastolic, and mean) Pulse pressure Heart rate ECG intervals (PR, QRS, QT, QTc) ECG waveforms Body temperature

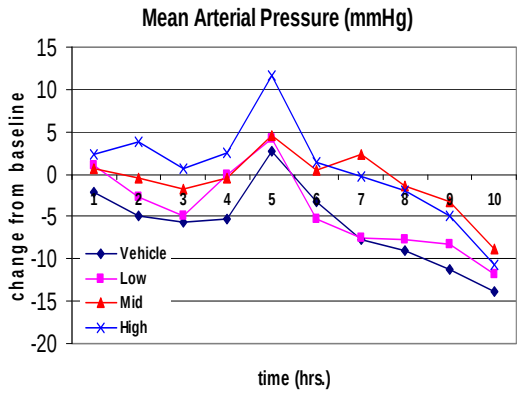
Table excerpted from sponsor

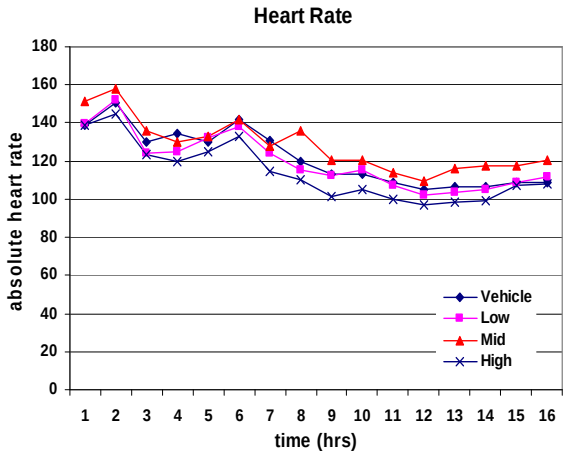
Dosing Schedule (5, 50 or 500 mg/kg GW786034B or Vehicle)*				
Animal				
Number	**Day 1**	**Day 8**	**Day 15**	**Day 22**
101	5	500	50	vehicle
102	50	5	vehicle	500
103	vehicle	50	500	5
104	500	vehicle	5	50
* Doses are expressed as GW786034X (free base).				

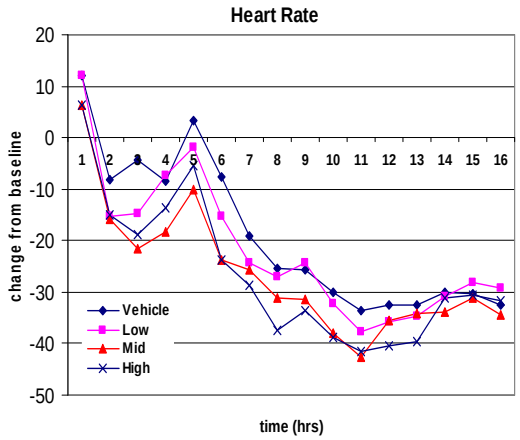
Measurements were taken in conscious unrestrained animals and no deaths occurred during the study. There was an increase in the baseline-adjusted mean arterial blood

pressure between 2 and 8 hours at doses ≥50 mg/kg, though there was no dose-proportional increase observed. At 2-5 hours this trend in increased mean arterial pressure was also evident in the 5 mg/kg dose group. There was a concurrent decrease in baseline adjusted heart rate for the 50 and 500 mg/kg doses which was proposed to be a baroreceptor reflex to the increased blood pressure. Once again this decrease was evident in the lowest dose group as well at the early time points (2-5 hours). The highest change in blood pressure was an 11% increase. No other remarkable changes were observed.









CD2006/00750/00 GW786034B: Acute Effects on Cardiovascular Function Following Intravenous Administration in the Conscious Cynomolgus Monkey (Safety Pharmacology Study)

Report #:	G05397 (GSK) N105929 (b) (4)
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	March 23, 2006
GLP Compliance:	Yes
QA Report:	Yes (X), No ()
Drug, lot #, and % purity:	GW786034B, 786034B-A4-02P-MIC-RTN, 98.5%

Doses:	0, 3.75 mg/kg
Species/strain:	Cynomolgus monkeys/ *Macaca fascicularis*
Number/sex/group or time point:	4 males/group
Route; formulation; volume; and infusion rate:	IV; 5 mg/mL suspension in 7% Captisol containing 25 mM sodium phosphate monobasic dihydrate and 15 mM sodium chloride in sterile water for injection, USP adjusted to a pH of approximately 5.0; 1 minute infusion
Age:	21-49 months
Weight:	3.8-4.9 kg
Parameters:	Systemic arterial blood pressures (systolic, diastolic, and mean) Pulse pressure Heart rate ECG intervals (RR, PR, QRS, QT, QTc) ECG waveforms Body temperature

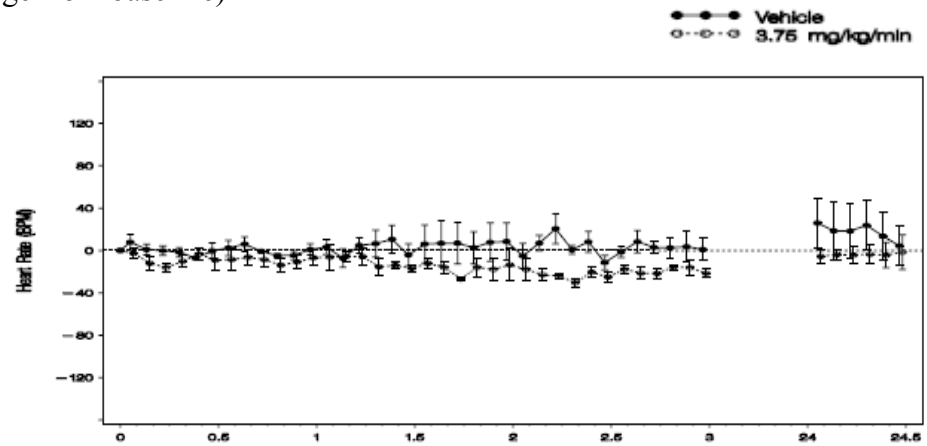
Excerpted from sponsor

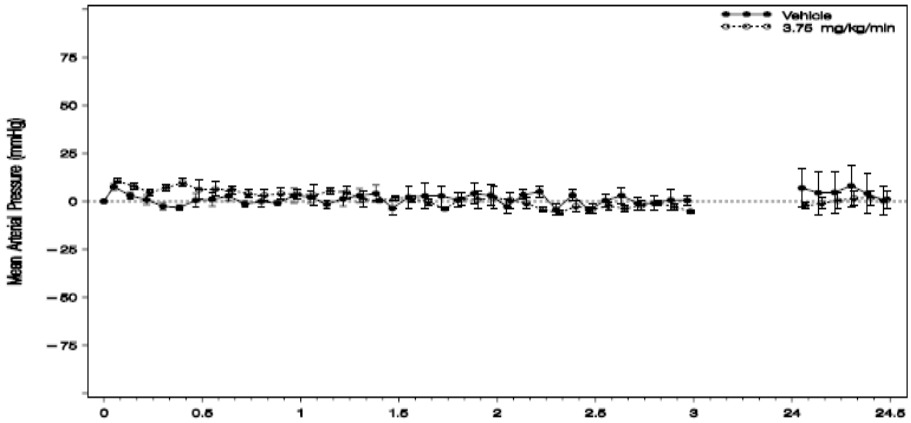
Dosing Schedule (3.75 mg/kg/min of Test Article or Vehicle)1		
Animal Number	**Day 1**	**Day 6**
101	3.75	Vehicle
102	Vehicle	3.75
103	3.75	Vehicle
104	Vehicle	3.75
105	3.75	NA
106	3.75	NA
107	3.75	NA
1. Dose is expressed as mg/kg/min of the parent compound. Study Day 1 is defined as the first day of dosing for each respective group (i.e., Day 1 for animal numbers 101 through 104 was April 6, 2006 and Day 1 for animal numbers 105 through 107 was March 31, 2006).		

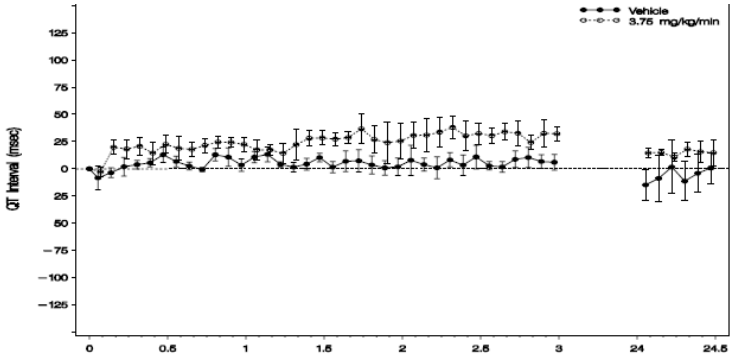
Decreased heart rate was observed between 75 minutes and 24.5 hours post dose. This was associated with prolonged QT and PR intervals. Prolongation of these intervals is inversely associated with heart rate and the QTc showed no significant change. There was also an increase in mean arterial pressure seen from 15 to 30 minutes after dosing. This change was more transient than the change seen in the oral dosing study and the sponsor did not mark this change as significant.

Excerpted from sponsor

(change from baseline)







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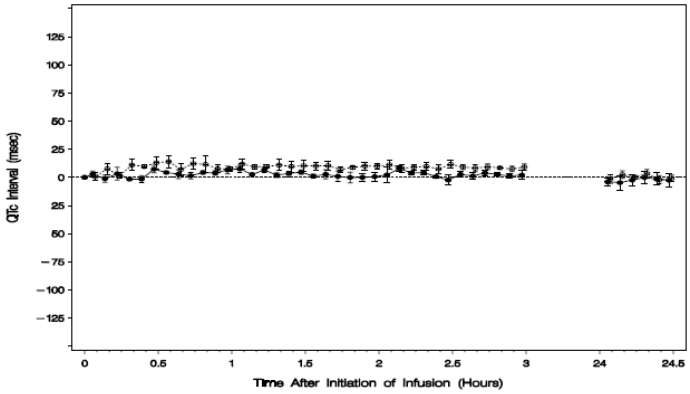

CH2002/00002/00 Hemodynamic Evaluation of GW-786034A in the Anesthetized Rat

Report #:	Not Assigned
Conducting Laboratory and Location:	GlaxoSmithKline CVU CEDD, Upper Merion King of Prussia, PA
Date of Study Initiation	September 11, 2001
GLP Compliance	No
QA Report	Yes (), No (X)
Drug, lot #, and % purity	GW-786034A

Doses:	1, 3, 10 mg/kg
Species/strain:	Sprague Dawley Rat
Number/sex/group or time point:	3 Males/group
Route; formulation; volume; and infusion rate:	IV 30% hydroxypropyl-B-cyclodextrin with the pH adjusted to 2.5
Weight:	420-490 g
Parameters:	Left ventricular pressure Arterial blood pressure Heart rate

Isoflurane anesthetized rats were injected with either isoproterenol or GW-786034A and results were recorded for unconscious animals. The effects of isoproterenol were as expected. Heart rate was slightly decreased for GW-786034A treated rats at every dose.

Rat ID	Plasma Concentration (ng/ml)
Rat 1	60822
Rat 2	45075
Rat 3	54838
Mean	**53578 ± 4589**

FD2002/00060/00 GW786034B: Effect on Action Potential Parameters in Dog Isolated Cardiac Purkinje Fibres.

Report #:	D23802
Conducting Laboratory and Location:	GlaxoSmithKline Safety Pharmacology Dept., The Frythe, Welwyn, Hertfordshire, UK
Date of Study Initiation	May 20, 2002
GLP Compliance	Yes
QA Report	Yes (X), No ()
Drug, lot #, and % purity	GW-786034B; 786034-A2-01M,

Beagle dogs were anesthetized by injection with sodium pentobarbitone and ventricular Purkinje fibers were isolated from the hearts. The fibers were transferred to gassed physiological salt solution (PSS) on ice. Transmembrane potentials were recorded after a 60 minute period for tissue equilibration/ sodium pentobarbitone wash-out. The Purkinje fibers were stimulated at rates of 0.5 and 1 Hz with an additional stimulation at 3 Hz performed to examine maximum rate of depolarization (MRD). Action potential duration at 60% and 90% repolarization (APD_{60} and APD_{90}), MRD, resting membrane potential (RMP), and upstroke amplitude (UA) were measured. One vehicle (1% DMSO) and two test groups (40nM and 80nM GW786034B) were analyzed to investigate the possible effect of the compound on sodium channels. The sponsor included analysis of *dl*-sotalol hydrochloride (50µM) as a positive control. Results were unremarkable suggesting that there was no significant effect of GW786034B on cardiac sodium channels.

Tables Excerpted from Sponsor

1 Hz

Action Potential Parameter	Control	GW786034B	GW786034B	DMSO	Sotalol
	100% PSS	40 nM	80 nM	1%	50µM
RMP (mV)	-89.6 ± 1.1	-90.2 ± 1.6	-90.5 ± 1.6	-92.1 ± 2.2	-92.7 ± 1.8
UA (mV)	126.0 ± 1.7	126.2 ± 1.3	126.5 ± 1.2	120.5 ± 2.4	121.0 ± 3.4
MRD (V/s)	789.9 ± 49.4	758.0 ± 31.3	747.6 ± 45.9		
APD60 (ms)	214.6 ± 12.7	213.4 ± 12.0	217.7 ± 10.6	202.9 ± 9.8	292.6 ± 8.5*

APD90 (ms)	264.0 ± 10.6	263.2 ± 8.4	269.2 ± 8.8	246.3 ± 13.0	352.2 ± 8.3*

0.5 Hz

Action	**Control**	**GW786034B**	**GW786034B**	**DMSO**	**Sotalol**
Potential	**100% PSS**	**40 nM**	**80 nM**	**1%**	**50µM**
Parameter					
RMP (mV)	-86.7 ± 0.6	-87.7 ± 1.1	-88.5 ± 1.2	-89.9 ± 2.1	-90.9 ± 1.9*
UA (mV)	123.2 ± 2.1	124.1 ± 1.1	124.3 ± 1.5	119.4 ± 2.6	119.3 ± 3.6
MRD (V/s)	760.0 ± 52.3	736.6 ± 37.1	751.3 ± 46.3		
APD60 (ms)	237.9 ± 17.2	242.1 ± 12.9	245.0 ± 11.3	221.0 ± 17.6	346.2 ± 18.5*
APD90 (ms)	295.8 ± 16.8	300.6 ± 9.9	302.8 ± 11.0	269.0 ± 21.9	415.1 ± 18.8*

FD2008/00125/00 GW786034B: Effect on hERG Tail Current Recorded from Stably Transfected HEK-293 Cells

Report #:	V28197
Conducting Laboratory and Location:	GlaxoSmithKline Safety Pharmacology Dept., The Frythe, Welwyn, Hertfordshire, UK
Date of Study Initiation	April 11, 2008
GLP Compliance	*Yes, *(in accordance with UK Good Laboratory Practice Regulations)
QA Report	Yes (X), No ()
Drug, lot #, and % purity	GW-786034B; 786034B-A4(RW)-02P-MIC, 99.7%
Methods	
Doses:	0, 1.241, and 4.137µM
Study Design:	Standard hERG assay

GW786034 was tested up to the maximum soluble concentration of 4.137µM. Inhibition of hERG tail current was 18.5% and 19.5% at the concentrations of 1.241 and 4.137µM respectively. There was insufficient inhibition to allow an estimation of the IC_{25} or IC_{50} value. This study does not predict a high risk of QT prolongation.

Pulmonary effects:

RD2001/01691/00: GW786034B: A Single Oral Dose Respiratory Study in Rats

Report #:	R41018 (GSK) MN103383 (b) (4)
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation	February 12, 2002
GLP Compliance	Yes

QA Report	Yes (X), No ()
Drug, lot #, and % purity	GW-786034B; 786034-A2-01M

Doses:	0, 3, 10, 100, and 300 mg/kg
Species/strain:	Rat/ CRL:CD IGS BR
Number/sex/group or time point:	6 males/group
Route; formulation; volume; and infusion rate:	Oral Gavage 0.5% hydroxypropyl methylcellulose (HPMC) with 0.1% Tween 80 in reverse-osmosis water, pH 1.29 10 mL/kg
Age:	Approximately 9 weeks
Weight:	258-304 g
Parameters:	Respiratory rate, tidal volume, and minute volume

No mortalities or abnormal clinical observations were noted during the study. The pulmonary ventilation of male rats treated with a single dose of GW786034B or the vehicle control was monitored predosing (to obtain baseline), through the times of Cmax (0-5 hours post dose), and at 24 and 48 hours post dosing. No significant differences were seen in respiratory rate, tidal volume, or minute volume until 24 hours post dose. At this time-point a significant decrease in minute volume was seen in the treated animals compared to control; however, this finding is not likely to be clinically significant as the control group animals appeared to have an abnormally high minute volume (compared to all other vehicle control time points recorded) and as the decrease was not dose dependent.

2.6.2.5 Pharmacodynamic drug interactions

UH2008/00107/00: Effect of pazopanib and bevacizumab on the delivery of chemotherapeutic agents in human tumor xenografts grown in mice.

The present study compared the effects of pre-treatment with two different anti-angiogenic agents GW786034B and bevacizumab (an antibody that binds to VEGF ligand) using tumor xenografts (HT-29 and NCI-H460). Mice bearing HT29 (colon) or NCI-H460 (lung) tumor xenografts were treated for 1 week with either GW786034B (100 mg/kg/day) or bevacizumab (10 mg/kg on Days 1, 3, and 6) and then given a single administration of chemotherapeutic agent (5-FU, CPT-11, paclitaxel or carboplatin) on Day 7. Blood and tumor concentration of the various chemotherapeutic agents and pazopanib were measured at 2 and 8 h after administration of chemotherapeutic agent.

Tumor volumes and body weights were measured over the seven days of dosing. Mice bearing HT-29 tumors showed no significant effect of any of the treatment combinations on tumor growth over the 7 days (Figure 1A). Minimal body weight loss (<10%) was noted in all groups by day 3 of dosing which improved by the end of the study. The group dosed with GW786034B plus CPT-11 lost the most body weight, 11.8% by day 7 of the study (Figure 1B). Similarly for the NCI-H460 tumor study, there was no significant

tumor growth inhibition observed in any of the groups by day 7 of dosing (Figure 1C), and no significant loss of body weight was observed in any of the groups (all < 7%) (Figure 1D).

Figure 1 Tumor Growth and Bodyweight Curves for Mice Bearing HT-29 (Colon) and NCI-H460 (lung) Tumor Xenografts

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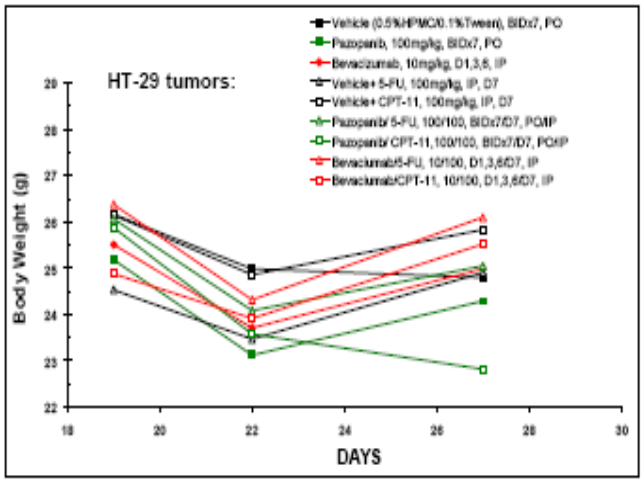

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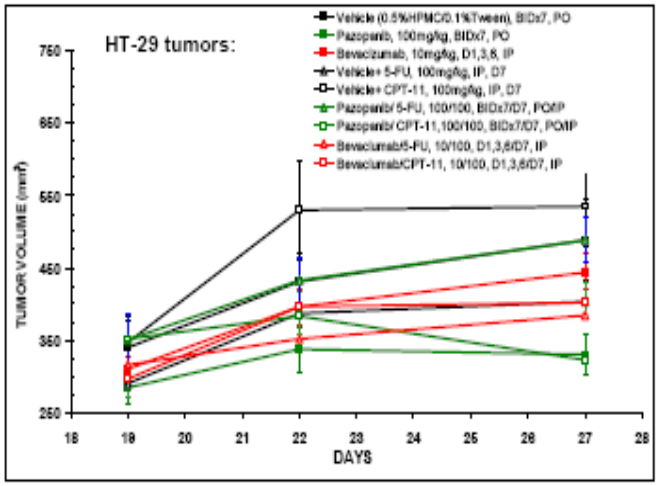

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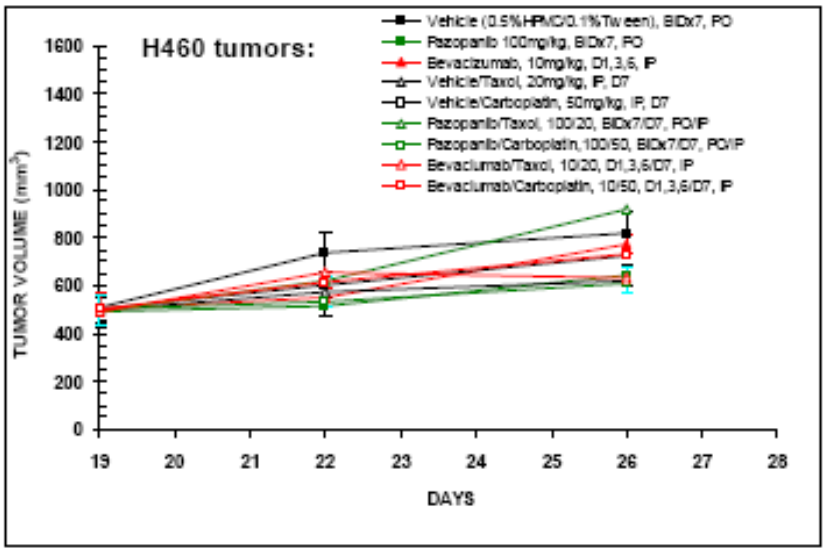
Figure 1 continued

C:



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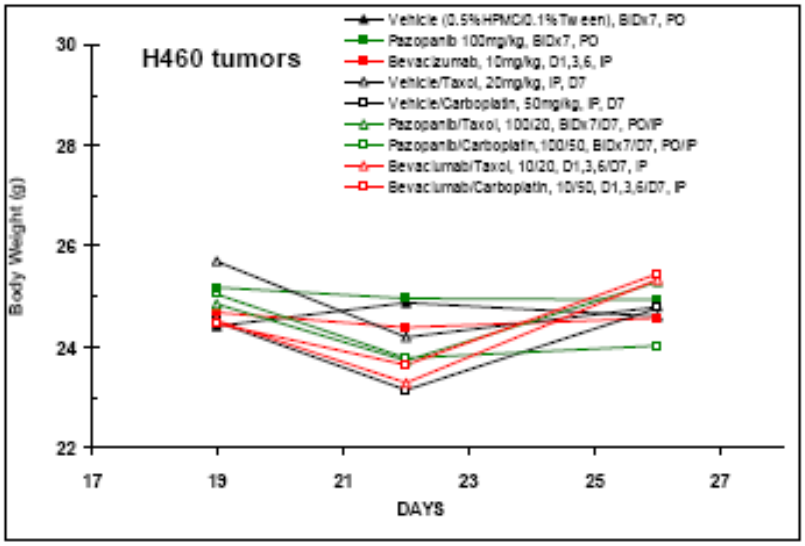

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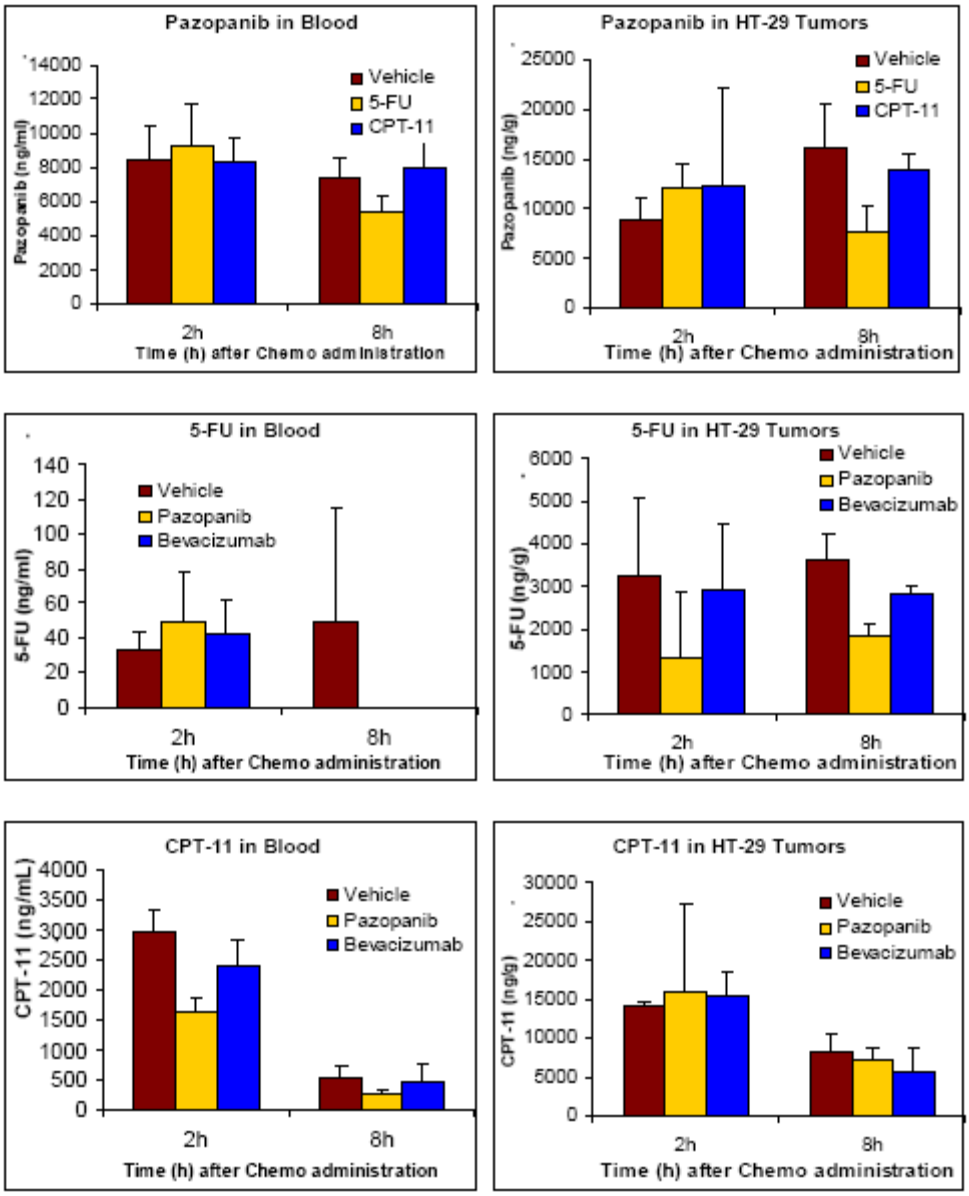
The blood and tumor concentrations of GW786034B, 5-FU and CPT-11 were analyzed in mice bearing HT-29 tumors. Similarly, the blood and tumor concentrations of GW786034B, paclitaxel, and carboplatin were analyzed in mice bearing H460 tumors. For both studies, there was no significant increase in the concentration of any of the 4 chemotherapeutic agents in the blood and tumor samples of mice pre-treated with either anti-angiogenic agent in both tumor models (Figures 2 and 3).

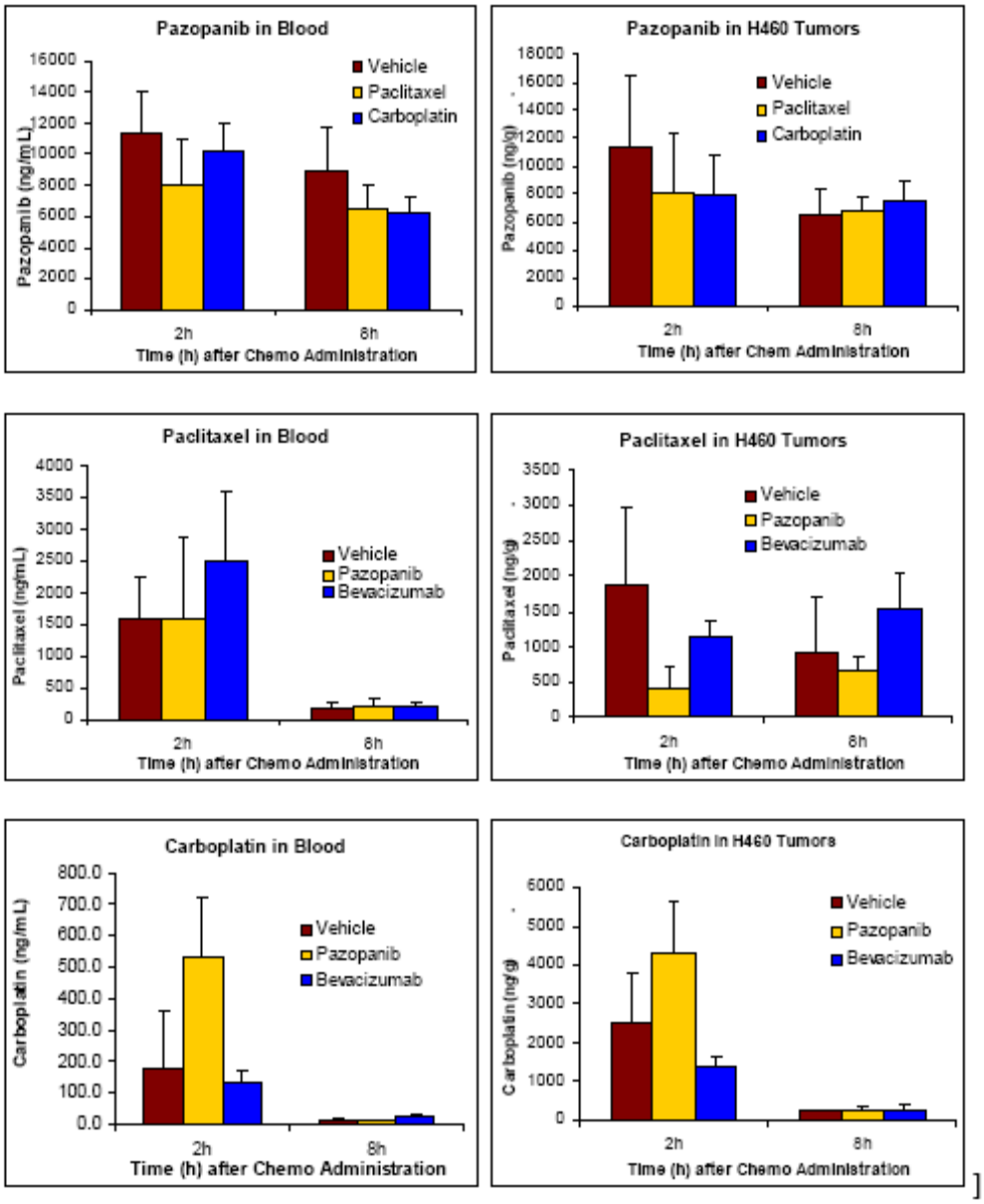
Figure 2 Analysis of Drug Concentration in the Blood and Tumor of Mice Bearing HT-29 (Colon) Tumor



Data represent mean ± standard deviation, N=4/group

[Figure excerpted from sponsor]

Figure 3 Analysis of Drug Concentration in the Blood and Tumor of Mice Bearing NCI-H460 (Lung) Tumor



Data represent mean ± standard deviation, N=4/group

[Figure excerpted from sponsor]

In summary, the study suggested that clinical administration of anti-angiogenic agents in combination with GW786034B is unlikely to affect the delivery of chemotherapeutic agents to the tumor tissue.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Study #/Type of study	Test system	Significant findings
Primary pharmacodynamics – in vitro		
RR2002/00010/01/ Activity and selectivity for vascular endothelial growth factors.	– Human VEGF1, VEGF2, and VEGF3. – VEGF-2 from mouse, rat, and dog. – Receptor tyrosine kinase (rbB-4, ErbB-2, EGFR, PDGFR1B, c-Fms, and Tie-2); cytoplasmic tyrosine kinases (Src, ITK, and Lck); proline-directed kinases (CDK-1/Cyclin A, CDK-2/Cyclin A, JNK1, JNK2 and TrJNK3); and serine/threonine directed kinases (GSK3, P38, AKT3, zPKC, PLK1 and PLK3	– GW786034B inhibited substrate phosphorylation catalyzed by human VEGFR1, VEGFR2, and VEGFR3 (IC50 = 30, 10, and 47 nM, respectively). – GW786034B inhibited mouse, rat and dog VEGFR2 (IC50 = 17, 42, and 17 nM, respectively). – C-Fms, ITK, Lck, P38, and PDGFR1B were most sensitive to GW786034B inhibition compared to other tyrosine kinases (IC50 = 146, 430, 411, 105.6, and 195 nM, respectively).
RH2003/00076/00/IC50 profiling using 16 protein kinases	ABL1, MET, PDGFR-α, PDGFR-β, IGF2-R, KIT, INS-R, WEE1, PKC- β1, PKC- β2, FAK, FGF-R4, FGF-R1, and FGF-R3, VEGFR-1 and VEGFR-2.	– GW786034B inhibited VEGFR-1 and VEGFR2 (IC50= 13 and 12 nM, repectively). – GW786034B inhibited PDGFR-α, PDGFR-β, and c-Kit with IC_{50} of 71, 84, and 80 nM, respectively. – GW786034B also inhibited FGFR-1 and 3 with IC_{50} of 14 and 13 nM, respectively.
UH2008/00035/00/ Inhibitory effect on kinase activity using apparent inhibition constant (Kiapp)	VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-α/β, Flt-3, c-Kit, B-Raf V600E mutant, B-Raf wild type and C-Raf	– GW786034B has weak inhibitory activity for Raf and FLT3 (Kiapp = 68-160 and 230 nM, respectively). – GW786034B inhibited the activity of VEGFR1, VEGFR2, and VEGFR3, PDGFR-α, PDGFR- β, and c-Kit (Kiapp = 15, 8, 10, 30, 14, and 2.4 nM, respectively).
RH2002/00042/00/ Effect on cellular proliferation and receptor phosphorylation	Human umbilical vein endothelial cells (HUVEC); human tumor cell lines (HT29, MDA-MB-468, PC3, A375P); and normal human fibroblasts (HFF).	– GW786034B inhibited proliferation of HUVEC cells stimulated with VEGF compared to HUVEC cells stimulated with bFGF (IC50 = 21.3 and 720 nM, respectively). – GW786034B had IC50>30 nM for HT29, MDA-MB-468, PC3, A375P. – GW786034B had IC50=1012.3 nM for HFF cell line.
UH2008/00110/00/ Effects on cellular proliferation in human tumor cell lines	Panel of 282 human cell lines.	GW786034B showed activity in the following cell lines (IC50<1 uM): GDM1 AML, ARH-77 myeloma, NCI-H716 colon carcinoma, G402 kidney leiomyoblastoma, CGTH-W-1 thyroid carcinoma, A204 rhabdomyosarcoma, CML-T1 CML blast phase, HUT78 lymphoma cutaneous T cell, and KATOIII stomach carcinoma.
UH2008/00016/00/ Activity and selectivity for VEGFR2, PDGFR-β, c-Kit and Flt-3 kinase	Human umbilical vein endothelial (HUVEC); human small cell lung cancer carcinoma (NCI-H526); human foreskin fibroblast (HFF);	– GW786034B inhibited autophosphorylation of VEGFR-2, c-Kit and PDGFR-β receptors (IC50=2.6, 3, and 8 nM, respectively). – GW786034B was much less active in blocking

Study #/Type of study	Test system	Significant findings
	human B-cell acute lymphoblastic leukemia (RS4-11) and human actue myelogenous leukemia (MV4-11)	Flt-3 receptor activation in RS4-11 cells (≥100 nM). – Sunitinib and sorafenib had more activity at inhibiting proliferation of RS4;11 and MV4-11 cells compared to GW786034B.
UH2008/00015/00/ Cellular activity against imatinib-resistant c-kit mutants	Imatinib-resistant c-kit mutants (V654A, T670I, Y816V, Y823D); imatinib-sensitive mutant, (K642E); and wild-type c-kit in transiently transfected CHO-K1 cells	– GW786034B showed more activity at inhibiting wild-type c-Kit activation compared to imatinib (IC50<0.01 and 0.230 uM, respectively). – GW786034B was moderately active against at inhibiting K642E (IC50 = 0.48 and 0.26 uM, respectively) compared to imatinib. – GW786034B was somewhat active against T670I gatekeeper mutation (IC50=0.31 uM). – GW786034B had weak activity against V654A mutant (IC50=1.45 uM) and was inactive against D816V and Y823D mutations (IC50=>10 and >5 uM, respectively).
Primary pharmacodynamics – in vivo		
RH2003/00005/00/ Inhibition of VEGFR2 phosphorylation	Female Swiss nude mice stimulated with exogenous VEGF ($VEGF_{121}$)	– GW786034B inhibited phosphorylation at 30 mg/kg at 8, 16, and 24-hours post-dose. – GW786034B inhibited phosphorylation in mice for up to 8 hours at a dose of 30 mg/kg. – GW786034B inhibited VEGF2 phosphorylation at doses ≥10 mg/kg.
RH2002/00043/00/ Anti-tumor activity in various human tumor xenografts	Colon carcinoma (HT29), head and neck (HN5), prostate (PC3), and melanoma (A375P) human tumor xenografts	Inhibition of tumor growth was slightly lower with the mono-salt form (GW786034B) compared to the di-salt form (GW786034A) when given orally at 10, 30, and 100 mg/kg either qd or bid in HT29, HN5, PC3, and A375P tumor xenografts models.
UH2008/00109/00/ Effects on growth and of renal cell tumors	CAKI-1, CAKI-2, ACHN, A498, and 786-O tumor xenografts in female CD-17 SCID and Swiss nude mice	– GW786034B inhibited CAKI-2 and A498 tumors at ≥10 mg/kg in CB-17 SCID mice compared to Swiss nude mice. – GW786034B inhibited tumors at a lesser extent in ACHN renal cell tumors in Swiss nude mice at ≥30 mg/kg.
UH2008/00114/00/ Anti-tumor activity against angiogenesis inhibitors	HT29 and Colo-205 (colon carcinoma) tumor xenografts in female CD-1 nude mice	GW786034B inhibited colon tumors to a lesser extent compared to sunitinib and sorafenib in HT-29 and Colo-205 tumor xenografts in CD-1 mice when given orally for 21 days.
RH2003/00006/00/ Effects on the growth of prostate tumors	Transgenic mouse model for metastatic prostate cancer, CR2-T-Ag	GW786034B had no effect on prostate tumor growth and organ weights (liver, lung, and abdominal lymph nodes) when compared to control.
RH2002/00048/00/ Effects of di-HCl salt in mouse model of angiogenesis	Basic fibroblast growth factor (bFGF)/Matrigel® model of angiogenesis in Swiss nu/nu mice	– GW786034A had no effect at inhibiting angiogenesis in mice in twice a day (bid) verses once a day (qd) dosing regimens. – The only exception, however, was at the low dose of 10 mg/kg where there was a greater effect with the twice a day compared to the once a day dosing regimen.
RH2002/00049/00/ Effects of mono-HCl salt in mouse model of	Basic fibroblast growth factor (bFGF)/Matrigel® model of angiogenesis in Swiss nu/nu mice	GW786034A showed greater inhibition of tumors compared to GW786034B at ≥30 mg/kg.

Study #/Type of study	Test system	Significant findings
angiogenesis		
Pharmacodynamic drug interactions		
UH2008/00107/00/ Effects of pazopanib and bevacizumab on delivery of chemotherapeutic agents in tumor xenografts in mice	HT-29 and NCI-11460 xenograft tumors	Both models did not support the hypothesis that treatment with anti-angiogenic agents enhances the delivery of chemotherapeutic agents to the tumor tissue.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Absorption:

The pharmacokinetic parameters of pazopanib have been determined in the rat, dog and monkey as outlined in sponsor's Table 4. Following intravenous administration of the dihydrochloride salt of pazopnaib (GW786034A), plasma clearance was low in all 3 species, with terminal half-life ranging from 2.2 hours (dog) to 4.7 hours (monkey). The volume of distribution was approximately 70%, 50% and 40% of total body water in the rat, dog, and monkey, respectively. In humans, pazopanib has low plasma clearance (0.21 to 0.45 L/hour) and a long half-life (26.7 to 39.3 hours) following intravenous administration (Information taken from human studies reviewed by Clinical Pharmacology). The oral bioavailability of pazopanib was moderate to high in male rats (72% at 10 mg/kg), dogs (47% at 1 mg/kg) and monkeys (49% or 53% at 5 mg/kg). Higher mean AUC values were observed when GW786034A was administered iv compared to oral dosing when administered to mice, rats, dogs, and monkeys. Dogs that were fasted prior to drug administration showed rapid decreases in plasma levels when following iv administration. Fed dogs showed similar findings following both iv and oral administration. No changes in plasma levels were seen in fasted and fed monkeys and rats following either iv and oral administration.

Table 4. Pharmacokinetic Parameters of Pazopanib after Single Oral and Intravenous Administration to Male Rats, Dogs and Monkeys

Pharmacokinetic Parameter[1]	Rat (n=3)	Dog (n=2)	Monkey (n=4)
Intravenous administration[2]:			
Dose (mg/kg):	10	1	5
Plasma clearance (mL/min/kg)	1.7	1.4	1.6
Volume of distribution at steady state (L/kg)	0.478	0.297	0.283
Terminal half-life (h)	3.6	2.2	4.7
Oral administration[3]:			
Dose (mg/kg):	10	1	5
Absolute bioavailability (%F)	72	47	49 53[4]

Key: Data shown are mean values. ND = Not determined.
1 = Rats were allowed food and water throughout the study. Dogs and monkeys were fasted overnight, then fed following the 8-hour blood collection time (unless otherwise noted).
2 = Pazopanib dihydrochloride salt, administered intravenously in 30% hydroxyl-beta-cyclodextrin in 0.05 M methanesulfonic acid.
3 = Pazopanib dihydrochloride salt, administered orally in 0.5% hydroxypropylmethylcellulose (HPMC) in 0.1% Tween 80.
4 = Monkeys were fed 30 to 45 minutes prior to dosing.

Best Available Copy

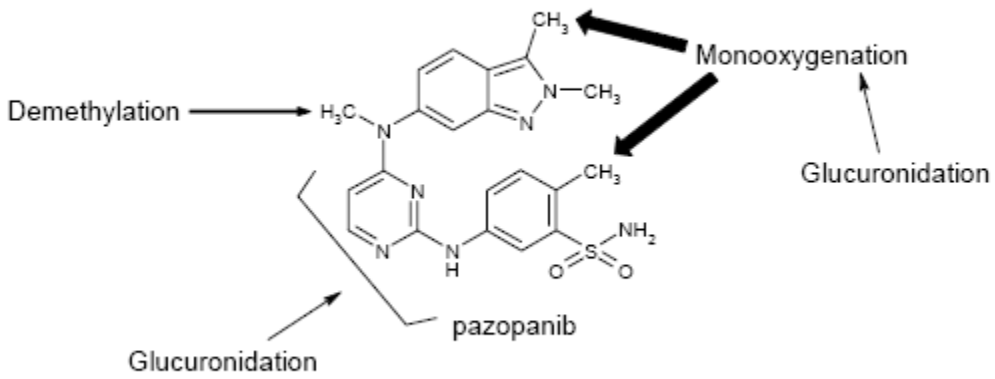
[Table excerpted from sponsor]

The (b) (4) of the monohydrochloride (GW786034B) form of pazopanib did not substantially change the systemic exposure following oral dosing in male rats at 300 mg/kg and in male monkeys at 50 mg/kg. In addition, oral administration of the monohydrochloride (GW786034B) or dihydrochloride (GW786034A) forms of pazopanib had no effect on exposure levels when given to male monkeys at 50 mg/kg. Metabolites of GW786034 caused no significant change in systemic exposure (AUC and Cmax) when administered daily for 7 days (at 100 mg/kg) in male mice. In 13-week repeat dose toxicokinetic studies in mice, systemic exposure either did not change or increase marginally over dose levels ranging from 300 mg/m^2 to 3000 mg/m^2. In oral repeat dose toxicokinetic studies of up to 26 weeks in rats (doses of 3, 30, and 300 mg/kg/day or 18, 180, and 1800 mg/m^2) and 52 weeks in the monkey (doses of 5, 50, and 500 mg/kg/day or 60, 600, and 6000 mg/m^2), there was a less than proportionally increase in systemic exposure with dose in both species. There were no marked differences in exposure over the dosing interval or between sexes for either species.

Distribution:
In distribution studies performed by the sponsor, pazopanib was highly protein bound. In mice, rats, dogs, monkeys, and humans > 99% of the drug was protein bound at all tested concentrations. Pazopanib was also widely distributed throughout the bodies of both male and female rats. *In vivo*, the drug associated strongly with melanin producing cells including the pigmented skin, the uveal tract, and the meninges. In all of these organs drug was detectable following a single dose administration for at least 35 days, the latest timepoint collected for either sex. Maximum concentrations of the drug were also high in these organs, particularly in the uvea. In addition, the liver had some of the highest drug concentrations detected and in males pazopanib was detectable for up to 7 days after administration of a single dose. Finally, pazopanib was found to be an inhibitor of the OATP1B1 transporter at an IC_{50} of 0.79 μM. This subfamily of uptake transporters in the liver is important in the transport of both xenobiotic and endogenous compounds. Disruption of this transport can lead to changes not only in drug elimination, but in general liver function due to changes in transcription caused by the failure to transport compounds that have direct interactions with nuclear receptors (1).

Metabolism:
Following *in vitro* incubation of radio-labeled pazopanib with hepatocytes and hepatic microsomes from mice, rats, rabbits, dogs, monkeys and humans, the route of metabolism was mainly mono-oxygenation, di-oxygenation and potential oxygenation of a methyl group to a carboxylic acid. Glucuronidation of a mono-oxygenated metabolite was a minor route detected in human hepatocytes (See sponsor Figure 2). No human specific Phase 1 metabolites were detected in human microsomal or hepatocyte incubations. In mice, rat, rabbit, dog, and monkeys hepatocytes, other routes of metabolism included glutathione conjugation and related pathways (minor route), demethylation and glucuronidation.

Figure 2. Overview of the Human Biotransformation of Pazopanib



Note: Thickness of arrows approximates relative significance of the pathways.

[Figure excerpted from sponsor]

Following oral administration of radio-labeled-pazopanib the principal radio-labeled component was unchanged pazopanib in mouse, rat, monkey and human plasma.

Pazopanib was excreted as unchanged parent compound via the feces following single oral administration of radio-labeled drug in both rats (54% and 46% of administered dose in male and female intact rats, respectively and 39% of administered dose in male bile duct cannulated (BDC) rats and monkeys (60.4% and 47% of administered dose in male and female intact monkeys, respectively and 45.2% of administered dose in BDC monkeys). The metabolites of pazopanib were also eliminated largely via feces instead of urine. Biliary metabolites accounted for a small amount in both rats (<2%) and monkeys (<4%). The fecal metabolites together accounted for 1.45% and 14% of administered dose in rats and monkeys, respectively, whereas the urinary metabolites together accounted for less than 1% of the administered dose in both species.

In studies with human liver microsomes, pazopanib showed moderate to marked inhibition of cytochrome P450 enzymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 with IC_{50} values ranging from 7.9 µM. The oxidiative metabolism of pazopanib was mediated mainly by CYP3A4 with minor contributions from CYP1A2 and CYP2C8. In addition, pazopanib was shown to a direct inhibitor of human UGT1A1 (IC_{50} = 1.2 µM) *in vitro*.

Excretion:
After administration of a single oral dose of radiolabelled pazopanib in intact and bile duct cannulated rats and monkeys, the majority of the parent drug was excreted via the feces with greater amounts of the parent drug in monkeys (intact = 85-87% and BDC = 60%) compared to rats (intact = 52-61% and BDC = 43%). Urinary excretion of the drug was higher in rats intact rats = 15-17% and BDC = 25%) compared to monkeys (intact monkeys = 0% and BDC = 2%). Biliary excretion, however, was present in monkeys only and accounted for 23% of the administered radioactivity. Most of the radio-labeled

drug was eliminated to a greater extent in rats (48 hours post dose) compared to monkeys (96 hours post-dose) with a total recovery of radioactivity in both species.

2.6.4.2 Methods of Analysis – No studies reviewed

2.6.4.3 Absorption

Pharmacokinetics/Toxicokinetics after single dose:

RD2001/01451/00: Exposure of GW786034X following oral administration of GW786034A at 10, 30, and 100 mg/kg to female CD-1 mice

Key Findings:
- GW786034A (dihydrochloride salt of pazopanib) showed rapid and prolonged absorption, as evidenced by dose-proportional increase in Cmax and AUC values, when given orally to mice at doses ranging from 10 to 100 mg/kg.
- A 10-fold increase in dose (10 mg/kg to 100 mg/kg) resulted in a 5-fold increase in exposure in terms of Cmax and AUC values.

Report #:	RD2001/01451/00
Module:	4.2.2.2
Conducting Laboratory and Location:	Memorandum Report – no information was provided
Date of Study Initiation:	January 29, 2002
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	GW786034A – no additional information was given

Doses:	Single Dose of 10, 30, and 100 mg/kg
Species/strain:	Mice/CD-1
Number/sex/group or time point:	27 females/dose
Route, formulation, volume, and infusion rate:	Oral Gavage; 0.5% HPMC and 0.1% Tween 80 10 mL/kg
Age:	No information was provided
Weight:	20-25 g
Sampling times:	0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 post-dose
Study design:	3 mice/time point

Results:

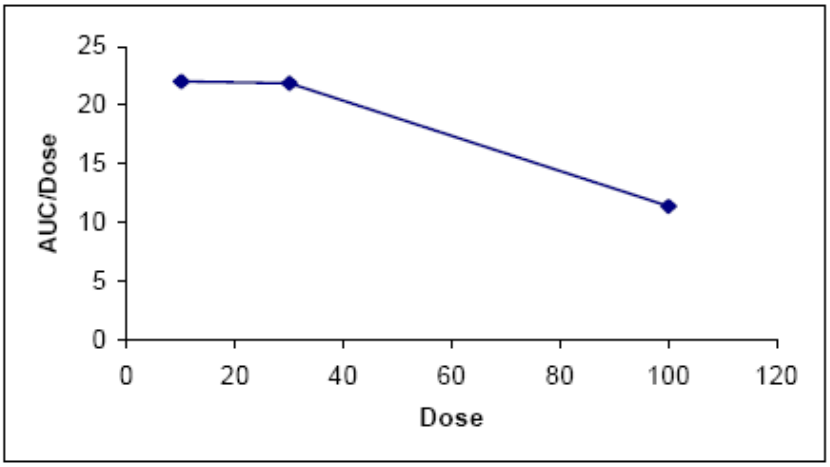
- Cmax and AUC values increased with increasing dose.
- Time to maximum plasma concentration (T_{max}) ranged from 0.5 to 6 hours.
- Dose linearity was observed from 10 to 30 mg/kg, however a less than proportional increase in systemic exposure was observed from 10 to 100 mg/kg (See sponsor's Figure 3).
- Similar half-lives were observed at each dose level and ranged from 3.8 to 4.6 hours.
- Summary of results are listed below in Sponsor's Table 3.

Table 3. Pharmacokinetic Parameters of GW786034X in Female CD-1 Mice Following Oral Administration at 10, 30 and 100 mg/kg

Dose	10 mg/kg	30 mg/kg	100 mg/kg
C_{max} (µg/mL)	19.5	58.0	127.8
T_{max} (h)	6	2	0.5
Half-life (h)	4.4	4.6	3.8
$AUC_{0\text{-}inf}$ (µg*h/mL)	220.2	656.8	1140.8
AUC/Dose	22.0	21.9	11.4

[Table excerpted from sponsor]

Figure 3. Dose Linearity of GW786034X in Female CD-1 Mice



[Figure excerpted from sponsor]

CD2002/00094/00: Preliminary investigation of the intravenous pharmacokinetic and oral bioavailability of GW786034B in conscious rat

Key Findings:

- GW786034B was quantifiable in the plasma for the entire 24 hours after iv and oral administration.
- Following iv administration, GW786034B was estimated to have a low clearance with a volume of distribution equal to total body water and a MRT of 301 minutes.
- Following oral administration, GW786034B had variable oral bioavailability (F%) with a mean value of 61%.

Report #:	CD2002/00094/00
Module:	4.2.2.2
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	April 3, 2007
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	GW786034B, 786034-A2-01M, 98.5%

Doses:	Single Dose of 2 and 10 mg/kg free base
Species/strain:	Rat/Sprague-Dawley
Number/sex/group or time point:	4 males
Route, formulation, volume, and infusion rate:	Oral Gavage and iv infusion 30% Encapsin in water 10 mL/kg (oral) and 4.0 mL/kg (iv)
Age:	~ no info. was provided
Weight:	~200-300 g
Sampling times:	0, 15, 30, 60, 120, 240, 480, and 1440 minutes
Study design:	4 rat/time point On study day 1, group 1 received GW786034B by oral gavage at a dose of 10 mg/kg of free base On study day 2, group 2 received GW786034B as a 60-minute iv infusion at a dose of 2 mg/kg of free base

Results:

Following 60 minute iv infusion (See Sponsor's Table 3):

- The mean maximal plasma concentration was 9.47 ug/mL and plasma clearance was low with a value of 2.14 mL/min/kg.
- The mean volume of distribution at steady-state was 582 mL/kg.
- Mean terminal phase half-life was about 4.0 hours.

Following oral administration(See Sponsor's Table 3):

- The mean maximal plasma concentration was 18.8 ug/mL were observed at 30 to 246 minutes.
- Oral bioavailability (F%) varied with a mean value of 61%

Table 3 Mean Pharmacokinetic Parameters of GW786034X in the Rat

Parameter	Intravenous	Oral Solution
Dose (mg/kg)	2.15 ± 0.058	10.3 ± 0.234
CL_p (mL/min/kg)	2.14 ± 2.61	a
AUC (0-inf) (min•ug/mL)	2116 ± 1294	6234 ± 8981
% Extrap AUC (0-inf)	1.42 ± 0.518	2.62 ± 1.26
AUC (last) (min•ug/mL)	2084 ± 1269	6027 ± 8643
Vss (L/kg)	0.582 ± 0.627	a
MRT (min)	301 ± 39.1	456 ± 73.7
$T_{1/2}$ (min)	244 ± 25.4	a
Cmax (ug/mL)	9.47 ± 5.29	18.8 ± 19.7
Observed Tmax (min)	a	146 ± 116
F (%)	a	61.4 ± 87.7

Values are the mean ± SD of n = 4 animals.

[a] Not calculated for this route of administration.

[Table excerpted from sponsor]

CD2003/00102/00: Preliminary investigation to evaluate the oral PK for GW786034B HCl salt ((b) (4) in male Sprague-Dawley rats.

Key Findings:

- (b) (4) GW786034B was quantifiable in the plasma for the entire 24 hours with a mean Cmax and AUC(0-t) values of 49.4 ug/mL and 541 ug.h/mL, respectively.
- Cmax values were variable between the animals (ranging from 49.4 ± 21.3 µg/mL).
- Mean Tmax was long with a value of 422 minutes.
- Summary of the results are listed in Sponsor's Table 1.

Report #:	CD2003/00102/00
Module:	4.2.2.2
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	January 21, 2003
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	(b) (4) GW786034B, 786034B-A5-01P-MIC, 91.6%

Doses:	Single Dose of 289 mg/kg
Species/strain:	Rat/Sprague-Dawley
Number/sex/group or time point:	4 males
Route, formulation, volume, and infusion rate:	Oral Gavage 0.5% hydroxypropyl-methylcellulose/ 0.1% Tween 80 10 mL/kg
Age:	~ no info. was provided
Weight:	~0.38-0.48 kg
Sampling times:	0, 15, 30, 60, 120, 240, 480, and 1440 minutes
Study design:	4 rat/time point

Table 1 Summary of the Mean Pharmacokinetic Parameters of GW786034 from Male Sprague-Dawley Rats Following Oral Dosing of GW786034B

PK Parameters	GW786034B suspension
Actual Dose (mg free base/kg)[1] (Parent)	289 ± 8.00
C_{max} (µg/mL)	49.4 ± 21.3
T_{max} (min)	422 ± 122
$AUC_{(0-t)}$[2] (µg*min/mL)	32440 ± 14206

n = 4 males

1. Calculated assuming the density of suspension is 1 g/mL, using nominal concentration of the dose solution (30 mg/mL)
2. $AUC_{(0-t)}$ refers to the area from time 0 to the last quantifiable concentration.

[Table excerpted from sponsor]

RD2001/01452/00: Pharmacokinetics and oral bioavailability of GW786034X following intravenous and oral administration of GW786034A to male Han Wistar rats

Key Findings:
- Single dose of GW786034A (dihydrochloride salt of pazopanib) showed higher AUC values after iv administration compared to oral administration in male rats.
- There was no change in plasma concentrations following either iv and oral administration.
- GW786034A was estimated to have a low clearance, high volume of distribution following iv administration.
- The oral bioavailability (F%) was also high (72%) when administered orally.

Report #:	RD2001/01452/00
Module:	4.2.2.2
Conducting Laboratory and Location:	Memorandum Report – no information was provided
Date of Study Initiation:	January 22, 2002
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	GW786034A, U17574/39/1, no additional information was given

Doses:	Single Dose of 10 mg/kg
Species/strain:	Rats/Han Wistar
Number/sex/group or time point:	3 males/group
Route, formulation, volume, and infusion rate:	Oral Gavage and bolus intravenous injection 0.5% HPMC and 0.1% Tween 80 10 mL/kg (oral and iv)
Age:	No information was provided
Weight:	200-250 g
Sampling times:	0.03, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours post-dose
Study design:	3 rats for iv and oral for PK

Results:
- Systemic plasma clearance (CLp) was 1.7 mL/min/kg
- Volume of distribution at steady state (Vdss) was large (478 mL/kg).
- GW786034X was eliminated from the systemic circulation after IV administration with a short half-life (3.6 hours).
- AUC values were higher after iv administration (98506 ngh/mL) compared to oral administration (70429 ngh/mL).
- Mean peak plasma concentration (C_{max}) after oral administration was 17270 ng/mL with a corresponding peak time (T_{max}) of 0.5 to 1 hour and plasma half-life of 4.2 hours.
- Oral bioavailability ranged from 49 to 93% with a mean value of 72%.

– Summary of results are shown in sponsor's Table 2.

Table 2. Pharmacokinetic Parameters of GW786034X in Male Han Wistar Rats Following IV and Oral Administration at 10 mg/kg

	Animal #	C_{max} (ng/mL)	T_{max} (h)	Half-life (h)	AUC_{0-inf} (ng*h/mL)	CL (mL/min/kg)	Vdss (mL/kg)	F (%)
	1	--	--	4.1	107,283	1.6	498	--
	2	--	--	3.5	109,350	1.5	432	--
	3	--	--	3.3	78,885	2.1	503	--
Mean				**3.6**	**98,506**	**1.7**	**478**	
SD				**0.4**	**17,024**	**0.3**	**40**	
	4	17,500	0.5	3.1	48,117	--	--	49
	5	17,700	1.0	5.2	91,744	--	--	93
	6	16,600	1.0	4.3	71,425	--	--	73
Mean		**17,267**	**0.8**	**4.2**	**70,429**			**72**
SD		**586**	**0.3**	**1.1**	**21,831**			**22**

[Table excerpted from sponsor]

RD2001/01514/00: Pharmacokinetics and oral bioavailability of GW786034X following intravenous and oral administration of GW786034A to male beagle dogs

Key Findings:

– Fasted and fed dogs had rapid decreases in plasma levels after iv and oral (fed only) administration.
– Single dose of GW786034A had a higher mean AUC value when administered via iv administration compared to oral administration.
– GW786034A had a low clearance and a moderate volume of distribution following iv administration.
– Oral bioavailability (F%) varied somewhat (30-64%) had a mean value of 47% when administered orally.

Report #:	RD2001/01514/00
Module:	4.2.2.2
Conducting Laboratory and Location:	Memorandum Report – no information was provided
Date of Study Initiation:	February 18, 2002
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	GW786034A, U17574/39/1, no additional information was given

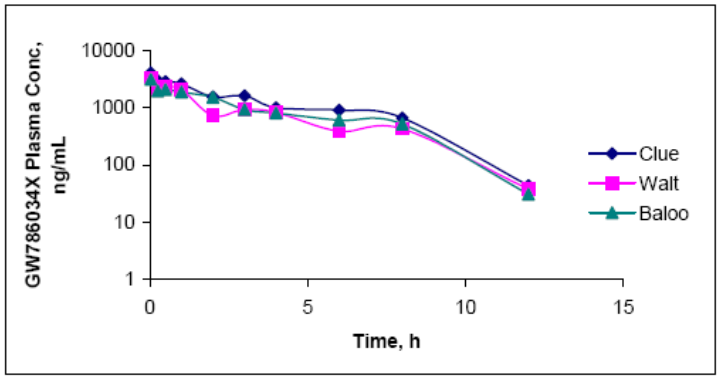
Doses:	Single Dose of 1 mg/kg
Species/strain:	dogs/beagle
Number/sex/group or time point:	2 males/group
Route, formulation, volume, and infusion rate:	Oral Gavage and bolus intravenous injection

	0.5% HPMC and 0.1% Tween 80 1 mL/kg (oral and iv)
Age:	No information was provided
Weight:	10-12 kg
Sampling times:	0.03, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours post-dose (iv) 0.08, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours post-dose (oral)
Study design:	Dogs were administered drug by iv first followed by a 2-week washout period before oral administration 3 dogs for iv and oral for PK

Results:

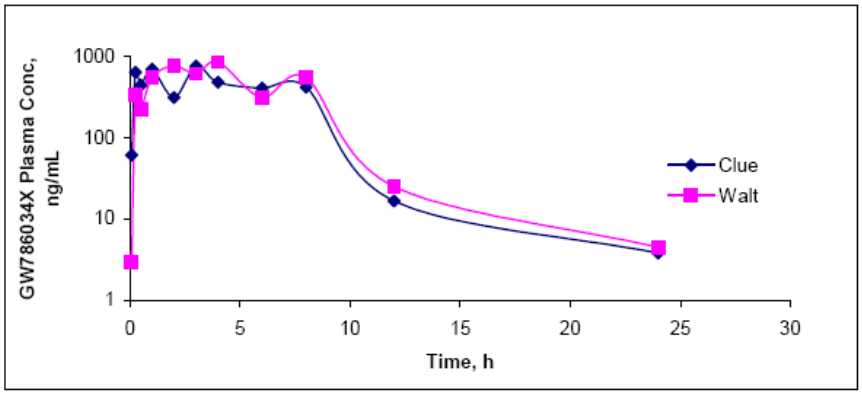
- A 15-fold decrease in plasma concentrations was observed between 8 and 12 hours while less than a 2-fold drop was observed between 4 and 8 hours. A similar profile was observed following oral administration (See Sponsor's Figure 2 and 3).
- The compound was rapidly absorbed following a single oral solution dose at 1 mg/kg.
- Systemic plasma clearance (CLp) was 1.4 mL/min/kg
- Volume of distribution at steady state (Vdss) was (279 mL/kg).
- GW786034X was eliminated from the systemic circulation after IV administration with a short half-life (2.2 hours).
- AUC values were higher after iv administration (98506 ngh/mL) compared to oral administration (70429 ngh/mL).
- Maximum plasma concentration after oral administration was observed 3 to 4 hours post-dose was 810 ng/mL with a Cmax value of 1.8 uM and a short corresponding peak time (T_{max}) of 3.5 hours.
- Oral bioavailability ranged from 30 to 64% with a mean value of 47%.
- Summary of results are shown in sponsor's Table 2.

Figure 2. Plasma Concentration-Time Profile of GW786034X in Male Beagle Dogs Following IV Administration



[Figure excerpted from sponsor]

Figure 3. Plasma Concentration-Time Profile of GW786034X in Male Beagle Dogs Following Oral Administration



[Figure excerpted from sponsor]

Table 2. Pharmacokinetic Parameters of GW786034X in Male Beagle Dogs Following IV and Oral Administration

	Animal ID	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	Half-life (h)	$AUC_{0\text{-}inf}$ (ng*h/mL)	CL (mL/min/kg)	Vdss (mL/kg)	F (%)
	Clue	0.8	--	--	2.2	13036	1.0	220	--
	Walt	0.95	--	--	2.2	8572	1.8	377	--
	Baloo	0.8	--	--	2.2	12122	1.4	295	--
Mean					**2.2**	**11243**	**1.4**	**297**	
SD					**0.0**	**2358**	**0.4**	**79**	
	Clue	1	765	3.0	NR[1]	4913	--	--	30
	Walt	1	855	4.0	NR	5751	--	--	64
Mean			**810**	**3.5**		**5332**			**47**

[1] NR = not reported

[Table excerpted from sponsor]

CD2002/00093/00: Preliminary PK evaluation of GW786034B following iv and oral administration in the male cynomolgus monkey.

Key Findings:
- GW786034B was estimated to have a low clearance and with a volume of distribution equal to approximately 40% of total body water following iv administration.
- The oral bioavailability (F%) was low with a mean value of 16% when administered orally.

Report #:	CD2002/00093/00
Module:	4.2.2.2
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	April 18, 2002
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	GW786034B, 786034B-A2-01M, 98.5%

Doses:	Single Dose of 2 and 10 mg/kg
Species/strain:	Cynomolgus monkeys
Number/sex/group or time point:	4 males
Route, formulation, volume, and infusion rate:	Oral Gavage and iv infusion 30% Encapsin in water 10 mL/kg (oral) and 4.0 mL/kg (iv)
Age:	~ no info. was provided
Weight:	~3-5 kg
Sampling times:	0, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720, 1440, and 2880 minutes
Study design:	4 monkey /time point On study day 1, group 1 received GW786034B by oral gavage at a dose of 10 mg/kg of free base On study day 2, group 2 received GW786034B as a 60-minute iv infusion at a dose of 2 mg/kg of free base

Results:

Following iv administration (See sponsor's Table 3):
- Mean Cmax was 10.3 ug/ml
- Plasma clearance was low with a value of 1.35 mL/min/kg
- Volume of distribution (Vss) was 268 mL/kg (approximately 40% of total body water)
- $T_{1/2}$ was short lasting ~ 3 hours .

Following oral administration (See sponsor's Table 3):
- Mean Cmax was 1.17 ug/ml (observed after 6-8 hours)
- AUC(0-inf) was 1225 min ug/mL

- MRT was 1042 minutes
- Oral bioavailability (F%) varied with a mean value of 16%.

Table 3 Mean Pharmacokinetic Parameters of GW786034X Following Administration of GW786034B to the Male Cynomolgus Monkeys

Parameter	Intravenous	Oral Solution
Dose (mg/kg)	2.30 ± 0.497	10.6 ± 0.0444
CL_p (mL/min/kg)	1.35 ± 0.236	a
AUC (0-inf) (min•ug/mL)	1755 ± 569	1225 ± 278
% Extrap AUC (0-inf)	0.427 ± 0.388	3.54 ± 1.37
AUC last (min•ug/mL)	1748 ± 570	1184 ± 279
Vss (L/kg)	0.268 ± 0.0504	a
MRT (min)	201 ± 43.5	1042 ± 20.3
$T_{1/2}$ (min)	175 ± 25.6	a
Cmax (ug/mL)	10.3 ± 3.75	1.17 ± 0.165
Observed Tmax (min)	60.5 ± 1.00	446 ± 57.2
F (%)	a	16.1 ± 6.28

Values are the mean ± SD of n = 4 animals.

[a] Not calculated for this route of administration.

[Table excerpted from sponsor]

RD2001/01169/00: Pharmacokinetics and oral bioavailability of GW786034X following intravenous and oral administration of GW786034A to non-naïve male cynomolgus monkeys

Key Findings:

- Similar half-lives were observed following iv and oral administration.
- A less than proportional increase in exposure was observed with an increase in dose. A 10-fold increase in dose resulted in a 6-fold increase in exposure.
- Unlike dogs, no change in plasma levels were observed after iv or oral administration of GW786034A in fed and fasted monkeys.
- Single dose GW786034A had a higher AUC when administered by iv administration compared to oral administration.
- GW786034A had a low clearance and a moderate volume of distribution following iv administration.
- Oral bioavailability (F%) varied to a great degree (20-92%) and had a mean value of 49% when administered orally.

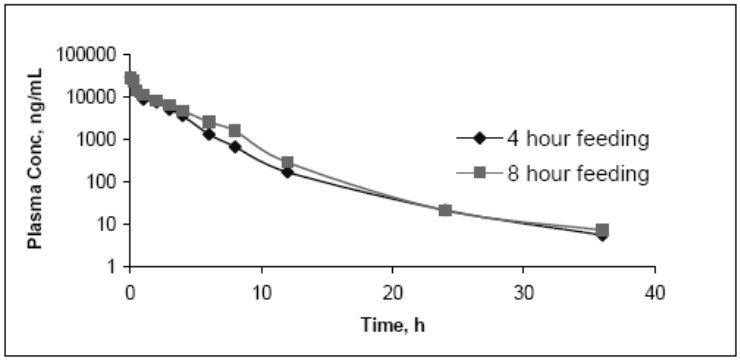
Report #:	RD2001/01169/00
Module:	4.2.2.2
Conducting Laboratory and Location:	Memorandum Report – no information was provided
Date of Study Initiation:	July 3, 2002
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	GW786034A, U17574/61/1, no additional information was given

Doses:	Single Dose of 5 and 50 mg/kg (oral only)
Species/strain:	monkeys/naïve male cynomolgus
Number/sex/group or time point:	4 males/group
Route, formulation, volume, and infusion rate:	Oral Gavage and intravenous injection 30% HPBCD in 0.05 M methansulfonic acid (iv) 0.5% HPMC and 0.1% Tween 80 (oral) 5 mL/kg (iv and oral)
Age:	No information was provided
Weight:	No information was provided
Sampling times:	2, 15 and 30 minutes, and 1, 2, 3, 4, 6, 8, 12, 24 and 36 hours postdose (iv) 5, 15 and 30 minutes, and 1, 2, 3, 4, 6, 8, 12, 24, 36, 40, 44 and 48 hours post-dose (oral)
Study design:	Group 4 monkeys received 50 mg/kg

Results:

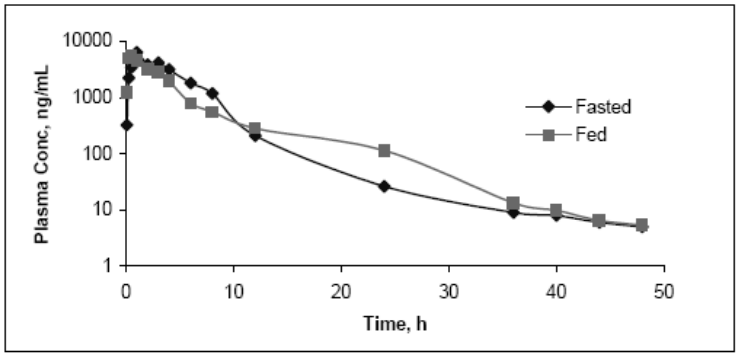
- No change in pharmacokinetics was observed in fed compared to fasted monkeys (See Sponsor's Figure 2 and 3).
- After oral administration at 5 and 50 mg/kg, a less than proportional increase in exposure was observed with an increase in dose (a 6-fold increase in AUC and 4-fold increase in C_{max} resulted from a 10-fold increase in dose).
- T_{max} and half-life values were similar at 5 and 50 mg/kg dose levels.
- Systemic plasma clearance (CLp) was 1.6 mL/min/kg
- Volume of distribution at steady state (Vdss) was (283 mL/kg).
- GW786034X was eliminated from the systemic circulation after IV administration with a short half-life (4.7 hours).
- Maximum plasma concentration after oral administration at 5 mg/kg was 800 ng/mL with a Cmax value of 1.8 uM and half life of 4.9 hours.
- Oral bioavailability ranged from 20 to 92% with a mean value of 49%.
- Summary of results are shown in sponsor's Table 3.

Figure 2. Mean Plasma Concentration-Time Profile of GW786034X in Male Cynomolgus Monkey after IV Administration at 5 mg/kg



[Figure excerpted from sponsor]

Figure 3. Mean Plasma Concentration-Time Profile of GW786034X in Male Cynomolgus Monkey after Oral Administration at 5 mg/kg



[Figure excerpted from sponsor]

Table 3. Mean Pharmacokinetic Parameters (SD) of GW786034X in Male Cynomolgus Monkey after IV and Oral Administration

Dose Session	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (h)	Half-life (h)	AUC_{0-inf} (μg*h/mL)	CL (mL/min/kg)	Vdss (mL/kg)	F (%)
1	5	--	--	5.5 (1.5)	46.7 (12.2)	2 (0.5)	285 (28)	--
2	5	--	--	4.7 (0.3)	58.9 (11.7)	1.6 (0.3)	283 (24)	--
3	5	8.0 (2.1)	1.4 (1.1)	4.9 (2.5)	28.4 (15.3)	--	--	49 (31)
4	50	33.7 (9.9)	2.0 (1.2)	6.2 (0.6)	174 (60.9)	--	--	30 (9)
5	5	5.5 (1.3)	0.5 (0)	5.5 (0.5)	22.7 (5.5)	--	--	53 (5)

[Figure excerpted from sponsor]

RD2002/00061/00: Exposure of GW786034X following oral administration of GW786034B to non-naïve male cynomolgus monkeys.

Key Findings:

– Oral exposure of the synthetic intermediate of pazopanib or GW786034X at 50 mg/kg is the same whether it is administered as the monohydrochloride or dihydrochloride salt forms.

Report #:	RD2002/00061/00
Module:	4.2.2.2
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	March 2, 2002
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	GW786034B, 786034B-A2-01M, 98.5%

Doses:	Single Dose of 50 mg/kg
Species/strain:	Cynomolgus monkeys
Number/sex/group or time point:	4 males
Route, formulation, volume, and infusion rate:	Oral Gavage 0.5% hydroxypropylmethylcellulose (HPMC)/0.1% Tween 80 5 mL/kg
Age:	~ no info. was provided
Weight:	~ no info. was provided
Sampling times:	0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24

	hours post-dose
Study design:	4 monkey /time point

Results:
- Mean Cmax value was 30.3 ± 10.4 µg/mL at 0.5 to 1 hours post-dose.
- Mean oral exposure (AUC0-inf) was 141 ± 13 µg*h/mL.
- Oral bioavailability (F) was determined to be 30 ±2%.
- Mean Tmax was 0.9.
- Summary of the results are listed in Sponsor's Table 2.

Table 2. Individual Animal Pharmacokinetic Parameters of GW786034X after Oral Administration

Animal ID	C_{max} (ng/mL)	T_{max} (h)	Half-life (h)	AUC_{0-inf} (ng*h/mL)	F (%)
1001	33000	1.0	3.6	146621	31
1002	42183	1.0	5.9	156191	33
1003	17089	0.5	6.4	130352	28
1004	28967	1.0	10.3	130905	28
Mean	30310	0.9	6.6	141017	30
SD	10405	0.3	2.8	12618	2

[Table excerpted from sponsor]

CD2003/00103/00: Preliminary investigation to evaluate the oral PK on GW786034 HCl salt in the male cynomolgus monkey.

Key Findings:
- Mean Cmax and AUC(0-t) values of 33.4 ug/mL and 177 ug.h/mL, respectively.
- Cmax values were similar between the animals while AUC(0-t) values varied.
- Mean Tmax values were variable ranging from 60-121 minutes.
- Summary of the results are listed in Sponsor's Table 1.

Report #:	CD2003/00103/00
Module:	4.2.2.2
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	January 14, 2003
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	(b) (4) GW786034B, 786034-A4-02P,

	98.9%

Doses:	Single Dose of 49.6 mg/kg
Species/strain:	cynomolgus monkey
Number/sex/group or time point:	4 males
Route, formulation, volume, and infusion rate:	Oral Gavage 0.5% hydroxypropyl-methylcellulose/ 0.1% Tween 80 10 mL/kg
Age:	~ no info. was provided
Weight:	~0.39-0.48 kg
Sampling times:	0, 15, 30, 60, 120, 240, 480, and 1440 minutes
Study design:	4 monkey/time point

Table 1 Summary of the Mean Pharmacokinetic Parameters for GW786034 Derived from Plasma Concentration-Time Results from Male Cynomolgus Monkeys Following Single Oral Gavage Administration of GW786034B Suspension.

PK Parameters	GW786034B suspension
Actual Dose (mg/kg)[1] (parent)	49.6 ± 0.3
Cmax (µg/mL)	33.4 ± 3.6
Tmax (min)	105 ± 30
AUC(0-t)[2] (µg.min/mL)	10596 ± 6360

n = 4 males

1. Calculated assuming the density of suspension is 1 g/mL, using nominal concentration (10 mg/mL)
2. $AUC_{(0-t)}$ refers to the area from time 0 to the last quantifiable concentration

[Table excerpted from sponsor]

Pharmacokinetics/Toxicokinetics after repeat doses:

CD2007/00494/01: Pharmacokinetics of GW786034 and its metabolites following daily oral administration of GW786034B for 7 days in male CD-1 mice

Key Findings:

– GW786034B and its metabolites (GSK1268992, GSK1268997, GSK1071306 and GW700201) caused no significant change in systemic exposure (AUC and Cmax) with repeated dosing between Day 1 and Day 7 when given to male mice at 100 mg/kg/day once daily for 7 days.

Report #:	CD2007/00494/01
Module:	4.2.2.2
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	March 27, 2007

GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	GW786034B, 786034B-A5-01P-MIC, 91.6%

Doses:	100 mg/kg/day for 7 days
Species/strain:	Mice/CD-1
Number/sex/group or time point:	4 males
Route, formulation, volume, and infusion rate:	Oral Gavage; 0.5% HPMC and 0.1% Tween 80 10 mL/kg
Age:	~ naïve was only info. given
Weight:	~30 g
Sampling times:	15, 30, 60, 120, 240, 360, 480 and 1440 minutes following dose administration on Days 1 and 7. Additional samples were taken at 0 (predose) on Day 7.
Study design:	4 mice/time point

Results:

- Following oral administration at 100 mg/kg/day, GW786034 was quantifiable in plasma for the entire 24 hour sampling period with the Tmax occurring at 28.50 minutes and 120.25 minutes after dosing on Days 1 and 7, respectively.
- Plasma concentrations were quantifiable for the entire 24 hour sampling period for metabolite GSK1268997 on Days 1 and 7. Tmax for this metabolite occurred at 120 and 240 minutes post-dose on Days 1 and 7, respectively.
- Metabolites GSK1268992, GSK1071306 and GW700201 were quantifiable up to the 480 minute time point on both Days 1 and 7. Tmax for GSK1268992 occurring at approximately 30 minutes on both Days 1 and 7. Tmax for GSK1071306 and GW700201 occurring at approximately 30 and 60 minutes post-dose on Days 1 and 7 respectively.
- A summary of the results are listed in Sponsor's Table 1.

Table 1 Summary of Composite Pharmacokinetic Parameters for GW786034 and Four Metabolites and Metabolite:Parent $AUC_{(0-t)}$ Ratios Following Oral Administration of GW786034 as a Suspension in 0.5% HPMC and 0.1% Tween 80 (pH 1.3) at a Nominal Dose of 100 mg/kg/day to Male Mice (n = 4/ time point)

Regimen (mg/kg/day)	Analyte	Study Day	C_{max} (ng/mL)	T_{max} (min)	$AUC_{(0-t)}$ (ng*min/mL)	$AUC_{(0-t)}$ Ratio Metabolite:Parent
100	GW786034 (parent)	1	120408	28.50	60995220	NA
		7	100895	120.25	59217885	NA
	GSK1268992	1	1218.5	28.50	421428	0.00691
		7	1544.5	31.00	445327	0.00752
	GSK1268997	1	5482.4	120.00	3578508	0.0587
		7	6496.1	240.00	3702814	0.0625
	GSK1071306	1	2804.0	28.50	738497	0.0121
		7	3091.5	60.33	935764	0.0158
	GW700201	1	514.7	28.50	167656	0.00275
		7	540.3	60.33	198843	0.00336

[Table excerpted from sponsor]

CD2007/00493/01: Pharmacokinetics of GW786034 and its metabolites following oral administration of GW786034B for 7 days in male Sprague Dawley rats.

Key Findings:

- There was a less than 2 fold difference in Cmax and AUC (0-t) between Days 1 and 7 for GW786034B and its 4 active metabolites.
- The only exception was for metabolite GSK1268997 at 3 mg/kg/day (2.32-fold increase in AUC(0-t) on Day 7 when compared to Day 1) and for metabolite GSK1268992 at 30 mg/kg/day (approximately 50% decrease in AUC(0-t) on Day 7 when compared to Day 1).

Report #:	CD2007/00493/01
Module:	4.2.2.2
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	March 1, 2007
GLP Compliance:	No
QA Report:	Yes

Drug, lot #, and % purity:	GW786034B, 786034B-A5-01P-MIC, 91.6%

Doses:	Repeat dose of 3 and 30 mg/kg/day for 7 days
Species/strain:	Rat/Sprague-Dawley
Number/sex/group or time point:	4 males
Route, formulation, volume, and infusion rate:	Oral Gavage 0.5% hydroxypropyl-methylcellulose/ 0.1% Tween 80 10 mL/kg
Age:	~ no info. was provided
Weight:	~295-328 g
Sampling times:	0.25, 0.5, 1, 2, 4, 6, 8, and 24 hours
Study design:	4 rat/time point

Results:

- For the 3 mg/kg/day group, metabolite concentration was quantifiable up to 6 or 8 hours for GSK1268992 (Day 7 only) and GSK1268997 (Days 1 and 7), up to 0.5 or 2 hours for GSK1071306 (Day 7 only). GW700201 was not quantifiable on Day 1 and sparsely quantifiable on Day 7 at the 3 mg/kg/day group.
- All four metabolites were quantifiable up to 8 hours on both Day 1 and Day 7 for the 30 mg/kg/day group.
- Median Tmax for GW786034 was approximately 1.12 to 4.09 hours on both Days 1 and 7.
- Median Tmax for GW786034 metabolites was between 1.12 to 4.09 hours on both Days 1 and 7.
- Summary of the results are listed in Sponsor's Table 1.

Table 1 Summary of Mean (SD) Pharmacokinetic Parameters of GW786034 and Metabolites and Metabolit Ratios Following Oral Administration of GW786034 as a Suspension in 0.5% HPMC and 0.1% Twe a Nominal Dose of 3 and 30 mg/kg/day to Male Rats (n = 4)

Regimen (mg/kg/day)	Compound	Study Day	C_{max} (µg/mL)	T_{max} [a] (hour)	$AUC_{(0-t)}$ (µg.hr/mL)	M
3	GW786034 (parent)	1	4.15 ± 0.588	3.99 [3.98 – 5.88]	43.9 ± 6.52	
		7	7.67 ± 1.46	4.09 [0.99 – 4.17]	88.1 ± 14.1	
	GSK1268992	1	NC	NC	NC	
		7	0.0468 ± 0.0137	0.61 [0.32-4.17]	0.256 ± 0.068	
	GSK1268997	1	0.0642 ± 0.019	4.93 [3.98-6.02]	0.368 ± 0.102	
		7	0.135 ± 0.038	4.09 [0.99-4.17]	0.852 ± 0.198	
	GSK1071306	1	NC	NC	NC	
		7	0.0926 ± 0.0313	0.46 [b] [0.32 – 0.62]	NC	
	GW700201	1	NC	NC	NC	
		7	0.0586 [c]	3.10 [c] [2.02 – 4.17]	NC	
30	GW786034 (parent)	1	42.5 ± 10.9	2.97 [1.03 – 5.97]	450 ± 128	
		7	33.4 ± 1.7	1.12 [1.00 – 2.20]	317 ± 28	
	GSK1268992	1	0.288 ± 0.050	2.52 [1.00-6.03]	2.10 ± 1.04	
		7	0.205 ± 0.061	1.00 [0.56-1.12]	1.05 ±0.18	
	GSK1268997	1	1.60 ± 0.56	4.01 [1.00-7.97]	14.9 ± 7.9	
		7	1.27 ± 0.42	2.03 [1.12-6.00]	8.59 ± 4.65	
	GSK1071306	1	0.387 ± 0.017	1.52 [0.50 – 4.00]	2.24 ± 0.42	
		7	0.350 ± 0.092	1.00 [0.56 – 1.20]	1.55 ± 0.22	
	GW700201	1	0.539 ± 0.232	4.00 [1.93 – 4.00]	4.63 ± 3.37	
		7	0.396 ± 0.127	1.66 [1.00 – 8.50]	2.65 ± 0.78	

NC = Not calculated due to limited data
SD = Standard Deviation
NA = Not applicable
a. T_{max} expressed as median and range
b. n=1
c. n=2

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[Table excerpted from sponsor]

CD2006/01538/01: Plasma exposure to GW786034 and metabolites following 7-day oral administration to male and female cynomolgus monkeys.

Key Findings:

- GW786034 was quantifiable up to at least 8 hours, with mean Tmax occurring at approximately 2 hours on both Days 1 and 7.
- The metabolite concentration for GSK1268992, GSK1268997 and GSK1071306 was quantifiable for up to 4-8 hours while the metabolite concentration for GW700201 was sparsely quantifiable on both study days.
- Mean Tmax of the four metabolites occurred at approximately 3 hours after dosing on both study days.
- No apparent gender differences in systemic exposure (AUC and C_{max}) for parent drug and its metabolites on between Days 1 and 7 with the exception of metabolite GSK1268992. This metabolite had a $AUC_{(0-t)}$ and C_{max} were 2.6- and 3.0-fold higher in males compared to females, respectively on Day 1.
- There was no difference in systemic exposure between Day 1 and Day 7 for the parent drug and its metabolites.
- Summary of the results are listed in Sponsor's Table 2.

Report #:	CD2006/01538/01
Module:	4.2.2.2
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	January 14, 2003
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	GW786034B, 786034-A4-02P, 98.5%

Doses:	50 mg/kg for 7 days
Species/strain:	cynomolgus monkey
Number/sex/group or time point:	3 males/ 3 females
Route, formulation, volume, and infusion rate:	Oral Gavage 0.5% hydroxypropyl-methylcellulose/ 0.1% Tween 80 10 mL/kg
Age:	~ no info. was provided
Weight:	~2-3 kg
Sampling times:	0, 15, 30, 60, 120, 240, 480, and 1440 minutes on Days 1 and 7
Study design:	3 monkey/sex/time point

Table 2 Mean (± SD) Pharmacokinetic Parameters of GW786034 and Metabolites Following Oral Administration of GW786034 at a Nominal Dose of 50 mg/kg/day to Male and Female Cynomolgus Monkeys on Days 1 and 7

		Males (n = 3)			Females (n = 3)		
Compound	Study Day	C_{max} (ng/mL)	T_{max} (h)	$AUC_{(0-t)}$ (ng*h/mL)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{(0-t)}$ (ng*h/mL)
GW786034 (parent)	1	20889 ± 4107	1.33 ± 0.58	76251 ± 22823	11127 ± 9692	2.00 ± 0.00	48794 ± 51923
	7	24965 ± 9706	2.67 ± 1.15	106525 ± 66023	17406 ± 21598	2.67 ± 1.15	84109 ± 115937
GSK1268992	1	638 ± 295	1.67 ± 0.58	2004 ± 1110	211 ± 127	2.67 ± 1.15	764 ± 714
	7	602 ± 264	2.67 ± 1.16	2141 ± 1263	342 ± 400	3.33 ± 1.16	1346 ± 1763
GSK1268997	1	2336 ± 958	2.00 ± 0.00	7657 ± 3233	1126 ± 1044	2.67 ± 1.16	4891 ± 5495
	7	2461 ± 1052	2.67 ± 1.16	9624 ± 5672	1652 ± 2113	2.67 ± 1.16	7976 ± 10947
GSK1071306	1	1633 ± 711	2.00 ± 0.00	5891 ± 2603	1021 ± 1060	2.67 ± 1.15	3994 ± 4513
	7	2337 ± 949	2.67 ± 1.15	9274 ± 4763	1803 ± 2277	3.33 ± 1.15	9213 ± 13087
GW700201	1	179 ± 81.5	2.00 ± 0.00	577 ± 143[1]	108 ± 75.0[1]	2.00 ± 0.00[1]	NA
	7	276 ± 157	2.67 ± 1.15	1191 ± 559[1]	420[2]	4.00[2]	1672[2]

1. n = 2.
2. n = 1.
NA: Not applicable due to limited quantifiable concentrations.

[Table excerpted from sponsor]

2.6.4.4 Distribution

CD2007/00356/00: Quantitative Tissue Distribution of Drug-Related Material Using Whole-Body Autoradiography Following a Single Oral Dose of [^{14}C]GW786034 (10 mg/kg) to Female Long-Evans Rats

Report #:	(b) 85-0623
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	December 12, 2006
GLP Compliance:	Yes
QA Report:	Yes (X), No ()
Drug, lot #, and % purity:	GW786034B, 786034B-A5-01P-MIC, [^{14}C]GW786034, 1 (CFQ15054), 98.3%

Doses:	Single Dose of 10 mg/kg
Species/strain:	Long-Evans partially-pigmented rats
Number/sex/group or time point:	7 females
Route, formulation, volume, and infusion rate:	Oral Gavage; [14C]GW786034 and non-radiolabeled GW786034 in 0.5% hydroxypropylmethylcellulose/0.1% Tween 80 in water, pH adjusted to approximately 1.3 with 0.1 N HCl; 10 mL/kg
Age:	~ 8 weeks
Weight:	~176.1 g
Sampling times:	2, 4, 8, 24 hrs., 3, 8, and 35 days
Study design:	1 rat/time point

Results: (Excerpted from Sponsor)

Tissue Type	Tissue	Concentration of Radioactivity (µg equiv/g)						
		Time Point						
		2 h	4 h	8 h	24 h	3 day	8 day	35 day
Vascular/ Lymphatic	Aorta	9.398	6.615	4.570	0.215	BLQ	BLQ	BLQ
	Blood (cardiac)	11.159	12.361	5.910	0.340	BLQ	BLQ	BLQ
	Bone Marrow	4.033	2.804	1.499	0.128	BLQ	BLQ	BLQ
	Mandibular Lymph Nodes							
	Spleen	3.842	2.772	1.723	0.125	BLQ	BLQ	BLQ
	Thymus	3.483	2.293	1.519	0.064	BLQ	BLQ	BLQ
		2.301	1.761	1.138	0.071	BLQ	BLQ	BLQ
Excretory/ Metabolic	Kidney	6.783	5.002	3.442	0.218	BLQ	BLQ	BLQ
	Renal Cortex	6.723	4.782	3.225	0.217	BLQ	BLQ	BLQ
	Renal Medulla	7.222	5.217	3.590	0.218	BLQ	BLQ	BLQ
	Liver	11.228	9.801	5.045	0.398	0.069	BLQ	BLQ
Nervous System	Brain	0.243	0.187	0.146	BLQ	BLQ	BLQ	BLQ
	Choroid Plexus	2.953	5.127	2.459	0.123	BLQ	BLQ	BLQ

Tissue Type	Tissue	Concentration of Radioactivity (µg equiv/g)						
		Time Point						
		2 h	4 h	8 h	24 h	3 day	8 day	35 day
	Meninges	11.880	8.704	4.302	0.312	1.769	0.563	0.758
Endocrine	Adrenal Cortex	8.560	6.494	4.316	0.315	BLQ	BLQ	BLQ
	Adrenal Medulla	9.917	7.171	3.931	0.189	BLQ	BLQ	BLQ
	Pineal Gland	6.037	3.968	2.677	0.128	BLQ	BLQ	BLQ
	Pituitary	4.416	3.338	2.324	0.188	BLQ	BLQ	BLQ
	Thyroid	3.571	4.588	2.432	0.175	BLQ	BLQ	BLQ
Secretory	Exorbital Lachrymal Gland	5.245	3.278	2.116	0.160	BLQ	BLQ	BLQ
	Intra-Orbital Lachrymal Gland	4.906	3.386	2.398	0.113	BLQ	BLQ	BLQ
	Harderian Gland	5.024	3.841	3.188	0.152	BLQ	BLQ	BLQ
	Pancreas	4.092	2.844	1.818	0.128	BLQ	BLQ	BLQ
	Salivary Gland	5.058	3.882	2.846	0.115	BLQ	BLQ	BLQ
Adipose	Fat (brown)	7.009	5.452	3.188	0.158	BLQ	BLQ	BLQ
	Fat (abdominal)	2.195	1.554	0.709	BLQ	BLQ	BLQ	BLQ
Dermal	Skin (non-pigmented)	2.944	2.397	2.267	0.304	0.144	BLQ	BLQ
	Skin (pigmented)	3.396	3.530	1.982	0.187	0.309	0.137	0.219
Reproductive	Ovary	6.576	9.913	4.406	0.723	BLQ	BLQ	BLQ
	Uterus	4.321	4.923	2.735	0.175	BLQ	BLQ	BLQ
	Vagina	4.852	5.620	5.210	0.142	BLQ	BLQ	BLQ
Skeletal/ Muscular	Muscle (skeletal)	2.027	1.536	0.822	0.075	BLQ	BLQ	BLQ
	Myocardium (heart)	5.339	3.612	2.480	0.145	BLQ	BLQ	BLQ
Respiratory Tract	Lung	9.702	7.856	4.849	0.240	BLQ	BLQ	BLQ
	Nasal Turbinates	2.853	1.513	1.147	0.758	0.055	BLQ	BLQ
Alimentary Canal	Cecum Mucosa	7.657	7.980	7.981	2.357	0.060	BLQ	BLQ
	Esophagus	7.722	5.880	3.098	0.270	BLQ	BLQ	BLQ
	Large Intestine Mucosa	6.114	5.745	6.368	0.253	BLQ	BLQ	BLQ
	Rectum Mucosa	3.952	5.329	2.833	0.248	BLQ	BLQ	BLQ
	Small Intestine Mucosa	5.337	5.389	5.539	0.328	BLQ	BLQ	BLQ
	Stomach Mucosa	7.988	3.757	2.722	0.155	BLQ	BLQ	BLQ
Alimentary Canal Contents	Cecum Contents	1331a	1473a	67.904	12.241	0.315	BLQ	BLQ
	Large Intestine Contents	BLQ	32.771	195.897	8.452	0.150	BLQ	BLQ
	Small Intestine Contents	1339a	347.006	30.893	2.725	0.051	BLQ	BLQ
	Stomach Contents	26.320	13.925	3.157	0.132	BLQ	BLQ	BLQ
Ocular	Lens	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Uveal Tract	11.837	12.214	29.687	15.828	8.341	4.996	3.572

BLQ: Below the limit of quantification (< 0.044 µg equiv/g) or tissue could not be visually identified because of non-detectable radioactivity;
a: Above the limit of quantification (> 423 µg equiv/g)

- GW786034 was widely distributed in tissues throughout the body.
- The largest amounts of radioactivity were found in the contents of the alimentary canal supporting the finding that excretion is mostly through the feces.
- Tissues in which there was long-term (up to 35 days) of retention included the meninges, pigmented skin, and uveal tract suggesting an association with melanin producing cells.
- Other tissues with longer retention (up to 3 days) include the liver, the non-pigmented skin, the nasal turbinates, and the cecum mucosa.
- The highest tissue levels of radioactivity were detected in the uveal tract, meninges, liver, lung, ovary, esophagus, and the mucosa of the cecum and large intestine.

- The highest levels of radioactivity were typically seen at 2 hours. In the reproductive tract (ovary, uterus, and vagina) the peak was at 4 hours. In the uveal tract the peak was at 8 hours.

CD2004/00153/01: GW786034 Quantitative Tissue Distribution of Drug-Related Material Using Whole Body Autoradiography Following a Single Oral Dose of [^{14}C]GW786034 (10 mg/kg) to Male Long-Evans Rats

Report #:	(b) 85-0337
Conducting Laboratory and Location:	(b) (4)
Date of Study Initiation:	August 18, 2003
GLP Compliance:	Yes
QA Report:	Yes (X), No ()
Drug, lot #, and % purity:	GW786034B, 786034B-A5-01P-MIC, [^{14}C]GW786034, 1 (CFQ15054), 98.3%

Doses:	Single Dose of 10 mg/kg
Species/strain:	Long-Evans partially-pigmented rats
Number/sex/group or time point:	6 males
Route, formulation, volume, and infusion rate:	Oral Gavage; [14C]GW786034 and non-radiolabeled GW786034 in 0.5% hydroxypropylmethylcellulose/0.1% Tween 80 in water, pH adjusted to approximately 1.3 with 0.1 N HCl; 10 mL/kg
Age:	~ 8 weeks
Weight:	~176.1 g
Sampling times:	2, 8, 24 hrs., 3, 7, and 35 days
Study design:	1 rat/time point

Results: (Excerpted from Sponsor)

Tissue Type	Tissue	Concentration of Radioactivity (µg equiv/g) Time Point 2 h	8 h	24 h	3 days	7 days	35 days
Adipose	Adipose (brown)	4.081	1.518	0.109	BQL	NI	NI
	Adipose (white)	0.757	0.258	BQL	BQL	NI	NI
Alimentary Canal	Cecum	2.308	2.622	0.177	BQL	NI	NI
	Cecum contents	AQL	80.052	22.028	0.784	NI	NI
	Esophagus	2.325	9.759	0.129	NI	NI	NI
	Large intestine	2.156	4.584	0.335	BQL	NI	NI
	Large intestine contents	1.296	135.233	24.102	0.176	NI	NI
	Stomach	2.127	1.106	0.094	BQL	NI	NI
	Stomach contents	14.127	163.133	5.845	0.158	NI	NI
	Small intestine	3.046	3.396	0.216	NI	NI	NI
	Small intestine contents	99.574	26.368	10.749	0.051	NI	NI

Tissue Type	Tissue	Concentration of Radioactivity (µg equiv/g)					
		Time Point					
		2 h	8 h	24 h	3 days	7 days	35 days
Central Nervous System	Brain, cerebrum	0.155	0.086	BQL	BQL	BQL	NI
	Brain, cerebellum	0.189	0.098	BQL	BQL	BQL	NI
	Brain, medulla	0.096	0.054	BQL	BQL	NI	NI
	Meninges	3.544	3.349	2.240	0.966	1.283	0.666
	Spinal cord	0.128	0.064	BQL	NI	NI	NI
Dermal	Skin – Pigmented	1.984	2.685	0.743	0.463	0.298	0.368
	Skin – Non-Pigmented	1.552	1.910	0.337	0.264	0.122	0.063
Endocrine	Adrenal gland	5.449	2.550	0.250	BQL	NI	NI
	Pitutitary gland	2.361	1.466	0.120	BQL	0.041	NI
	Thyroid	2.911	1.436	0.127	NI	NI	NI
Excretory/ Metabolic	Bile	21.183	18.771	0.891	NI	NI	NI
	Liver	8.225	3.663	0.461	0.111	0.050	NI
	Renal cortex	4.114	2.296	0.223	BQL	NI	NI
	Renal medulla	5.237	1.904	0.166	BQL	NI	NI
	Urinary bladder	3.888	3.315	0.088	NI	NI	NI
	Urine	1.263	0.753	0.132	BQL	NI	NI
Ocular	Eye -uvea	4.516	15.683	12.678	14.920	7.326	4.618
	Eye -lens	BQL	BQL	BQL	BQL	BQL	BQL
Reproductive	Epididymis	1.561	1.649	0.195	BQL	NI	NI
	Prostate gland	1.346	1.006	0.044	NI	NI	NI
	Seminal vesicles	0.205	0.368	BQL	BQL	NI	NI
	Testis	1.679	1.408	0.139	BQL	NI	NI
Respiratory Tract	Lung	7.226	2.493	0.181	BQL	NI	NI
	Nasal turbinates	1.127	0.581	0.096	NI	NI	NI
	Trachea	1.574	2.028	0.092	NI	NI	NI
Secretory	Exorbital lacrimal gland	2.390	1.185	0.093	BQL	NI	NI
	Intraorbital lacrimal gland	1.775	1.055	0.080	BQL	NI	NI
	Harderian gland	2.347	1.985	0.132	BQL	NI	NI
	Pancreas	2.740	1.223	0.113	BQL	NI	NI
	Salivary gland	2.899	1.440	0.105	BQL	NI	NI
Vascular/ Lymphatic	Blood	8.567	3.536	0.210	BQL	NI	NI
	Bone marrow	2.417	1.164	0.100	NI	NI	NI
	Lymph node	2.453	1.156	0.179	NI	NI	NI
	Spleen	1.839	0.902	0.078	BQL	NI	NI
	Thymus	1.193	0.701	0.058	BQL	NI	NI
Skeletal/ Muscular	Bone	0.219	0.121	BQL	NI	NI	NI
	Heart	3.915	1.620	0.113	BQL	NI	NI
	Skeletal muscle	1.063	0.490	0.049	BQL	NI	NI

BQL: Below the quantitation limit (<0.037 µg equiv/g)
AQL: Above the quantitation limit (>374.712 µg equiv/g)
NI: Not identifiable during quantitative analysis

- The drug was widely distributed throughout the body.
- The highest overall levels of radioactivity were seen in the contents of the stomach, small intestine, large intestine and cecum, consisitent with excretion through the feces.
- The highest tissue concentrations were seen in the blood, bile, liver, and, uvea.
- The peak concentration of radioactivity in the tissues was at the 2 hour time point with the exception of the uvea which peaked at the 8 hour time point.

- No radioactivity was detectable in tissues past Day 3 with the exceptions of the liver (Day 7), the meninges, skin, and uvea (Day35).

RH2002/00074/01: Interaction of GW786034B and GW771806A with human serum albumin

Report #:	RH2002/00074/01
Conducting Laboratory and Location:	GlaxoSmithKline Research and Development Research Triangle Park, North Carolina
Date of Study Initiation:	June 24, 2002
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	GW786034B HSA, 90K7604
Methods Assay for: Instrument: Temperature:	 Changes in intrinsic protein fluorescence of HSA upon binding to drug. λ ex=280 nm λem=340 nm Kontron SFM25 Spectrofluorometer 25°C

- Intrinsic fluorescence of HSA was quenched over 80% by GW786034B.
- GW786034B binds to HSA at an affinity of ~0.20 μM (low ionic milieu) 0.150 μM (more physiologically relevant milieu).
- The fraction of free GW786034B in human plasma with 700 μM HSA was predicted to be 0.000029.
- The presence of long chain fatty acids significantly reduces the ability of HSA to bind to GW786034B.

RD2002/00877/00: Preliminary In Vitro Protein Binding of GW786034B in Mouse, Rat, Dog, Monkey and Human Plasma

Report #:	02AVT0045
Conducting Laboratory and Location:	GlaxoSmithKline RTP CEDD DMPK Research Triangle Park, North Carolina
Date of Study Initiation:	July 22, 2002
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	GW786034B
Design:	Measured plasma protein binding of GW786034B at a concentration of 5μM

Results:

(Excerpted from Sponsor)

GW786034B Protein Binding in Mouse, Rat, Dog, Monkey andHuman Plasma	
Species	Average Fraction Bound (n = 3)
Mouse	>99.9%
Rat	>99.9%
Dog	>99.9%
Cynomolgus Monkey	>99.9%
Human	>99.9%

- GW786034B is highly bound to protein in plasma across multiple species.

CD2004/00451/00: Assessment of GW786034B Protein Binding in Mouse, Rat, Dog, Monkey and Human Plasma by Equilibrium Dialysis In Vitro

Report #:	04DMM013
Conducting Laboratory and Location:	GlaxoSmithKline R&D Drug Metabolism and Pharmacokinetics King of Prussia, PA
Date of Study Initiation:	March 23, 2004
GLP Compliance:	Yes
QA Report:	No
Drug, lot #, and % purity:	GW786034B, US0200305
Method:	Equilibrium dialysis to characterize protein binding of GW786034B in multiple species

Results:
(Excerpted from Sponsor)

Summary of In Vitro Protein Binding of GW786034B to Male Mouse, Rat, Dog, Monkey and Human Plasma (n=3) Using Equilibrium Dialysis

Nominal Conc. (µg/mL)	Percent Protein-Bound GW786034B in Plasma (±SD)[1]				
Species	Mouse	Rat	Dog	Monkey	Human
10	99.86 ± 0.10	99.58 ± 0.56	99.37 ± 0.08	99.88 ± 0.08	99.97 ± 0.01

20	99.73 ± 0.38	99.91 ± 0.02	99.33 ± 0.10	99.93 ± 0.01	99.98 ± 0.00
50	99.97 ± 0.00	99.90 ± 0.01	99.13 ± 0.15	99.91 ± 0.01	99.97 ± 0.00
100	99.95[2]	99.67 ± 0.01	98.87 ± 0.27	99.88 ± 0.00	99.94 ± 0.01

1. Plasma was pooled from at least three male subjects.
2. n=2, so SD was obtained

- GW786034B was highly bound to protein at all concentrations tested
- GW786034B was highly bound to protein in all species tested (mouse, rat, dog, monkey, and human)
- Recovery of GW786034B was high after equilibrium dialysis (>93% at all concentrations tested for mouse, rat, monkey, and human; for dogs >93% at 10 and 20 µg/mL, 86% at 50 µg/mL, and 73% at 100 µg/mL).

CD2006/00629/00: An In Vitro Investigation into the Inhibition by GW786034B of Xenobiotic Transport via Human OATP1B1 Heterologously Expresssed in CHO Cells

Report #:	06DMM047
Conducting Laboratory and Location:	GlaxoSmithKline R&D Drug Metabolism and Pharmacokinetics King of Prussia, PA
Date of Study Initiation:	March 8, 2006
GLP Compliance:	Yes
QA Report:	No
Drug, lot #, and % purity:	GW786034B, 786034B-A4-02P-MIC-RTN, 98.5%; Rifamycin sv, 014K2503; [^{3}H]-Estradiol 17β-D-glucuronide ([^{3}H]-EG), 33559944, >97% , specific activity 53.0 Ci/mmol
Methods:	Chinese Hamster Ovary cells expressing the human OATP1B1 transporter were used to determine the ability of GW786034B to prevent uptake of [^{3}H]-EG (probe). Rifamycin was included as a positive control.

Results:
(Excerpted from Sponsor)

Test compound	Concentration of GW786034 (µM)	Uptake rate of [3H]-EG (fmoles/cm2/min)	Uptake rate of [3H]-EG (% control)
[3H]-EG only	None	11[1] ± 0.42	100 ± 4.0
[3H]-EG + GW786034	0.1	8.7 ± 0.20	82 ± 1.9
	1	5.3 ± 0.23	50 ± 2.1

	3	3.1 ± 0.22	29 ± 2.1
	10	1.8 ± 0.071	17 ± 0.66
	30	1.3 ± 0.075	12 ± 0.70
[3H]-EG + 10 µM rifamycin	None	0.51 ± 0.069	4.7[2]± 0.64

Data is the mean ± standard deviation from 3 wells.

1. Aceptance criteria ≥ 6fmoles/cm2/min
2. Acceptance criteria < 15% of control [^{3}H]-EG value

- GW786034B is an in vitro inhibitor of the human OATP1B1 transporter at a concentration of 0.79 µM

2.6.4.5 Metabolism

CD2004/00901/00: A preliminary *in vitro* investigation into the oxidative metabolism of GW786034.

Key Findings:

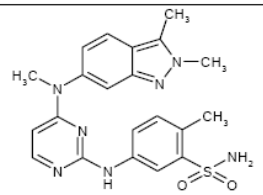
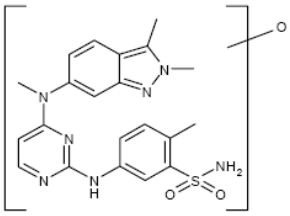
- Oxidative metabolism of GW786034 metabolites in human liver microsomes is primarily mediated by CYP3A4, with minor contributions from CYP1A2 and CYP2C8.
- Metabolite M24 and M26 was detected with CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4
- Metabolite M27 was detected with CYP1A2, CYP2D6, and CYP3A4
- Metabolite M12 and M16 were only detected with CYP3A4.
- A summary of results are presented in sponsor Table 1.

Report #:	CD2004/00901/00
Module:	4.2.2.4
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	March 18, 2004
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	[14C]-GW786034A, R1083667/3, >97.5%

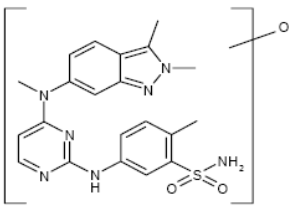
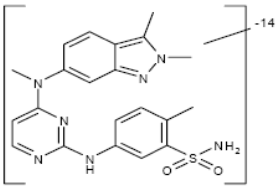
Methods:

- Radio-active GW786034 at concentrations of 5 and 50 uM were incubated in the presence of pooled human liver microsomes and Supersome™ expressing individual CYP enzymes.
- Incubates were analyzed by LC/MS/MS.

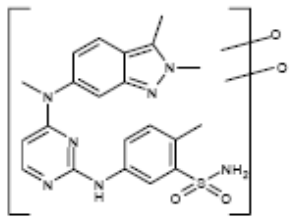
Table 1 Summary of [^{14}C]-GW786034 Related Metabolites Detected in Human Liver Microsomes using LC/MS

Peak ID	Retention Time (min.)	Proposed metabolite structures	Test std 5-μM	Test std 50-μM	HLM 5-min 5-μM	NCF-HLM 5-min 5-μM	HLM 5-min 50-μM	NCF-HLM 5-min 50-μM	1A2 15-min 50-μM	2C9 30-min 5-μM	2C19 30-min 5-μM	2D6 15-min 50-μM	3A4 2-min. 50-μM
P	43.9	 Parent	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
M24	32.1	 Mono-oxygenation	ND	ND	✓	ND	✓	ND	✓	✓	✓	✓	✓

[Table excerpted from sponsor]

Peak ID	Retention Time (min.)	Proposed metabolite structures	Test std 5-μM	Test std 50-μM	HLM 5-min 5-μM	NCF-HLM 5-min 5-μM	HLM 5-min 50-μM	NCF-HLM 5-min 50-μM	1A2 15-min 50-μM	2C9 30-min 5-μM	2C19 30-min 5-μM	2D6 15-min 50-μM	3A4 2-min. 50-μM
M26	36.6	 Mono-oxygenation	✓	✓	✓	ND	✓	✓	✓	ND	✓	✓	✓
M27	40.1	 Demethylation	ND	ND	ND	ND	✓	ND	✓	ND	✓	ND	✓

[Table excerpted from sponsor]

Peak ID	Retention Time (min.)	Proposed metabolite structures	Test std 5-µM	Test std 50-µM	HLM 5-min 5-µM	NCF-HLM 5-min 5-µM	HLM 5-min 50-µM	NCF-HLM 5-min 50-µM	1A2 15-min 50-µM	2C9 30-min 5-µM	2C19 30-min 5-µM	2D6 15-min 50-µM	3A4 2-min. 50-µM
M12 or M16	27.3	 Di-oxygenation	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	✓

[Table excerpted from sponsor]

RD2002/00874/00: Preliminary identification of *in vitro* and *in vivo* metabolites of GW78604.

Key Findings:

- 5 (M1-M5) identifiable metabolites were detected from the *in vitro* and *in vivo* metabolism of GW786034.
- Metabolites M3 and M4 (mono-oxygenated products) were present in both microsomal and plasma samples in mouse, rat, dog and monkey, and human microsomal samples.
- The proposed mechanism of M3 and M4 is through the oxidation of a methyl group to the carboxylic acid (See Figure 1).
- Metabolite M2 (a di-oxygenated product) was present all *in vitro* mouse, rat, dog, monkey and human microsomal samples but not in any *in vivo* samples.
- Metabolite M5 (N-demethylated prodcut) was present in *in vivo* mouse and monkey samples and *in vitro* mouse and rat microsomal samples.
- Metabolite M1 was only present in human microsomal samples.
- A summary of results are listed in sponsor Table 1.

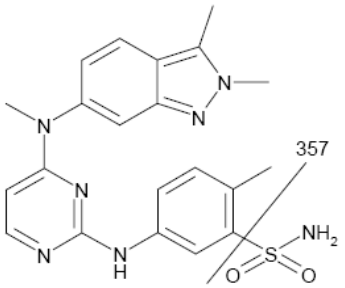
Report #:	RD2002/00874/00
Module:	4.2.2.4
Conducting Laboratory and Location:	GSK Labs, RTP, North Carolina 27709
Date of Study Initiation:	No information was provided
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	GW786034A, no other information is provided

Methods:

- GW786034A was incubated in the presence of pooled liver microsomes (CD-1 mouse, Sprague Dawley rat, beagle dog, cynomolgus monkey and human all obtained from (b) (4)
- Incubates were analyzed by LC/MS/MS.
- *In vivo* plasma samples collected from previous pharmacokinetic studies in mouse, rat, dog and monkey were also analyzed.

Figure 1

Proposed MS/MS Fragmentation Pattern for GW786034



GW 786034A
$[M+H]^+ = 438$

[Figure excerpted from sponsor]

Table 1 Preliminary In Vitro and In Vivo Metabolite Identification of GW786034

		Mouse		Rat		Dog		Monkey		Human
Component[1]		Micro[2]	Plasma[3]	Micro	Plasma	Micro	Plasma	Micro	Plasma	Micro
M1	P + 2O – 2H	ND	ND	ND	ND	ND	ND	ND	ND	++
M2	P + 2O	++	ND	++	ND	++	ND	+	ND	++
M3	P + O	++	+	++	+	++	+	+	+	++
M4	P + O	++	+	++	++	++	+	+	++	++
M5	P – CH_3	+	+	+	ND	ND	ND	ND	++	ND

1. Study conducted using non-radiolabelled material, thus quantification of metabolites was not possible. The metabolites are described as ND (not detected), + (at limit of detection), ++ (more abundant).
2. 10 μM GW786034 incubated for 90 min with liver microsomes from the indicated species.
3. Plasma obtained from various species dosed i.v. or orally with GW786034.

[Table excerpted from sponsor]

CD2007/00811/00: An *in vitro* investigation into the inhibition of human UDP-glucuronosyltransferase enzyme UGT1A1 by GW78604.

Key Findings:

– GW786034 was a direct inhibitor of human UGT1A1 *in vitro* with an IC_{50} = 1.2 μM.
– Summary of the results are listed in Sponsor's Table 1.

Report #:	CD2007/00811/00
Module:	4.2.2.4
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	April 25, 2007
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	GW786034B, 061128423, 99.3%

Methods:

- GW786034 (concentration range 0-250 μM) was incubated with 7-hydroxy-4-(trifluoromethyl)coumarin (HFC), human UGT1A1 Supersomes™ , and UDPGA for 10 minutes at 37°C.
- The production of 4-trifluoromethylumbelliferyl glucuronide (HFC-gluc) in each incubation was quantified by HPLC-UV and IC_{50} value for the direct inhibition of UGT1A1 activity was determined.
- Curcumin was used the positive control.

Table 1 Direct Inhibition of UGT1A1

UGT	Substrate	GW786034 IC_{50} (μM)	Positive Control Inhibitor	Positive Control IC_{50} (μM)
1A1	HFC	1.2	Curcumin	3.7

See Appendix 4 for further details

[Table excerpted from sponsor]

CD2003/00965/00: An *in vitro* investigation of the hepatic metabolism of [^{14}C]GW78604 in the mouse, rat, female rabbit, dog, monkey and man

Key Study Findings:
- The extent of metabolism of GW786034 was low in human liver microsomal and hepatocyte incubations.
- The magnitude of GW786034 metabolism varied with species but extensive metabolism occurred in mouse, dog and rabbit hepatocytes.
- There were no human specific phase 1 metabolites observed in either liver microsomal or hepatocyte incubations.
- Summary of results are shown in sponsor Table 1.

Report #:	CD2003/00965/00
Module:	4.2.2.4
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	April 15, 2002
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	[^{14}C]GW786034X, AS201420-028A1, 82.3%

Methods:
- Mouse, rat, female rabbit, dog, monkey and pooled human liver microsomes were incubated with 10 μM [^{14}C]GW786034X for 30 minutes in the presence of an NADPH regenerating system and UDP-glucuronic acid.
- Preclinical species and human hepatocytes were incubated with 10 μM [^{14}C]GW786034X for 4 and 24 hours.

- The incubation mixtures were centrifuged and supernatants analyzed by radio-HPLC, LC/MS and LC/MS/MS.

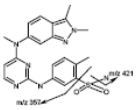
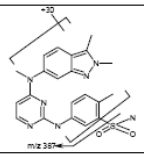
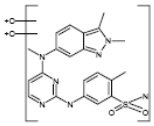
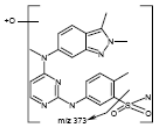
Results:
In human microsomes, the following routes of metabolism and metabolites were detected:
- Mono-oxygenation (M26 and M24)
- Di-oxygenation (M12)
- Oxidation to a carboxylic acid (M8)

In human hepatocytes, the following routes of metabolism and metabolites were detected:
- Mono-oxygenation (M26 and M24)
- Di-oxygenation (M12)
- Oxidation to a carboxylic acid (M8)
- Combination of mono-oxygenation and glucuronidation (M15)
- Conversion of methyl to carboxyl followed by glucuronidation (M9) – a phase 2 metabolite

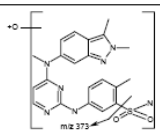
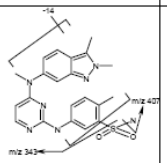
In preclinical microsomes, the following routes of metabolism and metabolites were detected:
- Mono-oxygenation, M24 and M26 in mouse, rat, rabbit, dog and monkey
- Di-oxygenation (M12) in rabbit and monkey microsomes

In preclinical hepatocytes, the following routes of metabolism and metabolites were detected:
- Mono-oxygenation, M24 and M26 in mouse, rat, rabbit, dog and monkey
- Mono-oxygenation followed by glucuronidation (M4) in rabbit hepatocytes
- Mono-oxygenation followed by glucuronidation (M15) in mouse, rat, rabbit and monkey
- Mono-oxygenation followed by glucuronidation (M17) in monkey
- Glutathione conjugation (M6) in rat and mouse
- Cysteine conjugation (M7) in rat and (M21) in dog and monkey
- Mercapturic acid conjugation (M13) in mouse and dog and (M23) in dog and monkey.

Table 1 Summary of GW786034 Metabolites Following 30-Minute Microsomal Incubations with 10 µM [^{14}C]GW7860

Metabolite #ID	Metabolite Identity	Metabolite Structure	[M+H]+	Human	Monkey	Rat	Mouse	Dog	Rabbit
P	GW786034		438	✓	✓	✓	✓	✓	✓
M8	Possible oxidation of CH_3 to COOH		468	✓	ND	ND	ND	ND	ND
M12	Di-oxygenation		470	✓	✓	ND	ND	ND	✓
M24	Mono-oxygenation		454	✓	✓	✓	✓	✓	✓

[Table excerpted from sponsor]

Metabolite #ID	Metabolite Identity	Metabolite Structure	[M+H]+	Human	Monkey	Rat	Mouse	Dog	Rabbit
M26	Mono-oxygenation		454	✓	✓	✓	✓	✓	✓
M28	Demethylation		424	ND	ND	ND	ND	ND	✓

ND = not detected by mass spectroscopy; ✓ = detected

[Table excerpted from sponsor]

Table 2 Summary of GW786034 Metabolites Following 24-Hour Hepatocyte Incubations with 10 µM [^{14}C]GW786034

Metabolite #ID	Metabolite Identity	Metabolite Structure	[M+H]+	Human	Monkey	Rat	Mouse	Dog	Rabbit
P	GW786034		438	✓	✓	✓	✓	✓	✓
M4	Mono-oxygenation+ Glucuronidation		630	ND	ND	ND	ND	ND	✓
M6	Glutathione conjugation		743	ND	ND	✓	✓	ND	ND
M7	Cysteine conjugation		557	ND	ND	✓	ND	ND	ND

[Table excerpted from sponsor]

Metabolite #ID	Metabolite Identity	Metabolite Structure	[M+H]+	Human	Monkey	Rat	Mouse	Dog	Rabbit
M14	Glucuronidation		614	ND	ND	ND	ND	ND	✓
M15	Glucuronidation + Mono-oxygenation		630	✓	✓	✓	✓	ND	✓
M17	Glucuronidation + Mono-oxygenation		630	ND	✓	ND	ND	ND	ND
M20	Demethylation + Mono-oxygenation		440	ND	✓	✓	✓	✓	ND

[Table excerpted from sponsor]

Metabolite #ID	Metabolite Identity	Metabolite Structure	[M+H]+	Human	Monkey	Rat	Mouse	Dog	Rabbit
M21	Cysteine conjugation		557	ND	✓	ND	ND	ND	ND
M22	MS/MS Unable to give definitive structural information		450	ND	✓	ND	ND	ND	ND
M23	Mercapturic acid conjugation		599	ND	ND	ND	ND	✓	ND
M24	Mono-oxygenation		454	✓	✓	✓	✓	✓	ND

[Table excerpted from sponsor]

Metabolite #ID	Metabolite Identity	Metabolite Structure	[M+H]+	Human	Monkey	Rat	Mouse	Dog	Rabbit
M25	Mono-oxygenation		454	ND	ND	ND	ND	✓	ND
M26	Mono-oxygenation		454	✓	✓	✓	✓	✓	ND
M27	Demethylation		424	ND	ND	✓	ND	ND	ND
M28	Demethylation		424	ND	ND	✓	ND	ND	ND

ND = not detected by mass spectroscopy; ✓ = detected

[Table excerpted from sponsor]

CD2003/00864/00: An *in vitro* investigation into the inhibition of human cytochrome P450 enzymes by GW78604.

Key Findings:
- GW786034 showed moderate to marked inhibition of human cytochrome P450 enzymes tested (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4) with the exception of CYP2A6 (IC_{50} of >100 μM)
- (GW786034 was shown not to be a time-and NADPH-dependent inhibitor in any of the cytochrome P450 enzymes investigated.
- Summary of the results are listed in Sponsor's Tables 1 and 2.

Report #:	CD2003/00864/00
Module:	4.2.2.4
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	December 12, 2003
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	GW786034B, 786034-A3-01P, 99.1%

Methods:
- The enzyme activities, phenacetin O-deethylase (CYP1A2), coumarin 7-hydroxylase (CYP2A6), bupropion hydroxylase (CYP2B6), paclitaxel 6α-hydroxylase (CYP2C8), diclofenac 4'-hydroxylase (CYP2C9), S-mephenytoin 4'-hydroxylase (CYP2C19), bufuralol 1'-hydroxylase (CYP2D6), chlorzoxazone 6-hydroxylase (CYP2E1), atorvastatin o-hydroxylase (CYP3A4), midazolam 1'-hydroxylase (CYP3A4) and nifedipine oxidase (CYP3A4) in human liver microsomes were tested in the presence and absence of GW786034.
- The metabolite was quantified by LC/MS/MS and IC50 values for the inhibition of each enzyme activity were determined.
- Time-and NADPH-dependent inhibition of P450 enzyme activity was also measured.

Table 1 ***In Vitro* Inhibition of Cytochrome P450 Activities by GW786034 and Positive Control Inhibitors**

P450 Enzyme	Substrate	GW786034 IC_{50} Value (μM)	Positive Control	Positive Control IC_{50} Value (μM)
1A2	Phenacetin	16	Fluvoxamine	0.041
2A6	Coumarin	> 100	Tranylcypromine	0.048
2B6	Bupropion	15	Orphenadrine	123
2C8	Paclitaxel	10	Quercetin	4.5
2C9	Diclofenac	7.9	Sulphaphenazole	0.90
2C19	S-Mephenytoin	11	Ticlopidine	0.71
2D6	Bufuralol	18	Quinidine	0.042
2E1	Chlorzoxazone	17	4-methylpyrazole	0.33
3A4	Atorvastatin	11	Ketoconazole	0.060
3A4	Midazolam	12	Ketoconazole	0.025
3A4	Nifedipine	14	Ketoconazole	0.027

[Table excerpted from sponsor]

Table 2 **Time- and NADPH-dependent In Vitro Inhibition of Cytochrome P450 Activities by GW786034 and Positive Control Inhibitors**

P450 Enzyme	Substrate	GW786034 IC_{50} Values (μM)		Positive Control	Positive Control IC_{50} Values (μM)	
		Control Pre-Inc[1]	NADPH Pre-Inc[2]		Control Pre-Inc[1]	NADPH Pre-Inc[2]
1A2	Phenacetin	21	12	Furafylline	3.6	0.23
2A6	Coumarin	> 100	> 100	None	ND	ND
2B6	Bupropion	25	30	None	ND	ND
2C8	Paclitaxel	14	13	None	ND	ND
2C9	Diclofenac	6.2	7.0	Tienilic Acid	2.6	0.28
2C19	S-Mephenytoin	12	13	Ticlopidine	1.0	0.44
2D6	Bufuralol	11	15	MDMA	9.7	1.5
2E1	Chlorzoxazone	21	15	None	ND	ND
3A4	Atorvastatin	10	6.8	Troleandomycin	13	1.1
3A4	Midazolam	10	8.9	Troleandomycin	4.5	0.50
3A4	Nifedipine	13	8.1	Troleandomycin	14	0.98

1. microsomes, buffer and GW786034B were pre-incubated for 20 minutes with probe substrate prior to initiation of the reaction by the addition of NADPH
2. microsomes, buffer and GW786034B were pre-incubated for 20 minutes with NADPH prior to initiation of the reaction by the addition of substrate

ND–Not Determined; no positive control time- and NADPH-dependent inhibitor identified

[Table excerpted from sponsor]

CD2005/00865/00: Investigation of GW78604 metabolites following a preliminary toxicity study by oral gavage administration to CD-1 mice for 13 weeks.

Key Findings:

- In mouse plasma, M24 and M26 (mono-oxygenation products) and M27 and M28 (oxidative products of N-demethylation) were metabolites identified.
- Each of these metabolites were detected at small amounts relative to the parent compound (2.5%, 8.4%, 6.1% and 0.6% for M24, M26, M27, and M28, respectively).
- Summary of the results are listed in Sponsor's Table 1.

Report #:	CD2005/00865/00
Module:	4.2.2.4
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	March 14, 2005
GLP Compliance:	No
QA Report:	No
Drug, lot #, and % purity:	Radiolabeled: [^{14}C]GW786034, R10836/67/3, 98.0% Non-radiolabeled: GW786034, 786034-A2-01M-WR, 99.7%

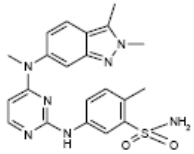
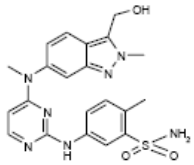
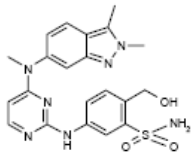
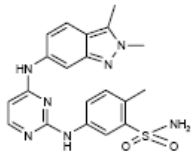
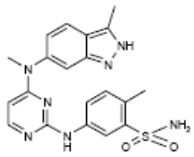
Methods:

- Mouse plasma samples from the 13 week-mice study at the 100 mg/kg dose administration was selected. The following table lists the time-points and pooled samples.

Time (hour)	Plasma	
	Subject number	Volume (mL)
0.5	115, 116, 117	0.100/subject
1	118, 119, 120	0.100/subject
2	121, 122, 123	0.100/subject
4	124, 125, 126	0.100/subject
8	127, 128, 129	0.100/subject
24	130, 131, 132	0.100/subject

- Structural characterization of selected metabolites was carried out by mass spectrometry (HPLC/MS).

Table 1 Summary of Metabolite Levels Reported Relative to Parent GW786034 in Pooled Mouse Plasma Using HPLC/MS

Peak ID	$[M+H]^+$ Based on ^{13}C	Retention Time (min)	Proposed Structure	% Relative to Parent
P	439	44.2		-
M24	455	32.2		2.5
M26	455	37.0		8.4
M27	425	40.3		6.1
M28	425	44.9		0.6

[Table excerpted from sponsor]

CD2003/00860/00: Quantification of the metabolites of GW786034 in the Sprague-Dawley rat following a single oral administration of [^{14}C]GW786034 at 10 mg/kg

Key Findings:

- No metabolites were detected in rat plasma and urine following a single oral administration of radio-labeled GW786034 to male and female rats,.
- GW786034 was eliminated via fecal route as the unchanged parent compound.
- The parent compound represented 54, 46, and 39% of the administered dose in male and female intact and bile duct cannulated (BDC) rats, respectively.
- Biliary metabolites accounted for a small amount of the administered dose (<2%).
- Bilary metabolites included the unchanged parent compound, products of mono-oxygenation and glucuronidation (M15 and M17), a glucuronide (M14), products of mono-oxygenation (M24 and M26), a glutathione conjugate (M6), a cysteine conjugate (M7), products of demethylation (M27 and M28) and a product of mono-oxygenation and demethylation (M20)

– Summary of results are listed in Sponsor's Table 2.

Report #:	CD2003/00860/00
Module:	4.2.2.4
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	May 10, 2002
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	GW786034B, 786034-A2-01M, 98.5%

Methods:

– In a separate study (Study No. CD2002/00088/00), intact male, female, and bile duct cannulated (BDC) male rats were orally dosed with [^{14}C]GW786034 at 10 mg/kg.
– Bile up to 24 hours was collected from BDC male rats.
– Radiolabeled components in plasma extracts, urine, bile, liver homogenate extracts and fecal homogenate extracts were quantified by radio-HPLC.

Table 2 Mean Quantification of Radiometabolites in Urine, Bile and Feces following a Single Oral Administration of [^{14}C]GW786034 to Male and Female Intact and Bile Duct-Cannulated Rats at a Nominal Dose Level of 10 mg/kg

Peak ID	Metabolite Identity	Mean % matrix radioactivity (mean % dose)[1]						
		Urine			Bile	Feces		
		Male Intact	BDC	Female Intact		Male Intact	BDC	Female Intact
A[2]	Mono-oxygenation	<LLQ	<LLQ	0.5 (0.1)	ND	2.1 (1.1)	<LLQ	0.6 (0.4)
B[2]	Mono-oxygenation	<LLQ	<LLQ	0.3 (0.1)	ND	2.6 (1.4)	<LLQ	<LLQ
C	Unknown	ND	ND	ND	<LLQ	ND	ND	ND
D	Unknown	ND	ND	ND	1.8 (0.2)	ND	ND	ND
M6[3]	Glutathione conjugate	ND	ND	ND	12.2 (0.7)	ND	ND	ND
M7	Cysteine conjugate	ND	ND	ND	6.0 (0.4)	ND	ND	ND
M14	Glucuronidation	ND	ND	ND	13.8 (0.9)	ND	ND	ND
M15	Mono-oxygenation and Glucuronidation	ND	ND	ND	16.6 (1.2)	ND	ND	ND
M17	Mono-oxygenation and Glucuronidation	ND	ND	ND	6.3 (0.4)	ND	ND	ND
M20	Mono-oxygenation and Demethylation	ND	ND	ND	0.9 (0.1)	ND	ND	ND
M24	Mono-oxygenation	ND	ND	ND	10.5 (0.7)	ND	ND	ND
M26	Mono-oxygenation	ND	ND	ND	0.9 (0.1)	ND	ND	ND

Peak ID	Metabolite Identity	Mean % matrix radioactivity (mean % dose)[1]						
		Urine			Bile	Feces		
		Male Intact	BDC	Female Intact		Male Intact	BDC	Female Intact
M27	Demethylation	ND	ND	ND	<LLQ	ND	ND	ND
P	GW786034	91.5 (13.9)	92.8 (23.2)	90.1 (15.0)	18.5 (1.3)	88.7 (54.1)	91.7 (38.6)	90.1 (46.4)
M28	Demethylation	ND	ND	ND	0.5 (0.1)	ND	ND	ND
Total		91.5 (13.9)	92.8 (23.2)	90.9 (15.2)	88.0 (6.1)	93.4 (56.7)	91.7 (38.6)	90.8 (46.7)
Mean % dose in matrix pool analyzed[4]		14.6	24.5	15.7	6.6	59.6	41.6	51.1
Mean % overall recovery[5]		97.1	98.0	93.7	97.3	98.8	99.0	100.0

ND = not detected; <LLQ = below lower limit of quantitation
1. Percent radioactivity recovered under each peak, corrected by the overall sample preparation recovery (data obtained from Winflow results).
2. Without definitive structural information, mono-oxygenation metabolites A and B in urine and feces cannot be directly correlated to mono-oxygenation metabolites M24 and M26 in bile.
3. Structure assignment by retention time comparison to *in vitro* rat hepatocytes.
4. Mean % dose in matrix pool analyzed corrected by sample preparation.
5. % recovery of radioactivity following centrifugation for urine and bile; % recovery of radioactivity following solvent extraction for feces.

[Table excerpted from sponsor]

CD2004/00028/00: Metabolism of GW786034 following a single oral administration of [^{14}C]GW78604 to male and female intact and bile duct-cannulated cynomolgus monkeys.

Key Findings:

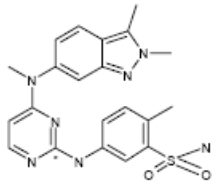
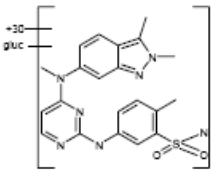
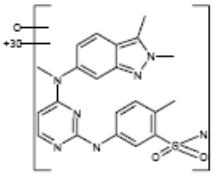
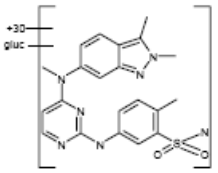
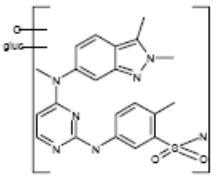
- The principal radiolabeled component in plasma was unchanged GW786034 following a single oral administration of [^{14}C]GW78604X to cynomolgus monkeys,
- Small concentrations of metabolites M26 (monooxygenation product) and M27 (product of demethylation) were present in the plasma.
- In intact and bile duct cannulated (BDC) monkeys, GW786034 is eliminated via fecal route as the unabsorbed parent compound. The parent drug represented 60.4 and 47.1% of administered dose in male and female intact monkeys, respectively and 45.2% of administered dose in BDC monkeys (See Sponsor's Table 2).
- Biliary metabolites of GW786034 accounted for a small amount of the administered dose (<4%) and included the following: M8 and M9 (products of oxidation) and M18 (product of di-demethylation plus glucuronidation) (See Sponsor's Table 2).
- The predominant route of metabolism is the oxidation of methyl to carboxyl. This route was followed by glucuronidation.
- Other pathways included monooxygenation, dioxygenation, demethylation, di-demethylation plus glucuronidation, glucuronidation plus monooxygenation, cysteine conjugation (See sponsor Figure 1).

Report #:	CD2004/00028/00
Module:	4.2.2.4
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	July 31, 2002
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	GW786034B, 786034-A2-01M, 98.5%

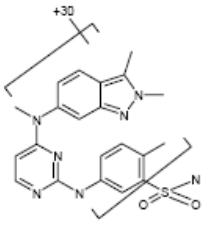
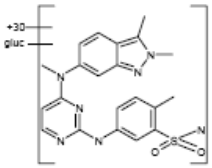
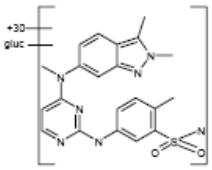
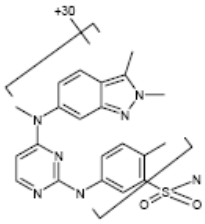
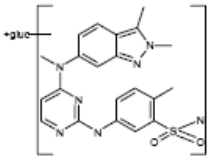
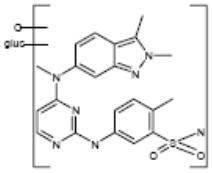
Methods:

- In a separate study (Study No. CD2002/00108/00), groups of three intact male, female, and bile duct cannulated (BDC) male monkeys were orally dosed with [^{14}C]GW786034 at 5 mg/kg.
- Monkey plasma from intact monkeys (1, 8, and 24 hours) as well as feces up to 48 and 96 hours were collected.
- Bile up to 48 hours was collected from BDC male monkeys.
- Radiolabeled components in plasma extracts, urine, bile, liver homogenate extracts and fecal homogenate extracts were quantified by radio-HPLC.

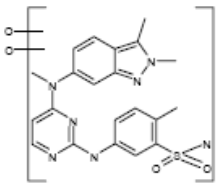
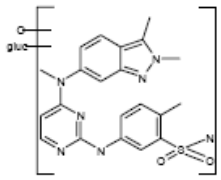
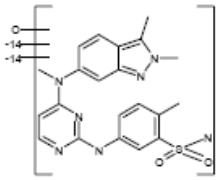
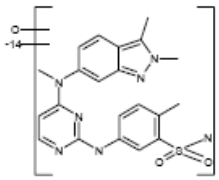
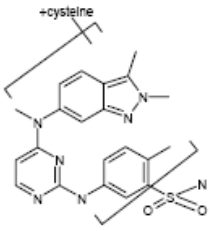
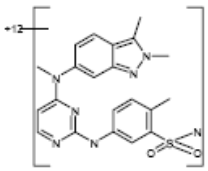
Table 2 Quantification of Radiometabolites in Bile and Feces following a Single Oral Administration of [^{14}C]GW786034 to Male and Female Intact and Bile Duct-Cannulated Monkey at a Nominal Dose Level of 5 mg/kg

Mean % matrix radioactivity (mean % dose)[1]					
Peak ID Metabolite Structure		Bile	Feces		
			Male Intact	Female Intact	BDC
P		1.5 (0.3)	73.1 (60.4)	54.5 (47.1)	77.1 (45.2)
M1		1.9 (0.4)	ND	ND	ND
M2		4.1 (0.9)	ND	0.4 (0.4)	ND
M3		3.4 (0.7) (as sum with M4)	ND	ND	ND
M4		3.4 (0.7) (as sum with M3)	ND	ND	ND

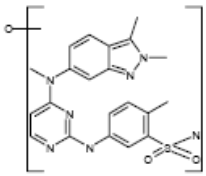
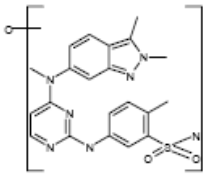
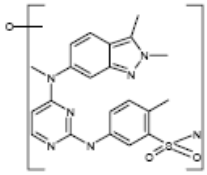
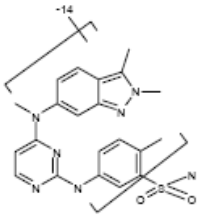
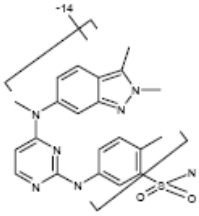
[Table excerpted from sponsor]

Mean % matrix radioactivity (mean % dose)[1]					
Peak ID Metabolite Structure		Bile	Feces		
			Male Intact	Female Intact	BDC
M8		17.2 (3.8) (as sum with M9)	7.1 (5.8)	9.1 (7.7)	ND
M9		17.2 (3.8) (as sum with M8)	ND	ND	ND
M10		3.2 (1.0) (as sum with M11)	ND	ND	ND
M11		3.2 (1.0) (as sum with M10)	ND	ND	ND
M14		6.7 (1.4) (as sum with M15)	ND	ND	ND
M15		6.7 (1.4) (as sum with M14)	ND	ND	ND

[Table excerpted from sponsor]

Mean % matrix radioactivity (mean % dose)[1]					
Peak ID Metabolite Structure		Bile	Feces		
			Male Intact	Female Intact	BDC
M16		6.5 (1.4) (as sum with M17)	ND	ND	ND
M17		6.5 (1.4) (as sum with M16)	ND	ND	ND
M18		10.8 (2.4)	ND	ND	ND
M20		4.4 (1.0) (as sum with M21 and M22)	ND	ND	ND
M21		4.4 (1.0) (as sum with M20 and M22)	ND	ND	ND
M22		4.4 (1.0) (as sum with m20 and M21)	ND	ND	ND

[Table excerpted from sponsor]

Mean % matrix radioactivity (mean % dose)[1]					
Peak ID Metabolite Structure		Bile	Feces		
			Male Intact	Female Intact	BDC
M24		3.0 (0.7)	3.6 (2.9)	5.6 (4.9)	2.2 (1.2)
M25		1.1 (0.2)	ND	ND	ND
M26		4.8 (1.1)	2.9 (2.4)	5.3 (4.6)	1.2 (0.7)
M27		1.6 (0.4)	NQ	ND	ND
M28		ND	ND	0.1 (0.1)	ND
Total		70.2 (15.7)	86.7 (71.5)	75.1 (64.8)	80.4 (47.1)
Mean % dose in matrix pool analysed[2]		21.9	83.1	86.1	58.5

[Table excerpted from sponsor]

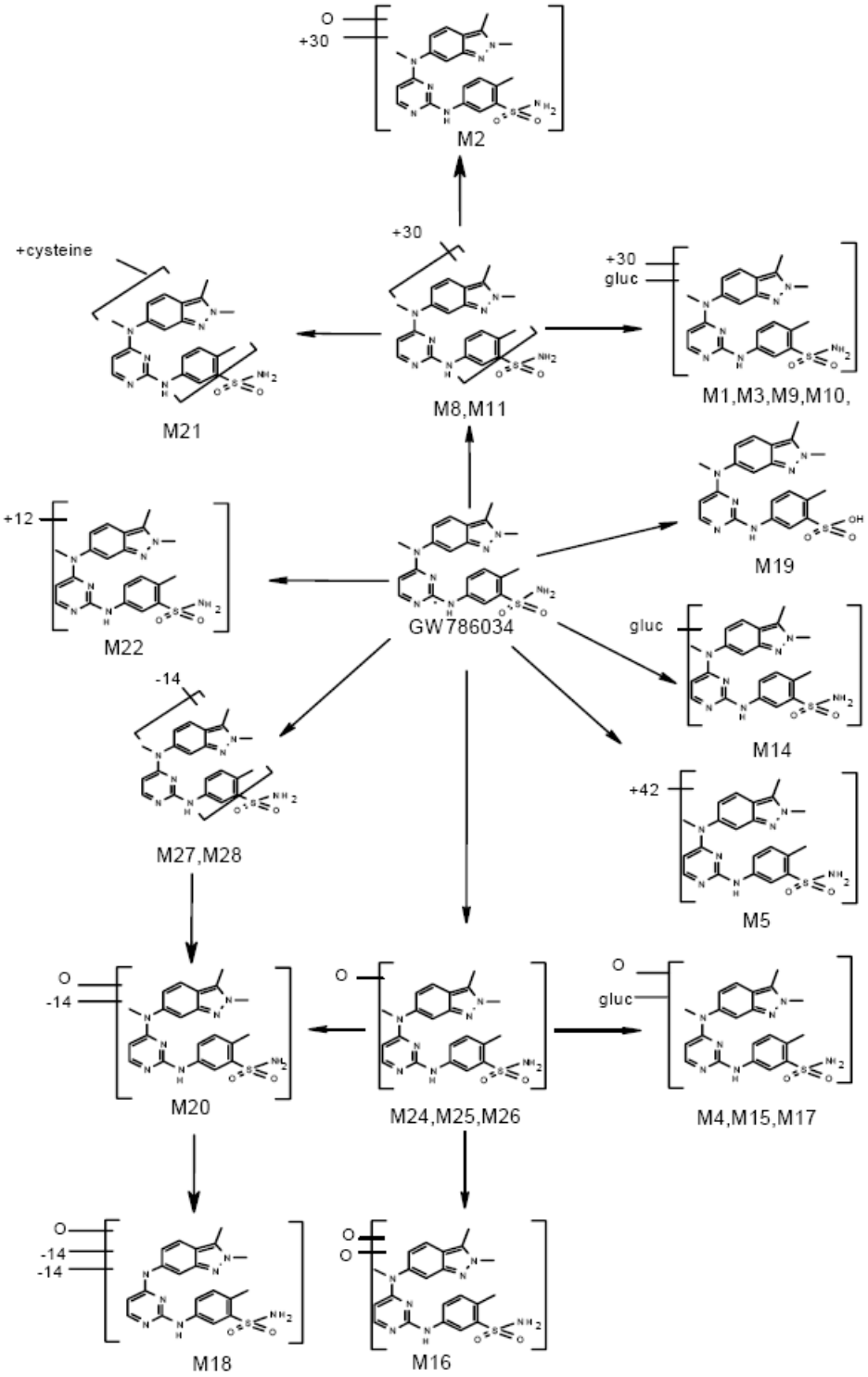
Mean % matrix radioactivity (mean % dose)[1]				
Peak ID Metabolite Structure	Bile	Feces		
		Male Intact	Female Intact	BDC
Mean % overall recovery[3]	100.6	98.0	84.3	83.0

ND = not detected; NQ = not quantifiable

1. Mean percent radioactivity recovered under each peak, corrected by the overall sample preparation recovery (data obtained from WinFlow results).
2. Mean percent of administered dose recovered in pooled samples (data obtained from [GSK Document Number CD2002/00108/00.
3. % recovery of radioactivity following centrifugation for bile; % recovery of radioactivity following solvent extraction for feces.

[Table excerpted from sponsor]

Figure 1 Proposed Metabolic Scheme for GW786034 in the Monkey



[Figure excerpted from sponsor]

CD2005/01355/00: Preliminary characterization of metabolites of GW786034 in human plasma and urine following oral administration in adults with solid tumors.

Key Findings:

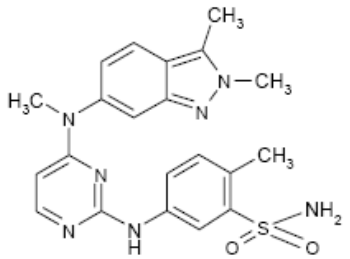
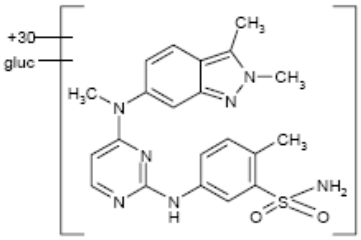
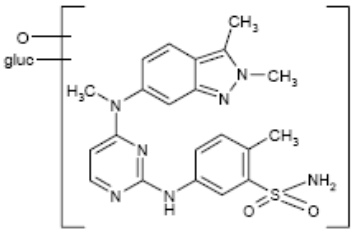
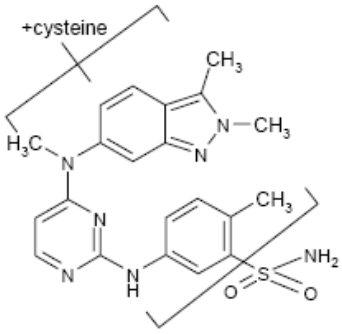
- In human plasma, metabolites M24 and M26 (mon-oxygenated prodcuts), M27 and M28 (demethylated products), and unchanged parent compound were detected on Day 1.
- The same metabolites detected on Day 1 were also identified on Day 22 with the addition of the following: M18 (mono-oxygentation and di-methylation product), M19(sulfonic acid product), M20 (mono-oxygenation product), and M21 (cysteine conjucate).
- Of all the metabolites identified in human plasma, M24 accounted for the highest amount of parent compound with 9 and 11% relative to parent compound on Day 1 and Day 22, respectively.
- In human urine, all metabolites detected on Day 1 were also detected on Day 22.
- In human urine, the following metabolites were identified: M1 and M10 (glucuronides of possible oxidation to carboxylic acid), M4, M15, M17, M17A, M33, and M34 (mono-oxygentated glucuronides), M7 and M21 (cysteine conjugates), M11 (oxidation of methyl carboxylic acid), M12 and M16 (di-oxygenated metabolites), M13 and 23 (mercapturic acids), M14 (glucuronide), M18 (mono-oxygenated and di-demethylated metabolite), M19 (sulfonic acid), M20 and M35 (mono-oxygenated and demethylated metabolites), M24, M26, and M36 (mono-oxygenated metabolites), M27 and M28 (demethylated metabolites) and unchanged parent compound.
- Summary of metabolites from plasma and urine are presented in sponsor's Table 1.

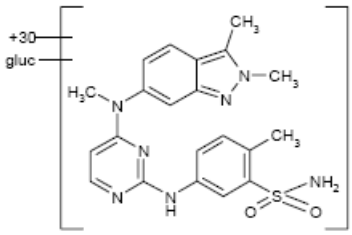
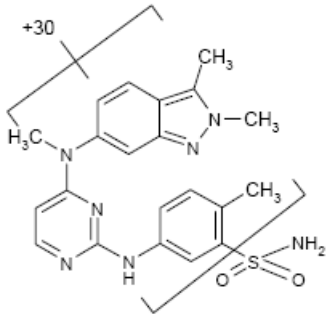
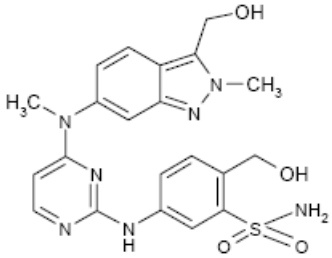
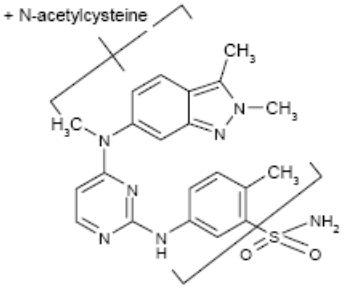
Report #:	CD2005/01355/00
Module:	4.2.2.4
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	July 9, 2004
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	Radiolabeled: [^{14}C]GW786034, R10836/67/3, 98.0% Non-radiolabeled: GW786034B, 786034-A2-01M-WR, 97.7%

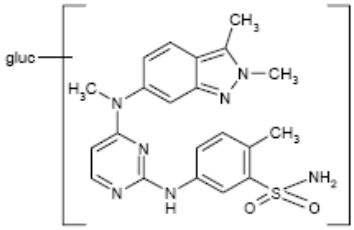
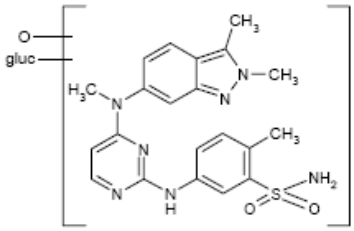
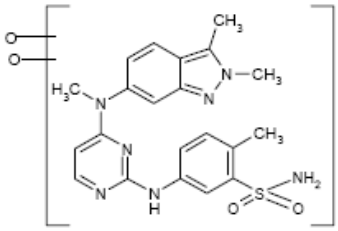
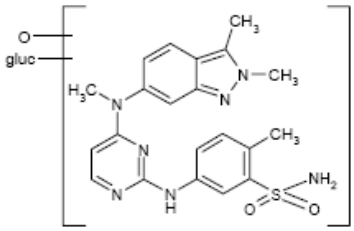
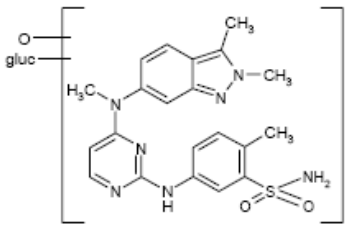
Methods:

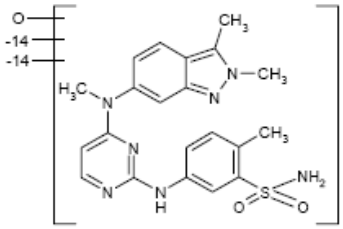
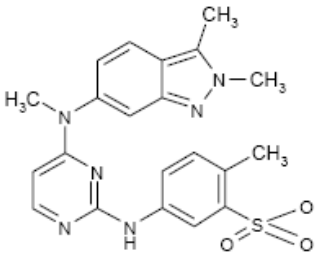
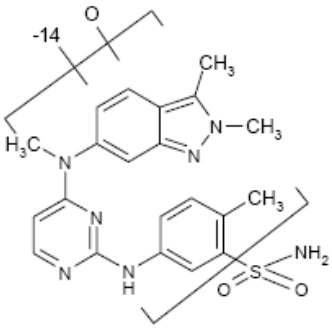
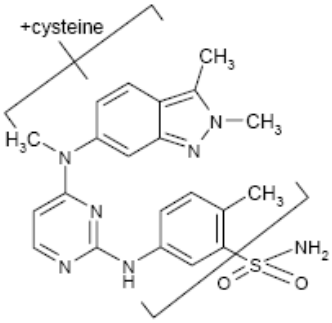
- Sample collection was obtained from a phase 1, open-label, non-randomized, multiple dose-finding clinical study in adult subjects with solid tumors (study identifier number VEG10003 and GSK Study No. RM2002/00345/07).
- Selected samples from human plasma and urine were collected from volunteers dosed with 2000 mg of GW786034 were used in this study.
- Selected samples of human urine and plasma were analyzed by LC/MRM to provide preliminary characterization of the metabolites.

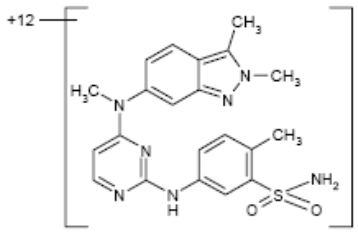
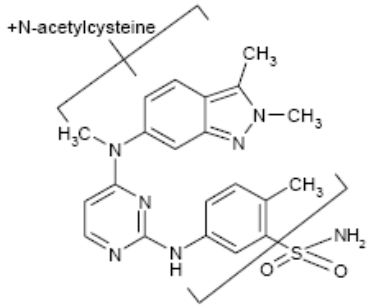
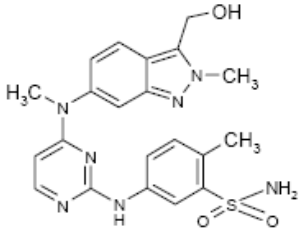
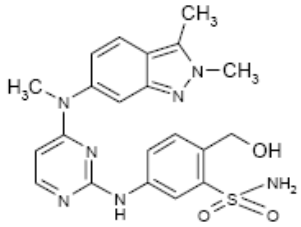
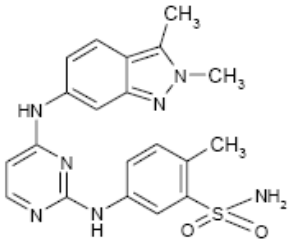
Table 1 Summary of GW786034-related Metabolites Detected in Human Urine and Plasma Using LC/MRM

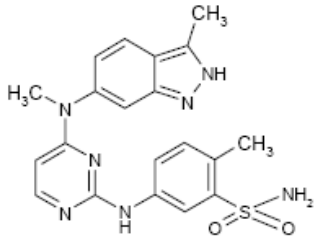
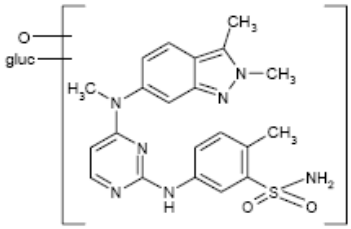
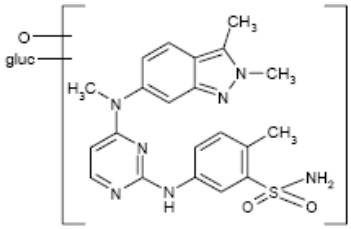
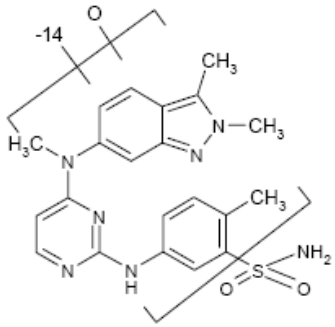
Metabolite ID (Retention time)	Proposed structure	MRM transition	Plasma (% Relative to Parent)[1]		Urine	
			Day 1	Day 22	Day 1	Day 22
P (46.2 min)		438→357	√ (100)	√ (100)	√	√
M1 (13.3 min)		644→468	ND	ND	√	√
M4 (21.3 min)		630→454	ND	ND	√	√
M7 (24.8 min)		557→436	ND	ND	√	√

Metabolite ID (Retention time)	Proposed structure	MRM transition	Plasma (% Relative to Parent)[1]		Urine	
			Day 1	Day 22	Day 1	Day 22
M10 (26.6 min)		644→468	ND	ND	√	√
M11 (25.7 min)		468→387	ND	ND	√	√
M12[2] (27.9 min)		470→452	ND	ND	√	√
M13 (28.1 min)		599→436	ND	ND	√	√

Metabolite ID (Retention time)	Proposed structure	MRM transition	Plasma (% Relative to Parent)[1]		Urine	
			Day 1	Day 22	Day 1	Day 22
M14 (27.0 min)		614→438	ND	ND	√	√
M15 (27.0 min)		630→454	ND	ND	√	√
M16 (28.8 min)		470→452	ND	ND	√	√
M17[3] (28.6 min)		630→454	ND	ND	√	√
M17A[3] (28.9 min)		630→454	ND	ND	√	√

Metabolite ID (Retention time)	Proposed structure	MRM transition	Plasma (% Relative to Parent)[1]		Urine	
			Day 1	Day 22	Day 1	Day 22
M18 (29.6 min)		426→408	ND	√ (NA)	√	√
M19 (29.6 min)		439→359	ND	√ (NA)	√	√
M20 (30.8 min)		440→422	ND	√ (NA)	√	√
M21 (30.5 min)		557→436	ND	√ (NA)	√	√

Metabolite ID (Retention time)	Proposed structure	MRM transition	Plasma (% Relative to Parent)[1]		Urine	
			Day 1	Day 22	Day 1	Day 22
M22 (32.3 min)		450→369	ND	ND	√	√
M23 (33.4 min)		599→436	ND	ND	√	√
M24[2] (33.7 min)		454→436	√ (9.4)	√ (10.9)	√	√
M26[2] (38.6 min)		454→436	√ (6.8)	√ (6.5)	√	√
M27[2] (42.1 min)		424→343	√ (2.8)	√ (3.1)	√	√

Metabolite ID (Retention time)	Proposed structure	MRM transition	Plasma (% Relative to Parent)[1]		Urine	
			Day 1	Day 22	Day 1	Day 22
M28[2] (47.0 min)		424→343	√ (0.9)	√ (1.2)	√	√
M33 (18.8 min)		630→454	ND	ND	√	√
M34 (19.8 min)		630→454	ND	ND	√	√
M35 (35.9 min)		440→422	ND	ND	√	√

[Table excerpted from sponsor]

2.6.4.6 Excretion

CD2002/00088/00: Elimination of radioactivity following a single oral (10 mg free base/kg) administration of [^{14}C]GW786034 (solution dose) to male and female intact and bile duct-cannulated rats.

Key Findings:
In intact male and female rats:
- Excretion of radioactivity was rapid, occurring largely within 48 hours of dosing.
- The major route of elimination was via the feces at approximately 52-61% of administered dose.
- Urinary excretion was moderate accounted for 15-17% of administered dose.
- Total recovery of radioactivity was similar in males and females (90% and 94% in males and females, respectively).
- There were no major gender differences in either the routes or rates of elimination.

In male bile duct-cannulated rats:
- The major route of elimination was via the feces at approximately 43% of administered dose.
- Urinary excretion was moderate accounted for 25% of administered dose.
- Absorption of drug-related material was estimated to be 35% of the dose.
- Total recovery of radioactivity was 92%.

In intact animals (in general):
- Mean plasma concentration of radioactivity was higher in females compared to males with male values of 936, 876, and 121 ng/g and female values of 1689, 1487 and 384 ng/g at 2, 8, and 24-hour post-dose, respectively.

Report #:	CD2002/00088/00
Module:	4.2.2.5
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	January 14, 2003
GLP Compliance:	No
QA Report:	Yes
Drug, lot #, and % purity:	Radiolabeled: [^{14}C]GW786034-A, AS201420-028A1, 98.5% Non-radiolabeled: GW786034-B, 786034-A2-01M, 98.5%

Doses:	Single dose of 10 mg free base/kg
Species/strain:	Sprague –Dawley
Number/sex/group or time point:	12 intact M / 12 intact F and 3 BDC M
Route, formulation, volume, and infusion rate:	Oral Gavage 30% hydroxypropyl β-cyclodextrin, pH 2.0 1.0 mg free base/mL
Age:	Male: 11 weeks/Female: 20 weeks
Weight:	266-362 g

Sampling times:	3/animals/timepoint Intact male (Group 4) and intact females (Group 5) at 2, 8, and 24 hours post dose
Study design:	See Methods section below

Methods:

The study design is summarized in the following table:

Type of Rats	No. of animals	Samples
Control (Intact)	2 male 2 female	Liver/Blood/Plasma G.I. tract/G.I. content/Carcass
Group I (Intact)	3 male	Urine/Feces Liver/Blood/Plasma G.I. tract/G.I. content/Carcass Cage-rinse, 96 hour sacrifice
Group II (Intact)	3 female	same as Group I
Group III (BDC)	3 male	same as Group I and bile
Group IV (Intact)	9 male	Liver/Blood/Plasma at 2, 8 and 24 hour
Group V (Intact)	9 female	Liver/Blood/Plasma at 2, 8 and 24 hour

[Table excerpted from sponsor]

- Total radioactivity was determined in urine and feces (up to 96 hours post-dose), from three intact male (Group 1) and three intact female (Group 2) animals.
- Total radioactivity was determined in urine, bile and feces up to 96 hours post-dose from three male BDC (Group 3) rats.
- For Groups 1, 2, and 3, the gastrointestinal tract (GI) and content, residual carcasses and cage rinse were also collected in order to assess the total radioactive dose recovered from these groups.

CD2002/00108/01: Elimination of radioactivity following a single oral (5 mg/kg) administration of [^{14}C]GW786034 to male and female intact and bile duct-cannulated monkeys

Key Findings:
In intact male and female monkeys:
- Excretion of radioactivity was gradual and was completed by 96-hours post-dose.
- The major route of elimination was via the feces at approximately 85% and 87% of administered dose in males and females, respectively.
- Urinary excretion was minimal.
- There were no major gender differences in either the routes or rates of elimination.

- The total recovery of radioactivity was similar between males and females (86% and 92% in males and females, respectively)
- Summary of results are shown in sponsor Figure 1.

In male bile duct-cannulated monkeys:

- The major route of elimination was via the feces at approximately 60% of administered dose.
- Biliary excretion accounted was moderate and accounted for 23% of administered dose.
- Urinary excretion was minimal and accounted for 2 % of administered dose.
- Absorption of drug-related material was estimated to be 25% of the dose.
- Total recovery of radioactivity was 91% (See sponsor Figure 2).

In intact animals (in general):

- Mean plasma concentration of radioactivity were observed 1 hour post dose in females and 8 hour post-dose in males.
- Mean plasma concentration of radioactivity declined by 168 hours post-dose.

Report #:	CD2002/00108/01
Module:	4.2.2.5
Conducting Laboratory and Location:	GSK Labs, King of Prussia, PA 19406
Date of Study Initiation:	June 13, 2002
GLP Compliance:	Yes
QA Report:	Yes
Drug, lot #, and % purity:	Radiolabeled: [^{14}C]GW786034 [SB-710468-B-[14C], AS201420-028A1, 96.7% and 96.0% Non-radiolabeled: GW786034-B, 786034-A2-01M, 98.5%

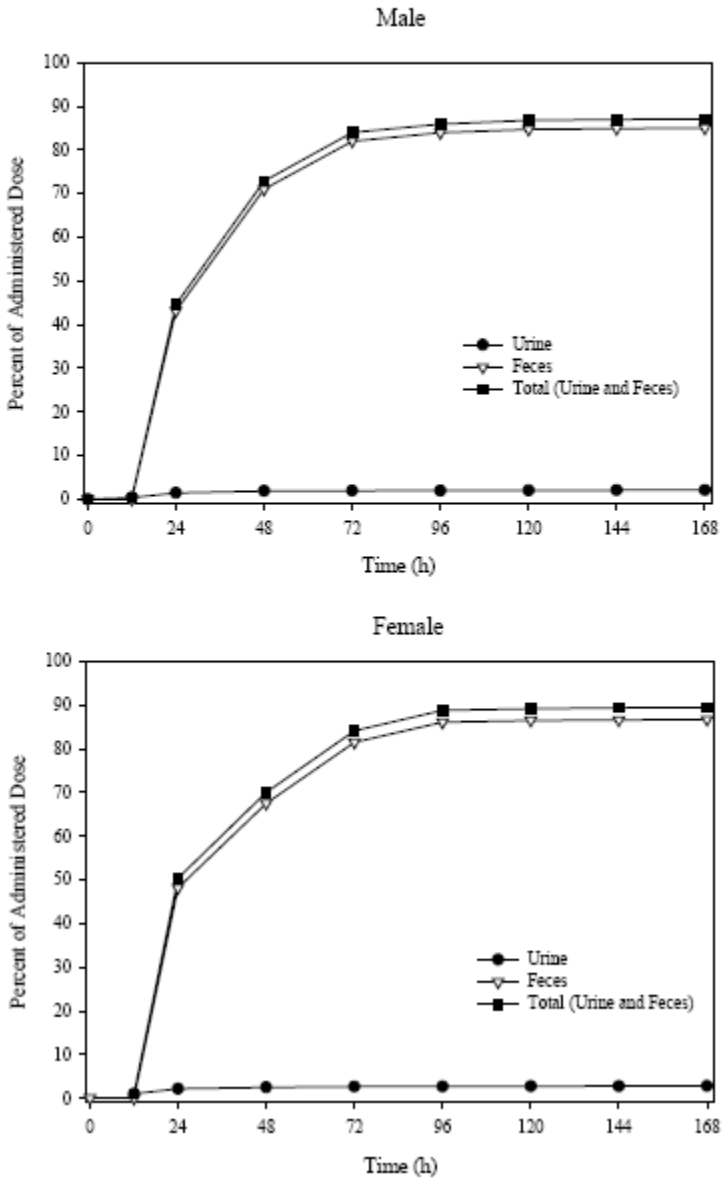
Doses:	Single dose of 5 mg/kg
Species/strain:	cynomolgus monkeys
Number/sex/group or time point:	3 intact males, 3 intact females and 3 BDC male
Route, formulation, volume, and infusion rate:	Oral Gavage 6% hydroxypropyl β-cyclodextrin, pH 2.3 5 mg/kg
Age:	Male: 11 weeks/Female: 20 weeks
Weight:	~4 kg
Sampling times:	3/animals/timepoint collected at 1, 8, 24 and 168 hours post-dose
Study design:	See Methods section below

Methods:

- Total radioactivity was determined from three intact male and three intact female monkeys (Group 1), urine and feces were collected pre-dose and at 0-12 h (urine only), 12-24 h (urine only) and at 24h intervals up to 168 h post-dose.

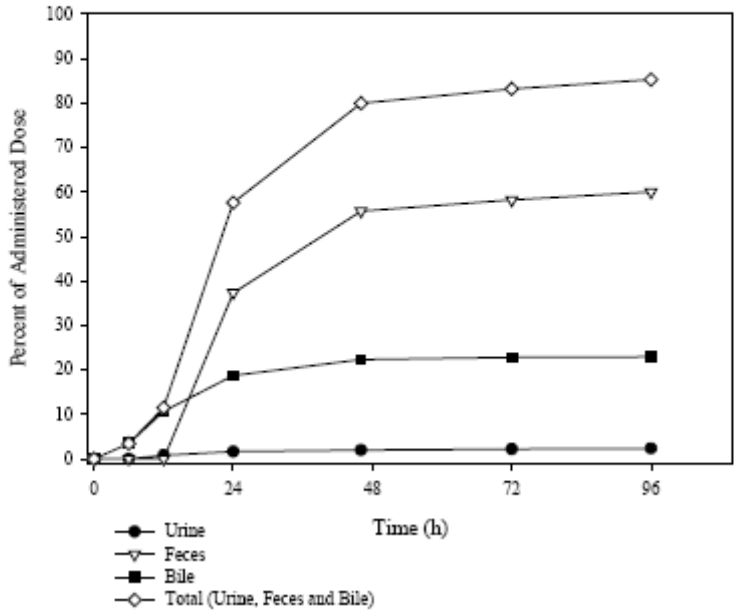
– From three male BDC monkeys (Group 2), urine, feces and bile were collected pre-dose and at 0-6 (bile only), 6-12 (bile only), 0-12 h (urine only), 12-24 h (bile and urine only) and at 24 h intervals through 96 h post-dose.

Figure 1 Mean cumulative elimination of radioactivity by intact male and female monkeys (Group 1) following an oral administration of [^{14}C]GW786034 at a target dose of 5 mg/kg.



[Figure excerpted from sponsor]

Figure 2 Mean cumulative elimination of radioactivity by bile duct-cannulated male monkeys (Group 2) following an oral administration of [^{14}C]GW786034 at a target dose of 5 mg/kg.



[Figure excerpted from sponsor]

2.6.2.7 Other Pharmacokinetic Studies – None

2.6.2.8 PHARMACOKINETICS TABULATED SUMMARY

Study #/	Method of Administration/ GLP?	Species	Doses	Gender/N	Findings
Absorption - Single dose					
RD2001/01451/00	Oral/ No	Mice	10, 30, and 100 mg/kg/day	Females/27	GW786034A showed rapid and prolonged absorption (Cmax and AUC values) at all doses levels. A dose increase from 10 to 100 mg/kg resulted in a 5-fold increase in Cmax and AUC values.
CD2002/00094/00	Oral and iv/ No	Rat	2 and 10 mg/kg free base	Males/4	Following iv dosing, GW786034B had a low clearance and volume of distribution equal to total body water. High oral bioavailability (61%) after oral administration.
CD2003/00102/00	Oral/ No	Rat	289 mg/kg	Males/4	(b) (4) GW786034B was quantifiable in the plasma for the entire 24 hours.

Study #/	Method of Administration/ GLP?	Species	Doses	Gender/N	Findings
					Cmax values were variable between the animals (ranging from 49.4 ± 21.3 µg/mL). Mean Tmax was long with a value of 422 minutes.
RD2001/01452/00	Oral and iv/ No	Rat	10 mg/kg/day	Males/3	No change in plasma concentrations following iv and oral administration of GW786034A. Low clearance (1.7 mL/min/kg) and high Vd (478 mL/kg) following iv administration of GW786034A. High oral bioavailability (72%) after oral administration.
RD2001/01514/00	Oral and iv/ No	Dog	1 mg/kg/day	Males/3	Fasted and fed dogs had rapid decreases in plasma levels after iv and oral (fed only) administration of GW786034A. Low clearance (1.4 mL/min/kg) and moderate Vd (297 mL/kg) following iv administration of GW786034A. Moderate oral bioavailability (47%) after oral administration.
CD2002/00093/00	Oral and iv/ No	Monkey	2 and 10 mg/kg	Males/4	Following iv administration, GW786034B was estimated to have a low clearance and with a Vd equal to approximately 40% of total body water following iv administration. Low oral bioavailability (16%) after oral administration.
RD2001/01169/00	Oral and iv/ No	Monkey	5 and 50 mg/kg/day	Males/4	No difference in plasma levels in both fasted and fed monkeys after iv and oral administration of GW786034A. Similar half-lives were observed following iv and oral administration (4.7-6.2 h) of GW786034A. Low clearance (~2 mL/min/kg) and moderate Vd (285 mL/kg) following iv administration of of GW786034A.. Moderate oral bioavailability (49%) after oral administration.
RD2002/00061/00	Oral/ No	Monkey	50 mg/kg	Males/4	Oral exposure of GW786034X at 50 mg/kg is the same whether it is administered as the

Study #/	Method of Administration/ GLP?	Species	Doses	Gender/N	Findings
					monohydrochloride or dihydrochloride salt forms.
CD2003/00103/00	Oral/ No	Monkey	49.6 mg/kg	Males/4	Cmax values were similar between the animals while AUC(0-t) values varied. Mean Tmax values varied ranging from 60-121 minutes.
Absorption - Repeat dose					
CD2007/00494/01	Oral/ No	Mice	100 mg/kg/day	Males/4	GW786034B and its metabolites caused no significant change in AUC and Cmax with repeat dosing between Day 1 and Day 7 at 100 mg/kg
CD2007/00493/01	Oral/ No	Rat	3 and 30 mg/kg/day	Males/4	A less than 2 fold difference in Cmax and AUC (0-t) between Days 1 and 7 for GW786034B and its 4 active metabolites.
CD2006/01538/01	Oral/ No	Monkey	50 mg/kg	Male and Female/3	GW786034 was quantifiable up to at least 8 hours, with mean Tmax occurring at approximately 2 hours on both Days 1 and 7. Metabolite concentration was quantifiable up to at least 4 or 8 hours for 3 metabolites identified (GSK1268992, GSK1268997, and GSK 1071306). Mean Tmax of the four metabolites occurred at approximately 3 hours after dosing on both study days. No apparent gender differences in AUC and Cmax and metabolites on both study days except GSK1268992. No difference in systemic exposure between Day 1 and Day 7 for the parent drug and metabolites
Distribution					
(b) (4) 85-0623	Oral/ Yes	Rat	60 mg/m^2	7F	Wide distribution throughout the body Long-term (up to 35 days) association with melanin producing cells (uveal tract, meninges, skin) There was quanitifiable drug present in the liver, cecum mucosa, and nasal turbinates for $\geq$ 3 days following single dose administration.
(b) (4) 85-0337	Oral/ Yes	Rat	60 mg/m^2	6M	Wide distribution throughout the body

Study #/	Method of Administration/ GLP?	Species	Doses	Gender/N	Findings
					Long-term (up to 35 days) assoication with melanin producing cells (uveal tract, meninges, skin) There was quanitifiable drug present in the liver at ≥ 7 days following single dose administration.
RH2002/0074/01	In vitro/ No	NA			GW786034B binds human serum albumin with high affinity.
RD2002/00877/00	In vitro/ No	NA	5 µM		Plasma protein binding was >99.9% for mouse, rat, dog, monkey and human.
CD2004/00451/00	In vitro/ Yes		10, 20, 50, and 100 µg/mL		Plasma protein binding was >99% for mouse, rat, dog, monkey and human at all concentrations. > 93% recovery after equilibrium dialysis for human plasma.
CD2006/00629/00 06DMM047	In vitro	NA	0, 0.1, 1, 3, 10, and 30 µM		GW786034B is an in vitro inhibitor of the human OATP1B1 transporter at a concentration of 0.79 µM.
Metabolism					
CD2004/00901/00	In vitro/No	Pooled liver microsomes	5 and 50 uM		Oxidative metabolism of GW78604 metabolites is mediated by CYP3A4 Metabolite M24 and M26 was detected with CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 Metabolite M27 was detected with CYP1A2, CYP2D6, and CYP3A4 Metabolite M12 and M16 were only detected with CYP3A4.
RD2002/00874/00	In vitro and in vivo/No	Pooled liver microsomes and plasma samples in mouse, rat, dog , monkey and human	NA		Mono-oxygenated metabolites (M3 and M4) were detected in microsomal and plasma samples in mouse, rat, dog, and monkey and human microsomal samples. Di-oxygenated metabolite (M2) was detected *in vitro* but not *in vivo* *N*-demethylated metabolite (M5) was detected *in vitro* mouse and rat and *in vivo* mouse and monkey The M1 metabolite was only present in human microsomal samples.
CD2007/00811/00	In vitro/No	NA	0-250 µM		GW786034 was a direct inhibitor of human UGT1A1 (IC50 = 1.2 µM)
CD2003/00965/00	In vitro/No	Pooled hepatocytes	10 µM		The routes of metabolism observed in human liver microsomes and

Study #/	Method of Administration/ GLP?	Species	Doses	Gender/N	Findings
		and hepatic microsomes from in mouse, rat, dog , rabbit, monkey and human			hepatocytes were mono-oxygenation, di-oxygenation, and possibly oxidation to a carboxylic acid. Glucuronidation of a mono-oxygenated metabolite was also detected in human hepatocytes. There were no human specific phase 1 metabolites observed in either liver microsomal or hepatocyte incubations. A phase 2 metabolite (M9), a glucuronide potentially derived from a carboxylic acid metabolite (M8), was observed only in human hepatocytes.
CD2003/00864 /00	In vitro/No	human liver microsomes			GW786034 showed moderate to marked inhibition of human cytochrome P450 enzymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 in human liver microsomes with IC50 values ranging from 7.9 µM (CYP2C9) to 18 µM (CYP2D6). GW786034 did not inhibit CYP2A6 (IC50 of >100 µM). GW786034 was not a time-and NADPH-dependent inhibitor in any of the cytochrome P450 enzymes investigated.
CD2005/00865 /00	In vivo/No	Mice	100 mg/kg	3 M/ 3 F	In mouse plasma, M24, M26, M27, and M28 were oxidative metabolites of GW78304. M24 and M26 are products of mono-oxygenation while M27 and M28 are oxidative products of N-demethylation. Each of these metabolites were detected at small amounts relative to the parent compound (2.5%, 8.4%, 6.1% and 0.6% for M24, M26, M27, and M28, respectively).
CD2003/00860 /00	Oral/No	Rat	10 mg/kg	3 M/ 3 F	Following a single oral administration of [^{14}C]GW78604 to male and female rats, no metabolites were detected in rat plasma. No metabolites were detected in rat urine.

Study #/	Method of Administration/ GLP?	Species	Doses	Gender/N	Findings
					GW786034 is eliminated via fecal route as the unchanged parent compound. GW786034 represented 54, 46, and 39% of the administered dose in male and female intact and bile duct cannulated (BDC) rats, respectively. Biliary metabolites accounted for a small amount of the administered dose (<2%).
CD2004/00028/00	Oral/No	Monkey	5 mg/kg	3 M/ 3 F	Minor metabolites in the monkey plasma included M26 and M27. In BDC monkeys, GW786034 is eliminated via fecal route as the unabsorbed parent compound. Biliary metabolites accounted for a small amount of the administered dose (<4%). Predominant route of metabolism is oxidation of methyl to carboxyl.
CD2005/01355/00	Oral/No	human plasma and urine	2000 mg		All metabolites detected on Day 1 were also detected on Day 22 in human and plasma samples
Excretion					
CD2002/00088/00	Oral/No	Rats	10 mg/kg	12 M/ 12 F and 3 M BDC	Major route of elimination was feces with unchanged drug representing 54% and 46% in male and female intact monkeys, respectively and 39% in BDC monkeys. Urinary excretion was the second major route of exposure accounting 42% and 15-17% of administered dose in intact and BDC rats, respectively. Total radioactivity recovery was similar in both types of rats (90 and 94% in males and females intact rats and 92% in BDC rats). Mean plasma concentration of radioactivity was higher in females compared to males. Biliary excretion was only present in BDC monkeys and accounted for <2% of administered dose.

Study #/	Method of Administration/ GLP?	Species	Doses	Gender/N	Findings
CD2002/00108 /01	Oral/Yes	Monkeys	5 mg/kg	3 M/ 3 F and 3 M BDC	Major route of elimination was feces with unchanged drug representing 60.4% and 47% in male and female intact monkeys, respectively and 45.2% in BDC monkeys. Urinary excretion was the second route of exposure and was minimal. Total radioactivity was at similar levels in both types of rats (86-92% in intact and 91% in BDC). Biliary excretion was only present in BDC monkeys and accounted for <4% of administered dose.

1. Meyer Zu Schwabedissen HE, Kim RB. Hepatic OATP1B Transporters and Nuclear Receptors PXR and CAR: Interplay, Regulation of Drug Disposition Genes, and Single Nucleotide Polymorphisms. Mol Pharm. 2009 Jul 10.

2.6.4.9 Tables and figures to include comparative TK summary

The following studies are reviewed in the Toxicology section (Section 2.6.6) including repeat-dose toxicity (2.6.6.3). These studies are the following: 13-week repeat dose in mouse (Study No. WD2005/00481/00), 26-week repeat dose in rats (Study No. RD2002/01337/01) and 52-week repeat dose study in monkeys (Study No. RD2002/01338/02).

Dose (mg/kg/day)	Sex	Cmax (µg/mL) range		$AUC_{0—t}$ (µg h/mL)		Ratio of animal to human exposure
		Week 4	End of study	Week 4	End of study	AUC
Mice 13-week						
100	M	ND	102	ND	818	0.79
	F	ND	132	ND	1434	1.4
300	M	ND	98	ND	1044	1.0
	F	ND	134	ND	1463	1.4
1000	M	ND	81	ND	932	0.90
	F	ND	114	ND	1607	1.5
Rat 26-week						
3	M	6.5	9	63	77	0.07
	F	11	10.5	90	100	0.10
30	M	37	45	351	374	0.36
	F	66	61	498	544	0.52
300	M	73	67	877	830	0.80
	F	114	94	1308	887	0.85
Monkey 52-week						
5	M	14	13	44	46	0.04
	F	10	13	33	36	0.03
50	M	28	32	151	235	0.22
	F	27	44	160	289	0.28
500	M	62	44	665	252	0.24
	F	49	57	345	670	0.64

Note:

- Human exposure AUC value was based on Study No. VEF10007 where pazopanib was given for 16 days at a dose of 800 mg pazopanib administered 1x/day.
- For all studies, ratio of animal to human exposure was calculated from end of study AUC values
- For 13-week mice study, end of study Cmax and AUC values were at taken at Week 13.
- For 26-week rat study, end of study Cmax and AUC values were at taken at Week 13 for high dose animals only. For LD and MD, values were taken at end of Week 26.
- For 52-week monkey study, end of study Cmax and AUC values were at taken at Week 13 for high dose animals only. For LD and MD, values were taken at end of Week 52.
- ND = not done

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

See section 2.6.4.10

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

The general toxicology of pazopanib has been examined in mice, rats, dogs, and monkeys. Single dose studies were conducted in the rat and dog using both the iv (rat) and po (dog) routes while repeat dose studies used the po route of administration. A 13-week mice study was designed to select doses for future carcinogenicity study. 1-month, 28-day investigational and 13-week oral toxicity studies were conducted in the rats to further characterize findings observed during the 1-month study (Study reviewed by Lilliam Rosario, PhD). Chronic toxicology studies consisted of a 26-week study in rats and a 52-week study in monkeys.

In addition, the toxicology studies discussed were conducted using the monohydrochloride salt of pazopanib or referred to as GW786034B throughout this section. (b) (4) Since the monohydrochloride salt (GW786034B) is the one intended for clinical use, studies using this formulation were primarily reviewed.

In rat studies, the dry pellet rodent diets were either moistened with water (1-month and 28-day study) or crushed to a powder form (26-week study) to improve animal's ability to eat food due to GW786034B effects on teeth. These effects lead to reductions in body weight and food consumption parameters. Additionally, a powdered diet was used in the 13-week studies in both mice and rat.

In the 13-week mice study (12 mice/sex/group), GW786034B was administered once/day for 13-weeks at doses of 0, 100, 300, and 1000 mg/kg/day (0, 300, 900, and 3000 mg/m^2). No mortality occurred in the study. An unusual finding that occurred during Week 8 was the clinical observation of overgrown and/or broken nails in all dose groups. Additional clinical findings were seen mostly at $\geq$300 mg/kg/day ($\geq$900 mg/m^2) and included white, broken, and overgrown teeth and longer fore and hind paws in a number of animals during Week 8. Pale/colored feces were observed only at 1000 mg/kg/day (or 3000 mg/m^2) at Week 8 until the end of the study (Week 13).

There was a significant increase in body weight gain in males (+17%) at 300 mg/kg/day (900 mg/m^2) while significant decreases in females at both 300 mg/kg/day or 900 mg/m^2 (-19%) and 1000 mg/kg/day or 3000 mg/m^2 (-25%). Food consumption values varied in that significant decreases were only observed in females at 300 mg/kg/day or 900 mg/m^2 (-9%) and 1000 mg/kg/day or 3000 mg/m^2 (-17%).

A dose-dependent inflammatory response was present in both males and females at $\geq$100 mg/kg/day ($\geq$300 mg/m^2) with the most significant increases occurring in females at 1000 mg/kg/day (3000 mg/m^2). Changes included increases in white blood count (+16 to +234%), neutrophil (+36 to +814%), lymphocytes (+12 to +140), eosinophils (+33 to +142%), and monocytes (+44 to +400). Also at $\geq$300 mg/kg/day ($\geq$ 900 mg/m^2), there

were slight non-significant increases (≤21%) in red cell mass as measured by: mean cell hemoglobin and mean corpuscular volume, hematocrit and hemoglobin levels compared to controls.

Clinical chemistry showed elevated levels of alanine aminotransferase (+130% at 900 mg/m^2 and +108% at 3000 mg/m^2) and aspartate aminotransferase (+67% at 900 mg/m^2 and +89% at 3000 mg/m^2) in males only. No significant changes occurred in females at all dose levels.

Gross pathology changes were limited to the duodenum (females only), ileum (females only), and jejunum (both males and females) at 1000 mg/kg/day (3000 mg/m^2). Findings included thick, pale and distended organ.

Microscopic findings across all dose groups were in the spleen (minimal to slight haemosiderosis), liver (minimal to slight reduced hepatocyte cytoplasmic rarefraction), ovaries (minimal to slight crystalline material accumulation, presence of corpora lutea and slight to moderate epithelial cysts), and upper incisor teeth (minimal to slight irregular/thickened predentine, irregular ameloblast layer, and thinning of dentine). Similar lesions in the teeth were also present in lower incisor region, however, only at ≥300 mg/kg/day (≥900 mg/m^2). Lesions in the digits (minimal to slight distortion of distal phalanx and subcutaneous inflammation) and liver (minimal to slight centrilobular aggregates of granular macrophages) were primarily observed in males at ≥300 mg/kg/day (≥900 mg/m^2). There was minimal to moderate basophilic tubules, and minimal to slight inflammation in the cortex at ≥300 mg/kg/day (≥900 mg/m^2) and minimal to slight partial fusion and cartilage of the growth plate at 300 mg/kg/day (900 mg/m^2). Correlating to gross pathology findings, there were minimal to moderate crystalline material in lamina propria and minimal inflammatory cells within the duodenum in most females at 1000 mg/kg/day (3000 mg/m^2). In addition, there were minimal to slight crystalline material in lamina propria of the jejunum. The mesenteric lymph node (minimal to slight crystalline material accumulation) was also affected in both males and females at 1000 mg/kg/day (3000 mg/m^2).

There was no difference in Cmax and AUC between male and female mice. Exposure was essentially equivalent across all doses. In conclusion, GW786034B was well tolerated at 1000 mg/kg/day (3000 mg/m^2) for 13-weeks. There were some hepatic toxicity especially in male rats at ≥300 mg/kg/day (≥900 mg/m^2).

The 28-day investigative study in rats (Study No. RD2002/00721/01) was conducted to determine if dental and nail findings observed macroscopically during recovery phase (at 300 mg/kg/day or 1800 mg/m2) in 1-month rat study (Study No. CD2002/00063/00 – reviewed by Lilliam Rosario, PhD) could be resolved if animals were given extended recovery periods. GW786034B was administered in powder diet form at 10, 30, and 300 mg/kg/day (60, 180, and 1800 mg/m^2) orally to male rats (1x/day) for 26-28 days. Recovery periods consisted of 2, 4, 6, and 10 weeks. Results of this study showed that treatment-related macroscopic findings in the paws (hindlimb red discoloration and short) and mandible and maxilla teeth (incisor pale discoloration, incisor broken, incisor elongated) were not resolved at the end of week 7 (Study Day 71), however, by week 10 (Study Day 99), these findings were fully recoverable.

The 13-week rat study (Study No VD2006/00544/00) concluded that using a powdered diet in younger rats (6-7 weeks of age compared to 12-13 weeks of age) improved body weight parameters, survival and overall clinical condition at the highest dose level, 300 mg/kg/day (or 1800 mg/m^2), but did not impact toxicity at 3 or 30 mg/kg/day (18 and 180 mg/m^2).

In the 26-week rat study (18 rats/sex/group for control and 300 mg/kg/day and 12 rats/sex/group for 3 and 30 mg/kg/day), GW786034B was administered once/day for 26 weeks at doses of 0, 3, 30, and 300 mg/kg (or 0, 18, 180, and 1800 mg/m^2). Due to severe toxicity at the high dose of 300 mg/kg/day (or 1800 mg/m^2), interim necropsies were conducted on Week 14 (6 controls and all surviving males at 300 mg/kg/day) and Week 20 (6 controls and all surviving females at 300 mg/kg/day). The terminal or final necropsy was conducted on Week 27 (remaining controls, males and females at 3 and 30 mg/kg/day).

Mortality occurred at the HD of 300 mg/kg/day (or 1800 mg/m^2) and at an earlier onset in males compared to females. Nine males and nine females were either found dead or euthanized in moribund condition during study day 53 to 96 for males and study day 66-134 for females. Due to mortality and toxicity at this dose, all remaining males and females (and 6 control males and 6 control females) were euthanatized on Week 14 or Study Day 98 (males only) and Week 20 or Study Day 140 (females only). All other animals survived to the end of the study with the exception of 1 female at the MD of 30 mg/kg/day (or 180 mg/m^2) which died during Week 18. The death of this female was considered treatment-related.

Early death was mostly attributed to significant decreases in body weight and food consumption due to animals inability to eat due to deteriorating clinical signs of broken, loose, and missing teeth (both in the upper and lower incisors). Animals showed this finding at approximately Week 6. Other drug-related clinical findings included thin, hunched appearance, pale skin, malocclusion, hypoactive behavior, coldness to the touch, squinted eyes, missing nail(s), rough, red, yellow, or brown haircoat, and few/non-formed/liquid, or discolored feces. Clinical signs for the 1 F that died at 30 mg/kg/day (or 180 mg/m^2) were similar to those observed at 300 mg/kg/day and included thin, hunched appearance, coldness to the touch, pale ears, and missing or excessively long incisor teeth. Macroscopic findings showed that most animals that died early and/or were euthanized had large lumen in the GI tract. Similar to the clinical signs mentioned above, almost all animals had missing/broken/worn maxilla and mandible teeth. Large, dark and red diffuse areas in the adrenal cortex were present in a greater number of females while 7/9 males had small, soft testes. The most notable microscopic effects were those in the mandibular and maxillary incisor teeth. Most findings were marked to severe and included inflammation; decreased thickness in the dentine and enamel; periodontal edema; ameloblastic, dental pulp, odontoblastic necrosis; and degenerative dentine and enamel. Other microscopic findings showed changes in the adrenal cortex (minimal to moderate to severe hemorrhage); femur bone (minimal to severe growth plate hypertrophy); sternum bone (moderate periosteal chondroid change); bone marrow (minimal to severe hypocellularity); kidney (minimal to slight chronic progressive nephropathy), pancreas (minimal and moderate acinar atrophy); jejunum (minimal to

slight crystalline pigment); ovary (slight to moderate atrophy); epididymis (minimal to slight cribiform change); testes (moderate to severe degenerative atrophy), trachea (decreased globule leukocytes).

For animals that survived study termination and/or those that were euthanized early (Week 14 for males and Week 20 for females) and were not found in moribund condition, clinical signs included thin appearance, excessively long, loose, broken and missing teeth (both in the upper and lower incisors), non-formed feces and rough hair coat at 30 mg/kg/day (180 mg/m^2). With the exception of excessively long and broken teeth, animals at 3 mg/kg/day (18 mg/m^2) did not show any other clinical signs.

Lower mean body weights and food consumption of males and females closely correlated with the appearance of drug-related effects in the teeth that began around the same time as those reported in 1-month and 13-week rat studies. Decreases in body weight were present in males at 30 (-8 to 10%) and 300 (-16 to -22%) mg/kg/day (or 18 and 180 mg/m^2) while decreases for females occurred only at the high-dose of 300 mg/kg/day or 1800 mg/m^2 (-8 to -14%). Decreases in both males and females at the high-dose (300 mg/kg/day or 1800 mg/m^2) continued until the animals were euthanized (Week 14 for males and Week 20 for females). Due to the test article-related effects on the teeth, all animals were switched to a crushed feed diet instead of pelleted diet beginning at Week 7. As a result, mean body weights for males at 30 mg/kg/day (or 180 mg/m^2) increased by Week 10 and were comparable to controls throughout the dosing period.

Similar to body weight changes, food consumption values were lower in males at and females at 300 mg/kg/day or 1800 mg/m^2 (males: 16-22% and females: 8-14%) and males at 30 mg/kg/day or 180 mg/m^2 (8 -10%). By Week 10, food consumption values were similar to control levels after animals were switched diets.

Most hematology effects were seen at the HD of 300 mg/kg/day (or 1800 mg/m^2) with a few changes at the MD of 30 mg/kg/day (or 180 mg/m^2). The most prominent changes occurred by Week 13 and included decreases in red blood cell count (-28% for both males and females); platelets (-20% for males and +31% for females); mean corpuscular volume (+31% for males and +26% for females) and mean corpuscular hemoglobin (+44% for males and +40% for females). Signs of inflammation were present at 300 mg/kg/day (or 1800 mg/m^2) and consisted of an increase in white blood counts (+31% for females only), neutrophils (+47% for males and +56% for females) and monocytes (+57% for males and +50% for females). There were a few changes at the mid-dose of 30 mg/kg/day (or 180 mg/m^2). Changes were slight in magnitude but similar observations at the high dose. All changes at 30 mg/kg/day (or 18 mg/m^2) were still present by the end of the study (Week 27). Animals at 300 mg/kg/day or 1800 mg/m^2 (males and females) that were euthanized at interim sacrifices (Week 14 and 20) showed hematology changes similar to Week 13 findings.

Most clinical chemistry changes occurred at 30 and 300 mg/kg/day (or 180 and 1800 mg/m^2). The majority of these effects were relatively mild and nonspecific, although some may have been related to reduced food consumption and increased incidence and severity of chronic progressive nephropathy.

At Week 13, changes at the high-dose of 300 mg/kg/day (or 1800 mg/m^2) included decreases in albumin (-13% males and -16% females) and increases in cholesterol (+45% males and +42% females). There were specific changes in females at 30 or 300 mg/kg/day (or 180 and 1800 mg/m^2) which were not present in male rats. These included increases in urea (+20% Week 13 and +21% Week 20), total bilirubin (+50% for both Week 13 and 20), and phosphorus (+17% Week 13, +18% Week 20, and +16% Week 27). Males, however, showed slight but consistent increases in globulin levels (+15% Week 13 and +15-19% Week 27) at 3 and 30 mg/kg/day (or 18 and 180 mg/m^2). Changes at the mid-dose of 30 mg/kg/day (or 180 mg/m^2) dose were small and included significant decreases in albumin-to-globulin ratio (-18% males and -15% females) occurred by Week 27. Albumin (+13 males and +12 females) and cholesterol (+40 males and +38 females) changes were also was also increased at this dose.

The liver enzyme, alanine aminotransferase, was elevated at 300 mg/kg/day (or 1800 mg/m^2) as early as Week 4 (+197% males and to +87% females) and remained elevated by Week 13 (+149% males and to +90% females). Levels remained elevated in HD females (114% at 300 mg/kg/day) by Week 20. The findings for alanine aminotransferase were of somewhat less magnitude than those usually accompanied by correlative microscopic findings.

There were marked changes in urine chemistry for animals at 300 mg/kg/day (1800 mg/m^2) by the time rats were euthanized. Changes included a significant increase in urinary volume (+251%) at Week 20 for females and a decrease in urinary creatinine excretion (-37%) at Week 14 for males. The decrease in urine creatinine excretion in males at Week 14 may have been related to decreased muscle mass. Females had significantly higher protein excretion (+2416% in F and +120% in M) and protein/creatinine ratio (+1708% in F and +237% in M) compared to males. These increases were still present in rats that survived until study termination at Week 27 at 18 and 180 mg/m^2. Protein excretion values were 2-fold higher in females compared to males at both 180 mg/m^2 (+781% in F and +386% in M) and 18 mg/m^2 (+281% in F and +146% in M). Similar fold increases were found in protein/creatinine ratio for both males and females at both 180 mg/m^2 (+802% in F and +427% in M) and 18 mg/m^2 (+200% in F and +109% in M).

Organ weight changes included significant decreases in absolute and relative testes (absolute: -24% and -53%; relative -22% and -50% at 30 and 300 mg/kg/day, respectively). Epididymis weights were decreased, however, only at 300 mg/kg/day (absolute: -45% and relative -19% at 300 mg/kg/day). A large significant increase in absolute (-197%) and relative (-198%) adrenal weights was observed in females at 300 mg/kg/day (1800 mg/m^2). No changes occurred in animals at 3 mg/kg/day (18 mg/m^2).

At study termination at Week 27, drug-related histopathology findings were mostly found at the MD of 30 mg/kg/day (180 mg/m^2). Similar to findings in animals that died early and/or euthanized by Week 14 and 20, a significant number of changes were observed in the mandible and maxilla incisor teeth (decreased dentine and enamel thickness, degenerative dentine and enamel thickness, and odontoblastic attenuation/atrophy). However, the incidence and severity of dental lesions were less for animals at 30 mg/kg/day (18 mg/m^2) compared to those that died early and/or were euthanized at 300

mg/kg/day (1800 mg/m^2). Other organs that were affected included the adrenal cortex (minimal to marked angiectasis and hemorrhage), femoral and sternal bone marrow (minimal to slight/moderate hypocellularity), kidney (minimal to slight chronic/progressive nephropathy), pituitary (minimal basophilic hypertrophy), testes (minimal and severe degenerative atrophy), and trachea (presence of decreased globule leukocytes).

A small number of changes occurred in animals at 3 mg/kg/day (18 mg/m^2). These included findings in the adrenal cortex (minimal to marked angiectasis and hemorrhage) in females only, kidney (minimal to slight chronic/progressive nephropathy), pituitary (minimal basophilic hypertrophy) in males only, and trachea (presence of decreased globule leukocytes).

GW786034B was detected in the plasma of all drug-treated animals on all sampling days and increased with dose. The maximum plasma concentration was reached approximately 0.5 and 4 hours post dosing. There was no gender related difference in systemic exposure (Cmax and AUC) between males and females. AUC values increased, on average, 12.4-fold for a 100-fold increase in dose between 3 and 300 mg/kg/day (18 and 1800 mg/m^2) across Weeks 4 and 13. There was no marked change in systemic exposure between Weeks 4 and 13 for animals given 3 to 300 mg/kg/day (18 and 1800 mg/m^2) or Week 26 for animals at 3 and 30 mg/kg/day (18 and 180 mg/m^2) .

In the 52-week monkey study (4 animals/sex/group for all dose groups), GW786034B was administered once/day for 52 weeks at doses of 0, 5, 50 and 500 mg/kg (0, 60, 600, and 6000 mg/m^2). The high-dose of 500 mg/kg/day (6000 mg/m^2) was not tolerated by monkeys and resulted in a drug-related mortality in 1 M during Week 24 (Study Day 171). Non-drug related mortalities included 1 M at 5 mg/kg/day and 1 M at 500 mg/kg/day during Week 52 and 90, respectively. Due to persistent and severe diarrhea that correlated with inappetence and weight loss, two monkeys (1 M and 1 F) at 500 mg/kg/day (6000 mg/m^2) were euthanized at Week 34. In addition to the clinical signs mentioned, these animals also exhibited group histological evidence of crystalloid material in the gastrointestinal tract. Based on these findings, all remaining animals at 500 mg/kg/day dose or 6000 mg/m^2 (1 M and 3 F) were not dosed after Week 35 to recover until the end of the study.

As mentioned, drug-mortality occurred in 1M at the 500 mg/kg/day dose (6000 mg/m^2). Mortality was attributed to clinical signs of persistent diarrhea, dehydration, body weight loss ($\geq$20%), and low to no food consumption. Histopathology showed findings in the mesenteric lymph node (anisotropic crystalloid material) and the duodenum and jejunum (lamina propria of the villar tips). Based on the in-life and post-mortem findings, the other mortalities (1 M at 500 mg/kg/day and 1 M at 5 mg/kg/day) do not appear to be drug related.

With the exception of green/red/orange/yellow and tan excretion and few/liquid/mucoid/non-formed feces, no other adverse clinical signs occurred in animals that survived until the end of the study. Most excretion occurred mainly at the 500 mg/kg/day (6000 mg/m^2) dose while feces (of varying types) occurred across all dose

groups including controls. The clinical findings, however, were reversible in animals at the 500 mg/kg/day (6000 mg/m^2) dose by the end of Week 52.

A significant decrease in body weight (-27%) was observed in the one male at 500 mg/kg/day (6000 mg/m^2) that died early during Weeks 21 through 25 of the study. This correlated with a decrease in food consumption ($\geq$20%). The remaining male animals at 500 mg/kg/day or 6000 mg/m^2 also showed a decrease in body weight (-16%) compared to controls during Weeks 28 through 29 of the study which correlated with food consumption. These changes, however, were reversible once dosing was terminated in animals from this group (Weeks 35-52).

Clinical chemistry changes included a consistent but slight decrease in albumin values during Weeks 4 (-11%), 13 (-11%), and 26 (-8%). These decreases occurred only in females at 500 mg/kg/day (6000 mg/m^2). At Week 26, this decrease was accompanied by a slight decrease in total protein values (-13%). No changes occurred when monkeys were taken off this dose during the recovery period. There were no drug-related changes in hematology, coagulation, urinalysis, gross pathology and organ weight at the terminal and/or recovery necropsies.

Histopathology findings for animals that died before study termination (males at 500 mg/kg/day or 6000 mg/m^2) during Weeks 13 and 25 correlated with clinical signs of liquid and/or non-formed feces. Findings were observed mainly in the GI tract and lymph nodes. There was a slight to minimal crystalline pigment in the duodenum, jejunum and mesenteric lymph node. These findings were not present in animals that survived until study termination (Week 52). At Week 52, significant histopathology findings were seen in females across all dose levels (including controls) and included the presence of an active/mature corpora lutea in the ovaries and increased ratio of endometrial stroma/glands in the uterus. These findings were still present in a fewer number of animals during the recovery period. In addition, females also had findings in the kidney (minimal lymphohistiocytic infiltrate) and salivary gland (minimal lymphohistiocytic infiltrate) which were recoverable.

There was no apparent gender differences in mean Cmax and AUC(0-t) values across dose groups. For a 100-fold increase in dose from 5 to 500 mg/kg/day (60 and 6000 mg/m^2), AUC(0-t) and Cmax values increased, on average, 12-and 4-fold, respectively, on Weeks 4, 13, and 26. For a 10-fold increase in dose from 5 to 50 mg/kg/day (60 and 6000 mg/m^2), AUC(0-t) and Cmax values increased, on average, 4-and 2-fold, respectively, on Weeks 4, 13, 26, 39, and 52. Overall, multiple dose administration of GW786034B resulted in no marked (>2-fold) increase in systemic Cmax and AUC(0-t) values.

Genetic toxicology:
Pazaponib was tested for mutagenicity in the *in vitro* Ames test and cytogenetics study using cultured human peripheral blood lymphocytes. The clastogenic potential of pazopanib was assessed *in vivo* using the micronucleus test performed by the oral route in the rat. Similar to toxicology studies discussed above, all genotoxicity studies were conducted with GW786034B, the monohydrochloride salt form of GW786034X, which is the drug-form intended for clinical use.

GW786034B did not induce an increase in structural chromosome aberrations, polyploidy or endoreduplication in the presence or absence of S9 metabolic activation in cultured human peripheral blood lymphocytes in the initial or the confirmatory assays at concentrations ranging from 10 to 100 µg/mL.

GW786034B was not cytotoxic to the bone marrow (i.e. there was no statistically significant increase in the PCE:NCE ratio) and produced no significant increase in the frequency of micronucleated PCEs at doses up to 2000 mg/kg/day. Therefore, GW786034B was not clastogenic *in vivo.*

Carcinogenicity:
Carcinogenictiy studies were not conducted and are not necessary to support the safety of pazapanib for the proposed metastatic cancer indication.

Reproductive toxicology:
The reproductive toxicology program included male and female fertility studies in the Sprague-Dawley rat and embryo-fetal development studies in both the rat and the rabbit. In the male fertility study rats were dosed for 105 days with 0, 3, 30, or 100 mg/kg (0, 18, 180, or 600 mg/m^2) of pazopanib and were mated to untreated females at both early and late timepoints. While overall male fertility was not affected by pazopanib in either phase, there were changes noted in sperm production, concentration and motility. These changes were dose dependent. Lower sperm concentration and production were noted even at the lowest dose (18 mg/m^2) of the drug while decreased motility was only seen at the highest dose (600 mg/m^2). In addition, at doses ≥ 180 mg/m^2 animals exhibited decreased organ weights for the testis, epididymis, and seminal vesicles; observations of small, soft testis and epididymis were also seen beginning at this dose.

In the study examining females dosed daily with 0, 3, 30, or 300 mg/kg (0, 18, 180, or 1800 mg/m^2) of pazopanib for 2 weeks prior to mating until Day 6 post-coital (pc) there were changes in fertility. At the highest dose examined, 1800 mg/m^2, there were decreases in body weight after Day 14 pc and in food consumption by Day 7 pc. These dams had a fertility index of only 32% and females who became pregnant were unable to maintain the pregnancy: 100% of the implants in this dose group were resorbed. At 180 mg/m^2 females had an increased percentage of resorbed implants as well, 25.6%, approximately 5 times higher than control. Male and female pups from these dams also had lower fetal body weights, (16% and 13%, respectively) and an increase in the number of malformations. There were single cases of agnathia, micrognathia with a dome-shaped head, and a cleft palate/cleft lip. Each case was from a separate litter.

Two embryo-fetal development studies were completed for rats. Pregnant females were dosed from Day 7-16 pc. In the first, a dose range study with only 6 females/dose group administered pazopanib at 0, 3, 10, 30, or 300 mg/kg (0, 18, 60, 180, or 1800 mg/m^2), the sponsor reported a 100% post-implantation loss at doses ≥ 180 mg/m^2. There was also an increase in post-implantation loss compared to control at 60 mg/m^2. A decrease in the percentage of live males produced at 60 mg/m^2 was also noted in this study, but the second, more comprehensive embryo-fetal development study in rats did not support this

finding and the conclusion was that the drug did not affect the male/female ratio. In the second study female rats were dosed with 0, 1, 3, or 10 mg/kg (0, 6, 18, or 60 mg/m^2) of pazopanib. This study confirmed that at 60 mg/m^2 there was an increase in post-implantation loss. At the same dose the average fetal weight was decreased by approximately 6.3%. Incomplete ossification was a frequently seen event, particularly in the thoracic vertebrae. Alterations in the thoracic vertebrae increased in a dose-dependent manner. The sponsor also reported malformations of the great vessels in this study. Increasing numbers of animals had a missing innominate at doses ≥ 6 mg/m^2. At the 60 mg/m^2 dose level 41% of litters had at least 1 animal with 1 or more great- vessel malformations (increased from 14% of litters at the 18 mg/m^2 level). Four of the 60 mg/m^2 animals had retroesophogeal subclavian arteries.

An oral dose range study performed in nonpregnant rabbits showed that doses ≥ 100 mg/kg (1180 mg/m^2) led to maternal moribidity with frequent anorexia. Mortalities occurred at doses ≥ 3540 mg/m^2. In the pivotal rabbit study females were dosed daily (0, 3, 10, 30 and 100 mg/kg or 0, 35.4, 118, 354, and 1180 mg/m^2) from Days 7-19 pc and euthanized on Day 29. All animals in the 1180 mg/m^2 group were euthanized early due to morbidity. No fetuses were examined from this group. Decreased fetal birth weight was observed in all treatement groups or doses ≥ 35.4 mg/m^2. At 354 mg/m^2 there was a single mortality, the mother was euthanized early after aborted fetuses were found in the cage. A second rabbit in this group had 100% pre-implantation loss. A third rabbit from this group produced only 2 offspring. Few malformations were found in this study. At 35.4 mg/m^2 there was a single incidence of gastroschisis and at 354 mg/m^2 there was a single incidence of microtia. Finally, the sponsor included a toxicokinetic assessment of the female rabbits. At the dose of 118 mg/m^2 the AUC was 1722.6 ng*hr/mL or 1.723 ug*hr/mL with a Cmax of 1063.4 ng/mL or 1.06 ug/mL. This dose in rabbits showed a slight increase in the percentage of post-implantation loss compared to control. At the more frankly toxic dose of 354 mg/m^2 the AUC and Cmax were 7.561 ug*hr/mL and 2.253 ug/mL respectively. Included below is a table excerpted from the sponsor including the exposure of patients given a daily dose of pazopanib at the proposed treatment dose. The AUC and Cmax of pazopanib in these patients is significantly greater than the reported exposure in rabbits (138-fold increase in AUC and 26-fold increase in Cmax) indicating that pazopanib given to patients at a clinically relevant dose is likely to significantly and negatively impact pregnancy.

Summary of Derived Pharmacokinetic Parameters for Pazopanib and Metabolites After a Single Dose in Study VEG10005 and After 16 Days of 800 mg Pazopanib Administered Once Daily in Study VEG10007

Analyte	**AUC(0-t)**		**Cmax**	
	(μg*h/mL)1		**(μg/mL)1**	
	Single Dose	Repeated Dose**,2**	Single Dose	Repeated Dose
	(VEG10005)	(VEG10007)	(VEG10005)	(VEG10007)
Pazopanib	669 (526.3, 851.4)	1,040 (879, 1,230)	20.4 (16.0, 25.9)	58.1 (49.5, 68.3)

*Exceprted from sponsor

Special toxicology: No studies reviewed

2.6.6.2 Single-dose toxicity:
Two single dose toxicity studies were conducted with pazaponib in the rat and dog using the IV and oral routes of administration. For both studies, GW786034B, the monohydrochloride salt form of GW786034X, was used. These studies are briefly summarized below.

Study title: **GW786034B: Single Dose Intravenous Injection Toxicity Study in Rats**

Key study findings:
- This study examined the toxicity and toxicokinetics of an intravenous formulation of GW786034B following single intravenous doses of 1.1 and 5.4 mg/kg (6.6 and 32.4 mg/m^2) to rats (18 sex/dose).
- No drug-related changes were observed at each dose.
- Systemic exposure (AUC) increased proportionally to the increase in dose. For a 4.9 fold increase in dose, mean AUC values increased approximately 4.1 fold in both male and female rats.
- At the highest dose (5.4 mg/kg or 32.4 mg/m^2), mean Cmax value was 102 µg/mL and mean AUC0-24 value was 242 µg.hr/mL (sexes combined).

Study no	RD2006/00221/00
Volume #, and page #:	Module 4.2.3.1
Conducting laboratory and location:	GlaxoSmithKline Five Moore Drive Research Triangle Park, NC 27709 USA
Date of study initiation:	February 21, 2006
GLP compliance:	GLP
QA reports:	yes (X) no ()
Drug, lot #, and % purity:	GW786034B, 786034B-A4-02P-MIC-RTN, 98.5% purity

Methods

Doses:	1.1 and 5.4 mg/kg
Species/strain:	Rat/ CD™ IGS [Crl:CD(SD)]
Number/sex/group or time point (main study):	3/sex/dose
Route, formulation, volume, and infusion rate:	IV via tail vein, 7% SBE7β-CD with 25 mM sodium phosphate/15 mM sodium chloride adjusted pH 5 with 0.1 N sodium hydroxide, 0.22 (LD) and 1.08 mg/kg/min (Cont. and HD)
Satellite groups used for toxicokinetics or recovery:	3/sex/dose
Age:	12 weeks
Weight:	368 -388 g M 237- 271 g F
Schedule:	Single dose

Unique study design or methodology (if any): None

Dose justification: Doses selection of 1.1 and 5.4 mg/kg (to be administered over an approximate 1 minute period) were selected for this study. These doses were estimated to represent approximately 1 and 5 times, respectively, the maximum estimated exposure (AUC) levels in humans.

Observations and times:

Mortality:	2x/day
Clinical signs:	2x/predose, 5 min after dosing, and prior to necropsy
Body weights:	Predose, Day 1, and on the day of necropsy (Day 2, fasted body weight).
Food consumption:	Not done
EKG	Not done
Ophthalmoscopy:	Not done
Hematology:	Day 1
Clinical chemistry:	Day 1
Coagulation	Day 1
Urinalysis:	Not done
Gross pathology:	Conducted on all animals including those found dead, euthanized *in extremis*, and those euthanized on Day 2
Organ weights:	Not done
Histopathology:	All animals Adequate Battery: yes (X), no () Peer review: yes (X), no ( )
Toxicokinetics:	Day 1 at 0 (predose), 0.5, 1, 2, 4, 8, and 24 immediately after the end of dosing.

Mortality: None

Clinical signs: Unremarkable

Body weights: Unremarkable

Food consumption: Not conducted

Ophthalmoscopy: Not conducted

EKG: Not conducted

Hematology: Unremarkable

Clinical chemistry: Unremarkable

Urinalysis: Not conducted

Gross pathology: Unremarkable

Organ weights: Not conducted

Histopathology: Unremarkable

Toxicokinetics:
The sponsor's table below presents the toxicokinetic data for Days 1 for rats. At the highest dose (5.4 mg/kg, 1.08 mL/kg/min), the mean Cmax value was 102 µg/mL and the mean AUC0-24 value was 242 µg.hr/mL (sexes combined).

Day 1 Results	Male		Female	
Dose (mg/kg/day)	Mean C_{max}[1] µg/mL	Mean AUC_{0-24}[1] µg.h/mL	Mean C_{max}[1] µg/mL	Mean AUC_{0-24}[1] µg.h/mL
1.1	21.4 [20.8 – 22.5]	51.8 [39.8 – 59.7]	56.3 [21.0 – 125]	68.5 [54.5 – 95.6]
5.4	79.7 [68.6 – 88.8]	226 [213 – 235]	124 [74.4 – 182]	257 [189 – 324]

1. Results are reported as mean and [range].

Study title: GW786034B: Single Dose Investigative Toxicity Study in Beagle Dogs (Study No. RD2001/01637/00):

This non-GLP study was conducted to examine the systemic exposure of GW786034B (monohydrochloride salt of GW786034X) following single dose oral administration to beagle dogs. Two male beagle dogs were administered a single dose of either 150 mg/kg or 450 mg/kg (3000 and 9000 mg/m^2) of GW786034B. Blood was collected for toxicokinetic analysis. There was no mortality and/or drug-related changes at both doses. As this was a single dose investigative study, insufficient details were provided regarding animal data. The toxicokinetic data is currently being issued as a separate report and will be submitted within 120 days of the NDA submission date.

2.6.6.3 Repeat-dose toxicity

Study title: GW786034B: Toxicity Study by Oral Gavage Administration to CD-1 Mice for 13-Weeks (Study No. WD2005/00481/00)

Key study findings:
- Study designed to select doses for future carcinogenicity study.
- No drug-related mortality occurred throughout the study.
- Dose-dependent increase in WBC, NEUT, LYMPH, ESO, and MONO at ≥300 mg/kg/day (≥900 mg/m^2) in both males and females.
- Elevated liver enzyme levels in males only at 300 and 1000 mg/kg/day (900 and 3000 mg/m^2). However, both males and females had histological evidence of hepatic toxicity.
- Main target organ of toxicity across all dose levels included: kidney, liver, teeth (incisors), spleen, and ovary.

- Additional target organs at 300 and 1000 mg/kg/day (900 and 3000 mg/m^2) included digits (mainly males), teeth (lower incisor), femur, and small intestine (mostly at 1000 mg/kg/day or 3000 mg/m^2).
- TK data showed that exposure was essentially equivalent across the dose range.

Study no.:	WD2005/00481/00
Volume #, and page #:	Module 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Nov. 22, 2003
GLP compliance:	GLP
QA reports:	yes (X) no ()
Drug, lot #, and % purity:	GW786034B, 786034B-A4-02P-MIC-RTN, 91% purity

Dose justification: Dose selection was based on a 14-day dose range finding study in the mouse (GSK Study No. WD2004/00517/00). In this non-GLP-study, GW786034B (monohydrochloride salt of GW786034X) was administered to mice via oral gavage at doses of 0, 200, 1000, and 2000 mg/kg/day. No mortality occurred in the study. Drug-related changes occurred at 1000 and 2000 mg/kg/day and included findings in the heart (mineralization in females only) and kidney (nephropathy). The sternum/bone and stifle joint (growth plate hypertrophy) was affected at all doses. No increase in systemic exposure occurred at ≥1000 mg/kg/day. Based on these findings, doses below 2000 mg/kg/day were chosen in the 13-week study and additional doses of 100 and 300 mg/kg/day were included to provide an assessment of a dose response.

Methods

Doses:	0, 100, 300, and 1000 mg/kg/day
Species/strain:	Mouse/Crl: CD-1™ (ICR) BR
Number/sex/group or time point (main study):	12/sex/dose
Route, formulation, volume, and infusion rate:	Oral gavage, 0.5% hydroxpropyl methylcellulose/ 0.1%Tween (pH adjusted to 1.3 with 0.1M HCl)/suspension, 10 mL/kg
Satellite groups used for toxicokinetics or recovery:	18/sex/dose (TK)
Age:	6-8 weeks old
Weight:	28.3-38.7 g M 21.6-30.3 g F
Schedule:	once daily x 13 weeks
Unique study design or methodology (if any):	None

Observations and times:

Mortality:	Twice daily
Clinical signs:	At least once daily
Body weights:	Predose, weekly during treatment
Food consumption:	Predose and weekly during treatment
EKG	Not done
Ophthalmoscopy:	Predose and at Study Termination (Week 13)
Hematology:	At study termination (Week 13)
Clinical chemistry:	At study termination (Week 13)
Coagulation	Not done
Urinalysis:	Not done
Gross pathology:	Conducted on all animals including those found dead, euthanized *in extremis*, and those euthanized on Week 14 (HD males) and 20 (HD females) and at end of terminal sacrifice, Week 27
Organ weights:	Weeks 14 (interim for HD males), 20 (interim for HD females), and 27 (Control, LD and MD males and females)
Histopathology:	All early deaths at HD (300 mg/kg/day) Week 14 (6 control males and 9 males at HD) Week 20 (6 control females and 9 females at HD) Week 27 (all remaining animals in Control, LD and MD) Adequate Battery: yes (X), no () Peer review: yes (X), no ( )
Toxicokinetics:	Week 13. 18 animals/sex/group were bled predose from posterior vena cava approximately 0.5, 1, 2, 4, 8, and 24 hours after dosing.

Results:

Mortality:

- No drug-related mortality
- On Week 5, a TK animal (1M) in Control group was killed for humane reasons.

Clinical signs:

- 100 mg/kg/day (300 mg/m^2)
  - Overgrown and broken nails in both males and females
  - Longer fore and hind paw nails in males at Week 13
- 300 mg/kg/day (900 mg/m^2)
  - White teeth in both males and females at Week 5, 8, and 13
  - Broken teeth at in females only at Week 8 and 13
  - Overgrown teeth both males and females at Week 8 and 13
  - Overgrown and broken nails in both males and females at Week 8 and 13
  - Longer fore and hind paw nails in males and females at Week 13
- 1000 mg/kg/day (3000 mg/m^2)
  - Pale colored feces in both males and females at Week 8 and 13
  - White teeth in both males and females at Week 5, 8, and 13
  - Broken teeth at in males (Week 13 only) and females only at Week 8 and 13

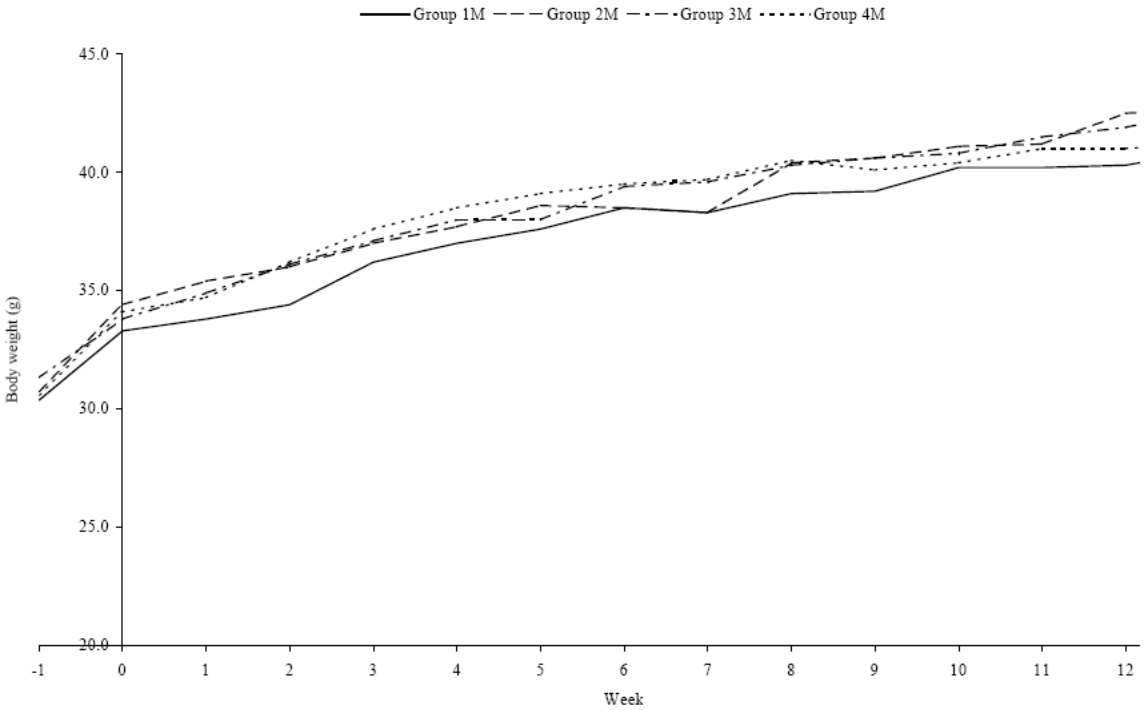
- Overgrown teeth both males (Week 13 only) and females at Week 8 and 13
- Overgrown and broken nails in both males and females at Week 8 and 13
- Longer fore and hind paw nails in males and females at Week 13
- Absent nails in males at Week 13.

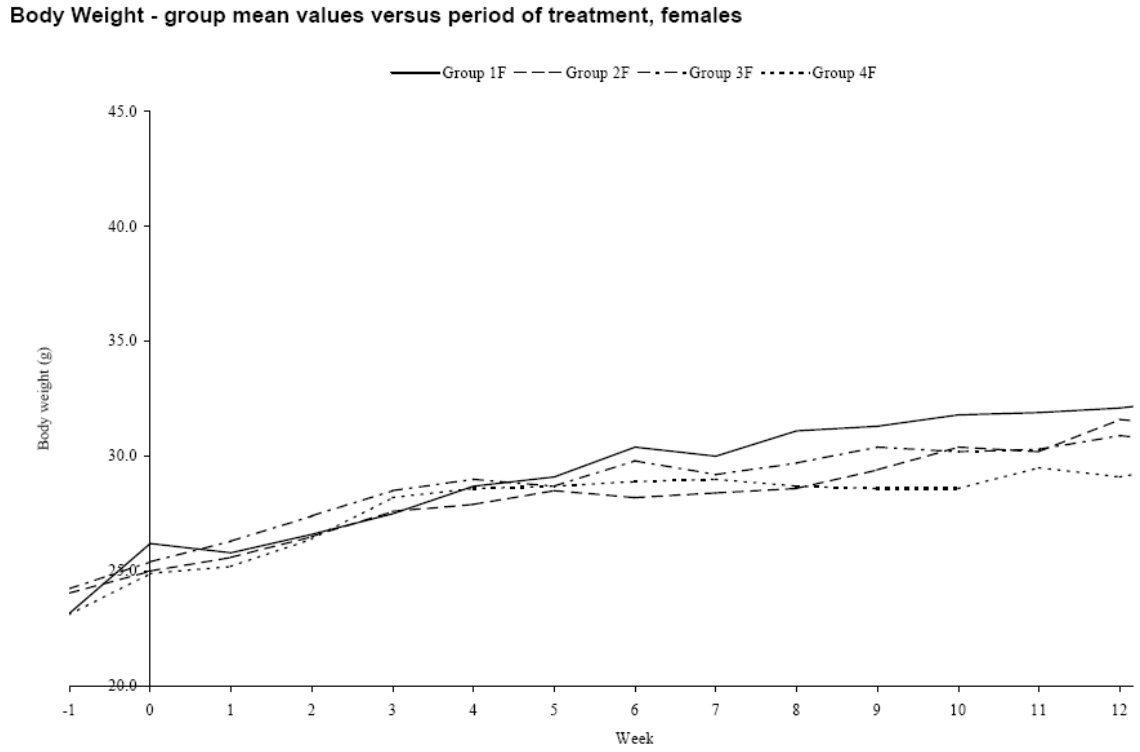
Body weight (See graphs excerpted from the sponsor's submission)**:**

Gender	**Dose (mg/kg/day)**	**Observations**
Males	100	Unremarkable
	300	Statistically significant ↑ (17%) in BW gain during weeks 1-13.
	1000	Statistically significant ↓ (4%) in BW gain during weeks 1-13.
Females	100	Unremarkable
	300	Statistically significant ↓ (19%) in BW gain during weeks 1-13.
	1000	Statistically significant ↓ (25%) in BW gain during weeks 1-13.

Note:
– Animals (N=30) includes 12 main study animals and 18 TK animals.

Body Weight - group mean values versus period of treatment, males





Food consumption**:**

Gender	**Dose (mg/kg/day)**	**Observations**
Males	100	Unremarkable
	300	Unremarkable
	1000	Unremarkable
Females	100	Unremarkable
	300	Statistically significant ↓ (9%) during weeks 1-13.
	1000	Statistically significant ↓ (17%) during weeks 1-13.

Note:
– Animals (N=30) includes 12 main study animals and 18 TK animals.

Ophthalmoscopy: Unremarkable.

EKG: Not done

Hematology:

Index	% difference from control group							
Dose (mg/kg/day)	0		100		300		1000	
Gender	M	F	M	F	M	F	M	F
Week 13								
HCT							+13	
HGB					+15		+15	
MCH					+13	+16	+18	+21
MCV							+13	+18
WBC			+39	+16	+46	+60	+119	+234
NEUT			+55	+36	+47	+56	+139	+814
LYMPH			+33	+12	+45	+55	+113	+140
ESO				+33	+60	+142	+53	+67
MONO			+114	+44	+114	+133	+150	+400

Note:
- All values were statistically significant, $p \leq 0.05$
- Animals (N=30) includes 12 main study animals and 18 TK animals.

Clinical Chemistry:

Index	% difference from control group							
Dose (mg/kg/day)	0		100		300		1000	
Gender	M	F	M	F	M	F	M	F
Week 13								
ALT					+130		+108	
AST					+67		+89	

Note:
- All values were statistically significant, $p \leq 0.05$
- Animals (N=30) includes 12 main study animals and 18 TK animals.

Coagulation: Not done

Urinalysis: Not done

Gross Pathology:

Organ	Observation	Macroscopic Observations							
		0 mg/kg		100 mg/kg		300 mg/kg		1000 mg/kg	
		M	F	M	F	M	F	M	F
	No. of animals	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**
Duodenum	Thick								3
	Pale								5
	Distended								3
Ileum	Pale							2	3
Jejunum	Pale							4	3

Note:
- Animals (N=30) includes 12 main study animals and 18 TK animals.

Organ weights: Unremarkable

Histopathology (Study Termination):

Organ	Observation	Microscopic Observations							
		0 mg/kg		100 mg/kg		300 mg/kg		1000 mg/kg	
		M	F	M	F	M	F	M	F
	No. of animals	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**
Kidney	Cortex-basophilic tubules – *minimal to moderate*	4	3	3	3	2	6	2	10
	Cortex-inflammatory cell infiltration – *minimal to slight*	5	4	7	2	3	8	4	9
Liver	Hepatocytes-reduced cytoplasmic rarefaction – *minimal to slight*	4	5	10	8	12	10	10	6
	Centrilobular aggregates of granular macrophages – *minimal to slight*					10		12	
	Pigmented macrophages – *minimal*						4		5
	Hepatocyte hypertrophy, generalized – *minimal to slight*	1				3		4	2
	Eosinophilic foci – *slight*								2
	Hepatocellular adenoma – *present*								1
Digits	Distortion of distal phalanx – *minimal to slight*					6		8	
	Subcutaneous inflammation – *minimal to slight*					3		6	
Teeth (upper incisor)	Irregular/thickened predentine – *minimal to slight*			8	8	12	12	12	11
	Thinning of dentine – *slight to moderate*			4	5	9	11	12	10
	Irregular ameloblast layer – *minimal to slight*			5	7	8	12	7	9
Teeth (lower incisor)	Irregular/thickened predentine – *minimal to slight*					4	5	2	4
Femur	Growth plate-cartilage – *minimal to slight*					2	2	4	8
	Growth plate-focal cartilage degeneration – *minimal to slight*					3		4	
	Growth plate-partial fusion – *minimal to moderate*	2		3		3		4	12
	Haemosiderosis – *minimal to slight*	2	10	5	11	10	12	12	12
Small intestine	Duodenum-crystalline material in lamina propria – *minimal to moderate*							1	8
	Duodenum-inflammatory cells							2	5

Organ	Observation	Microscopic Observations							
		0 mg/kg		100 mg/kg		300 mg/kg		1000 mg/kg	
		M	F	M	F	M	F	M	F
	No. of animals	**30**	**30**	**30**	**30**	**30**	**30**	**30**	**30**
	in lamina propria – *minimal*								
	Jejunum-crystalline material in lamina propria – *minimal to slight*							2	6
Mesenteric lymph node	Crystalline material accumulation – *minimal to slight*							8	6
Ovary	Sparse corpora lutea – *present*	--	3	--	4	--	7	--	6
	Epithelial cyst(s) – *slight to moderate*	--	3	--	4	--	5	--	6

Note:
– Animals (N=30) includes 12 main study animals and 18 TK animals.

Toxicokinetics:

Toxicokinetic Parameters in Mice Following 13 Weeks of GW786034B Administration						
Dose (mg/kg/day)	**Males**			**Females**		
	100	**300**	**1000**	**100**	**300**	**1000**
AUC_{0-t} (µg*h/mL)						
Week 13	818	1044	932	1434	1463	1607
C_{max} (µg/mL)						
Week 13	102	97.9	80.7	132	134	114

- Exposure was essentially equivalent across the dose range with an AUC_{0-24} at 100 mg/kg/day of 1126 µg.h/mL for males and females averaged. The AUC0-24 at 300 or 1000 mg/kg/day was only 11 and 12% higher.
- There were no apparent gender-related differences.
- T_{max} values were not reported by the Sponsor.

Study Title: GW786034: 1-Month Oral Toxicity and Reversibility Study in Rats (Study No. Report CD2002/00063/00 - Review by Lilliam Rosario, Ph.D; under IND 65747) See Attachment.

Study Title: GW786034B: 28-Day Oral Investigative Toxicity Study in Rats (Study No. RD2002/00721/01)

The aim of this GLP-investigative study was to better characterize teeth and nail changes as previously reported in the 1-month toxicity study in rats (GSK Study No. RD2002/01337/01). This study also wanted to better assess the reversibility of these changes using recovery periods at 2, 4, 6, and 10 weeks post-dose.

GW786034B was administered via oral gavage to male rats (5/dose/group) at 10, 30, and 300 mg/kg/day for approximately 28 days (rats receiving 300 mg/kg/day were dosed for 26 days). During the recovery period, rats were provided moistened diet to improve the reduced body weight and food consumption which lead to teeth abnormalities which subsequently reduced their ability to eat dry pelleted food.

No mortality occurred during the study. Adverse clinical observations of broken/missing teeth, striations in teeth, red area around the gums, swollen lower gum, malocclusion requiring clipping of teeth, and short nails were noted in animals given 300 mg/kg/day starting at week 5 (Study Day 35). There was a decrease in body weight on Days 36 to 42 (-15 to -17%) and food consumption on Days 29-40 (-30 to -46%), 57-64 (-28 to -15%), and 92-98 (-30 to -18%) at 300 mg/kg/day due to adverse clinical signs observed in teeth and paws. These parameters were similar to control values during or shortly after week 6.

At terminal necropsy (Day 29), macroscopic findings were observed in the teeth (incisors elongated and pale discoloration) and paw (hindlimb/forelimb nail short) at all doses. Microscopic findings were limited to doses ≥30 mg/kg/day and included findings in the teeth (dentine degeneration/thinning; enamel degeneration/thinning; atrophy of ameloblasts and odontoblasts; dental pulp necrosis, and periodontal edema of the incisors). At recovery week 6 (Study Day 71), macroscopic findings including pale discoloration and broken incisors were still present at 300 mg/kg/day. However, during recovery week 10 (Study Day 99), all teeth findings were completely resolved.

Study title: **GW786034B: 13-Week Oral Investigative Study in the Male Sprague Dawley Rat (Study No. VD2006/00544/00)**

This GLP-study further characterized the toxicity and toxicokinetics of GW786034B using younger rats (6-7 weeks of age) at the start of dosing and assessing the impact of using a powdered diet compared to a pelleted diet on weight gain, survival and general animal condition. This study was used to compare the results obtained from the previous 1-month study with 6-week recovery in rats (GSK Study No. RD2002/01337/01). In the 1-month study, older rats (12-13 weeks of age at the start of dosing) received a pelleted diet before being switched to powdered diet during Week 5 after having adverse clinical signs of severe tooth trauma.

In the present study, male rats (12/dose/group) received daily oral doses of GW786034B at 0, 3, 30, and 300 mg/kg/day for 13-weeks. An additional 6/dose/group was included at each dose level for toxicokinetic evaluation. No recovery period was included in this study.

Drug-related mortality occurred at 300 mg/kg/day on Week 12 (Study Day 83) in one TK male. Early death was attributed to a period of emaciation, changes in body weight, and food consumption. Since this animal was a toxicokinetic animal, no pathology and/or histopathology was conducted by the Sponsor. A non-drug related mortality occurred in 1 M at 300 mg/kg/day during Week 4 (Study Day 26). Macroscopic findings presented this animal with lesions in the heart and lung.

Clinical signs were predominately observed at 30 mg/kg/day and 300 mg/kg/day in the teeth and nails. Teeth (incisor) findings included overgrown, loose, broken, lost, pale and/or mottled. There was also some regrowth of incisor teeth. Nail findings included long, missing lose, and/or broken nails. These signs were observed during Week 6 at the 30 and 300 mg/kg/day groups but from Week 7 the incidence was higher for rats at 300 mg/kg/day. There was a small but pronounced effect on nails during Week 8 at 300 mg/kg/day. The nails were long, loose, broken and/or missing. Similar changes were noted macroscopically at necropsy, however, no microscopic changes were observed.

There were as a marginal, but consistent and dose-related, reduction in food consumption and a reduction in body weight gain in rats receiving 3 or 30 mg/kg/day throughout the study, but this was insufficient to be considered adverse. At 300 mg/kg/day, however, these changes were more pronounced and, coinciding with the appearance of dental abnormalities at around Week 6, food consumption decreased further and weight gain essentially ceased for several weeks, although there was some improvement during the final two weeks of the study. The reduced food consumption and suppression of weight gain at 300 mg/kg/day (and occasional individual weight loss, leading to sporadically thin appearance and, in one case, preceding death) was sufficient to be considered adverse.

Starting at Study Day 22, there were marginal reductions ($\leq$20%) in body weight at 300 mg/kg/day (18 mg/m^2). Starting at Study Day 57, $\geq$20% decease was observed which correlated with the appearance of pronounced appearance dental abnormalities. By the end of the study (Day 91), the decrease remained at -24%. Similar to body weight changes, food consumption also showed varying differences which correlated with dental changes. Starting at Study Day 43 until Study Day 78, $\geq$20% decease in food consumption was noted. By the end of the study (Day 91), there was a decrease of 20%.

Histopathology changes at 30 and 300 mg/kg/day included the following tissues: teeth (decreased thickness and degeneration of dentine, atrophy/necrosis of odontoblastic and ameloblastic cell layers, necrosis of the dental pulp, reactive bone at the border of the incisor alveolus, edema in the dental pulp, acute inflammation in the dental pulp, and fracture), femur and/or sternum (hypertrophy of the growth plate - only at 300 mg/kg/day in the sternum), bone (chondroid change), bone marrow (hypocellularity), adrenals (cortical hypertrophy), pituitary (basophilic cell hypertrophy) and kidneys (tubular degeneration/regeneration and chronic progressive nephropathy). Sinus histiocytosis, histiocyte foci and lymphangiectasis were also seen in the mesenteric lymph nodes at $\geq$30 mg/kg/day. Additional target organs of toxicity only at the 300 mg/kg/day dose included: adrenal glands (necrosis, angiectasis), testes (atrophy/degeneration, with small testes occasionally reported at necropsy), epididymides (severe hypospermia), duodenum (Brunner's gland inflammation and Brunner's gland dilation, mucosal hypertrophy,

mucosal erosion/ulceration, serosal inflammation with duodenal dilation at necropsy) and in the larynx and trachea (reduced globule leukocytes).

Results of this study concluded that using a powdered diet in younger rats improved the body weight performance, survival and clinical condition only at the HD of 300 mg/kg/day. However at the MD of 30 mg/kg/day, there were similar effects on clinical and body weight parameters from both studies.

Study title: GW786034B: 26-Week Oral Gavage Dose Toxicity Study in Rats (Study No. RD2002/01337/01)

Key study findings:

- The high-dose of 300 mg/kg/day caused severe toxicity and mortality with males being affected earlier in the study compared to females. Drug related mortalities occurred in 9 M (during Study Days 53-96) and 9 F (during Study Days 66-134) at this dose and 1 F (Study Day 131) at the 30 mg/kg/day dose.
- ↓ in BW and FC of males and females at the 30 and 300 mg/kg/day groups closely correlated with the appearance broken/missing/loose teeth that began at approximately Week 6 and affected their ability to eat the pelleted diet.
- BW and FC parameters increased by Week 10 when animals were switched to a powdered diet.
- As a result of the high mortality, decreased body weight and food consumption and associated clinical signs, all remaining males and females at the 300 mg/kg/day group were euthanized at interim sacrifices during Week 14 (males) and Week 20 (females).
- Main target organ of toxicity at 30 and 300 mg/kg/day dose groups include: femur, sternum, femoral and sternal bone marrow, incisor teeth (mandibular and maxillary), kidney, trachea, adrenal cortex, pituitary, testes, and ovaries.
- Additional target organs of toxicity at the HD (300 mg/kg/day) include pancreas, duodenum, jejunum, mesenteric lymph node, and epididymis.
- At the end of study, Week 26, animals from Control, LD (3 mg/kg/day) and MD (30 mg/kg/day) were evaluated.

Study no.:	RD2002/01337/01
Volume #, and page #:	Module 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Jan. 28, 2003
GLP compliance:	GLP
QA reports:	yes (X) no ()
Drug, lot #, and % purity:	GW786034B, 786034B-A4-02P-MIC, 91% purity

Dose justification: Dose selection was based on a 1-month oral toxicity with 6-week recovery study in rats (Study No. CD2002/00063/00). In this GLP-study, GW786034B (monohydrochloride salt of GW786034X) was administered to rats via oral gavage at doses of 0, 3, 10, 30, 100 or 300 mg/kg/day. Drug-related changes occurred in sternebrae, femur/stifle, and testis of rats at ≥100 mg/kg/day. After 10-week recovery

period, testicular and marrow changes had reversed and 1/4 treated male rats had resolving hypertrophy in the epiphyseal growth plate in the femur/stifle. Excessive growth and apparent brittleness of teeth and/or nails was observed for rats in the 300 mg/kg/day group approximately 2-4 weeks off-treatment. A minimal decrease in reticulocyte count and increases in ALT and AST, without liver histopathologic changes, occurred at 300 mg/kg/day. Based on these findings, doses of 3, 30, and 300 mg/kg/day were chosen for the 26-week study. These doses were expected to provide separation in exposure and allow assessment of the toxicity.

Methods

Doses:	0, 3, 30, and 300 mg/kg/day
Species/strain:	Rat/ CD®(SD)IGS BR Virus Antibody
Number/sex/group or time point (main study):	12/sex/dose
Route, formulation, volume, and infusion rate:	Oral gavage, 0.5% hydroxpropyl methylcellulose and 0.1%Tween 80 in water – pH to 1.3 with HCL, 10 mL/kg
Satellite groups used for toxicokinetics or recovery:	3/sex/dose (TK)
Age:	12-13 weeks
Weight:	222-276 g M 160-198 g F
Schedule:	Once daily x 26 weeks
Unique study design or methodology (if any):	1. Due to high mortality in both M and F at the HD (300 mg/kg), interim sacrifices were performed on Week 14 for all remaining M (N=9) and Week 20 for all remaining F (N=9) at the HD. 2. Terminal sacrifice for all surviving animals (Groups 1, 2, and 3) was performed on Week 26. 3. At week 10, animals were administered powdered diet.

Observations and times:

Mortality:	Twice daily
Clinical signs:	At least once daily
Body weights:	Predose, weekly during treatment until Day 61 and twice weekly thereafter.
Food consumption:	Predose, weekly during treatment until Day 96 and twice weekly thereafter.
EKG	Not done
Ophthalmoscopy:	Predose, during Weeks 13 and 26
Hematology:	Weeks 4, 13, 14 (HD males) , 20 (HD females), and prior to study termination
Clinical chemistry:	Weeks 4, 13, 14 (HD males) , 20 (HD females), and prior to study termination

Coagulation	Weeks 4, 13, 14 (HD males) , 20 (HD females), and prior to study termination
Urinalysis:	Weeks 4, 13, 14 (HD males) , 20 (HD females), and prior to study termination
Gross pathology:	Conducted on all animals including those found dead, euthanized *in extremis*, and those euthanized on Week 14 (HD males) and 20 (HD females) and at end of terminal sacrifice, Week 27
Organ weights:	Weeks 14 (interim for HD males), 20 (interim for HD females), and 27 (Control, LD and MD males and females)
Histopathology:	All early deaths at HD (300 mg/kg/day) Week 14 (6 control males and 9 males at HD) Week 20 (6 control females and 9 females at HD) Week 27 (all remaining animals in Control, LD and MD) Adequate Battery: yes (X), no () Peer review: yes (X), no ( )
Toxicokinetics:	Weeks 4, 13, and 26. 3 animals/sex/group were bled predose and at approximately 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing.

Results:

Mortality:

Gender	Animal No.	Dose (mg/kg/ day)	Day of death (Week)	Clinical signs and In-life observations
Males	C94932	300	90 (13)	Clinical signs: thin appearance; missing tooth/teeth at upper and lower incisors; rough hair coat Observations: missing/broken/worn bilateral mandible and maxilla teeth; multiple tan areas in liver; cyst in adrenal cortex
	C94935	300	66 (10)	Clinical signs: thin appearance; few feces; rough and brown hair coat; broken tooth/teeth at lower incisors; missing tooth/teeth at upper incisors Observations: missing/broken/worn bilateral mandible and maxilla teeth; mottled dark brown, red and tan kidney; large adrenal cortex; small, soft testes
	C94938	300	96 (14)	Clinical signs: thin appearance; broken tooth/teeth at upper and lower incisors; rough and yellow hair coat; swollen appearance Observations: missing/broken/worn bilateral mandible and maxilla teeth; red area in nails; dark brown/soft adrenal cortex; red focus and mucosa on stomach; small, soft testes; large lumen in GI tract
	C94939	300	60 (9)	Clinical signs: pale ears; broken tooth/teeth at upper and lower incisors; few feces; hypoactive; rough hair coat; cold to touch; Observations: missing/broken/worn bilateral mandible teeth; mottled maxilla teeth; small, soft testes; large lumen in GI tract; diffusely red adrenal cortex
	C94940	300	92 (14)	Clinical signs: rough, brown, and yellow coat; thin appearance; missing tooth/teeth at upper and lower incisors; few /non-formed feces Observations: missing/broken/worn bilateral mandible and maxilla teeth; dark focus area in mucosa of stomach; large lumen in GI tract
	C94941	300	64 (10)	Clinical signs: broken tooth/teeth at upper and lower incisors; missing nails; hypoactive; cold to touch; yellow hair coat and hunched; pale ears Observations: missing/broken/worn bilateral mandible and maxilla teeth; diffusely dark and brown adrenal cortex; small, soft testes
	C94942	300	82 (12)	Clinical signs: broken tooth/teeth at upper and lower incisors; thin appearance; liquid feces; brown, red and rough hair coat Observations: missing/broken/worn bilateral mandible and maxilla teeth;

				small thymus; diffusely red adrenal cortex ; small seminal vesicle; small, soft testes; large lumen in GI tract
	C94944	300	78 (12)	Clinical signs: missing tooth/teeth at upper incisors; broken tooth/teeth at lower incisors; thin appearance; few feces missing/broken/worn bilateral mandible and maxilla teeth; missing nail Observations: red focus in mucosa of stomach; small, soft testes; large lumen in GI tract
	C94947	300	53 (8)	Clinical signs: missing tooth/teeth at upper incisors; broken tooth/teeth at lower incisors; thin appearance; no feces; brown, red and rough hair coat Observations: missing/broken/worn bilateral mandible and maxilla teeth; missing nails; small, soft testes; large lumen in GI tract
Females	C94988	30	131 (19)	Clinical signs: missing tooth/teeth at upper incisors; excessively long teeth at upper incisors; thin and hunched appearance, cold to touch, pale ears. Observations: missing/broken/worn bilateral mandible teeth; mottled mandible teeth, large pelvis and granular material in kidney; dark focus and multiple brown areas in mucosa of stomach, calculus in urinary bladder; also lost 24% of BW.
	C94991	300	123 (18)	Clinical signs: missing tooth/teeth at upper and lower incisors; thin and hunched appearance; few feces; yellow and brown hair coat; liquid feces Observations: missing maxilla teeth; missing/broken/worn mandible teeth; diffusely dark red and large adrenal cortex; large lumen in GI tract
	C94992	300	134 (20)	Clinical signs: missing tooth/teeth at upper and lower incisors; thin appearance; few feces; broken nails; Observations: missing/broken/worn bilateral mandible and maxilla teeth; diffusely dark red and large adrenal cortex; large clitoral gland; large lumen in GI tract
	C94993	300	134 (20)	Clinical signs: broken tooth/teeth at upper incisor; missing tooth/teeth at lower incisors; rough, red and yellow hair coat; few and non-formed feces Observations: missing/broken/worn bilateral mandible and maxilla teeth; diffusely dark red and large adrenal cortex; missing nail
	C94994	300	78 (12)	Clinical signs: broken tooth/teeth at lower incisor and loose tooth/teeth; missing tooth/teeth at upper incisors; rough hair coat; thin and pale appearance; cold to touch Observations: missing/broken/worn bilateral mandible and maxilla teeth; diffusely dark red and large adrenal cortex; large lumen in GI tract
	C94999	300	66 (10)	Clinical signs: broken tooth/teeth at upper and lower incisors ; thin and pale appearance; rough and yellow hair coat Observations: missing/broken/worn bilateral mandible and maxilla teeth; diffusely dark and brown adrenal cortex; large bile duct filled with yellow fluid
	C95002	300	96 (14)	Clinical signs: broken tooth/teeth at lower incisor; missing tooth/teeth at upper incisors; thin and pale appearance ; brown, yellow and rough hair coat; squinted eyes; non-formed feces Observations: missing/broken/worn bilateral mandible and maxilla teeth; diffusely dark brown and large adrenal cortex; large lumen in GI tract
	C95003	300	78 (12)	Clinical signs: missing tooth/teeth at upper and lower incisors; thin and pale appearance; yellow hair coat; few feces Observations: missing/broken/worn bilateral mandible and maxilla teeth; diffusely dark and large adrenal cortex; large lumen in GI tract
	C95004	300	99 (15)	Clinical signs: missing tooth/teeth at upper and lower incisors; brown and red hair coat; thin appearance; squinted eyes; few feces Observations: missing/broken/worn bilateral mandible and maxilla teeth; diffusely red and large adrenal cortex; thickened wall and large bile duct
	C95008	300	75 (11)	Clinical signs: broken tooth/teeth at lower incisor; missing tooth/teeth at

				upper incisors; few feces; thin and hunched appearance; red and yellow hair coat Observations: missing/broken/worn bilateral mandible and maxilla teeth; large pelvis in right kidney; diffusely dark, large, mottled, red and tan adrenal cortex

Note: Table includes early deaths/unscheduled deaths which are animals that died or were euthanatized in moribund condition during Study Days 53 to 96 in males and Study Days 66-134 for females.

Clinical signs:

Index	**No. of animals affected**			
Dose (mg/kg/day)	**0**	**3**	**30**	**300**
No. of animals	**18**	**12**	**12**	**18**
Appearance				
– malocclusion		2F	3F	7F
– thin			7M/4F	14M/12F
Mouth				
– swollen			7M/4F	14M/12F
Excessively long teeth				
– upper incisors		2M	12M/11F	18M/18F
– lower incisors	8M/8F	10M/10F	11M/10F	18M/13F
Loose tooth/teeth				
– top			3M	10M/7F
– bottom			4M/2F	7M/17F
Broken tooth/teeth				
– upper incisors		1M	8M/10F	9M/16F
– lower incisors		1M/10F	10M/12F	18M/16F
Missing tooth/teeth				
– upper incisors			9M/10F	13M/18F
– lower incisors			2M/2F	14M/15F
Excretion				
– few	1M/1F	1M/1F	2M/5F	14M/15F
– non-formed	6M	1M/1F	7M/2F	9M/4F
Hair coat				
– rough	2F		5M/1F	14M/6F
– yellow				4M/7F

Body weight (See graphs excerpted from the sponsor's submission):

Gender	**Dose (mg/kg/day)**	**Observations**
Males	3	Unremarkable
	30	Statistically significant ↓ (8 to 10%) compared to control from Week 7 through Week 9. Beginning on Week 9 or Day 68 (when body weights for this group were approximately 7% lower than the controls), values were comparable to the control due to animals being placed on a crushed food beginning on Week 10.

	300	Statistically significant ↓ (16 to 22%) compared to control from Week 8 through Week 14 (when males at this dose were euthanized).
Females	3	Unremarkable
	30	Unremarkable
	300	Statistically significant ↓ (8 to 14%) compared to control from Week 6 through Week 20 (when females at this dose were euthanized).

Figure 1 Mean Body Weight Data (g) - Males

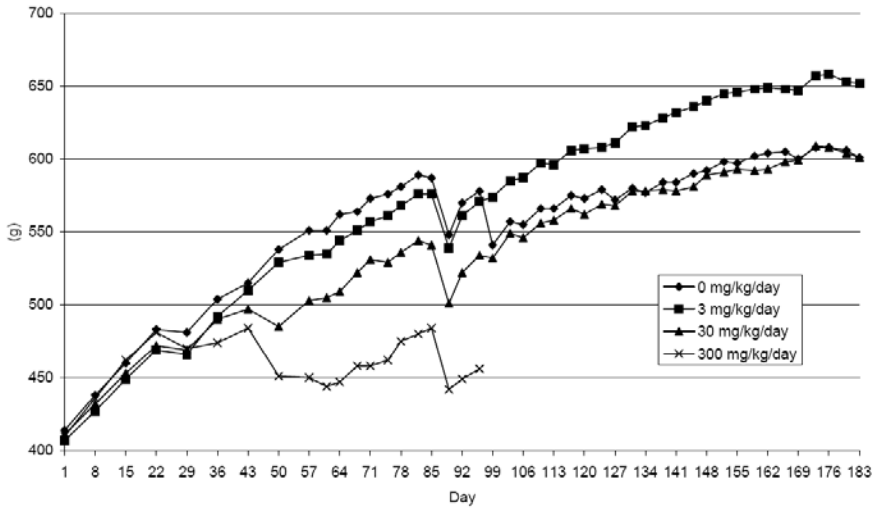
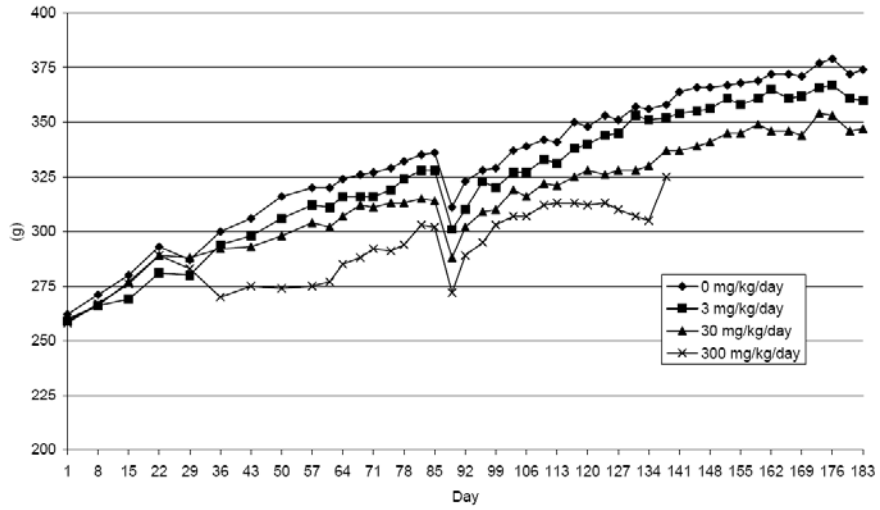


Figure 2 Mean Body Weight Data (g) - Females



Food consumption (See graphs excerpted from the sponsor's submission)**:**

Gender	Dose (mg/kg/day)	Observations
Males	3	Unremarkable
	30	Statistically significant ↓ (≥20%) compared to control from Week 7 through Week 9. Statistically significant ↓ (≥20%) compared to control from Week 6 through Week 11. Beginning on Week 9 or Day 68, values were comparable to the control due to animals being placed on a crushed feed beginning on Week 10.
	300	Statistically significant ↓ (≥20%) compared to control from Week 8 through Week 14 (when males at this dose were euthanized).
Females	3	Unremarkable
	30	Unremarkable
	300	Statistically significant ↓ (≥20%) compared to control from Week 6 through Week 20 (when females at this dose were euthanized).

Figure 3 Mean Food Consumption Data (g) - Males

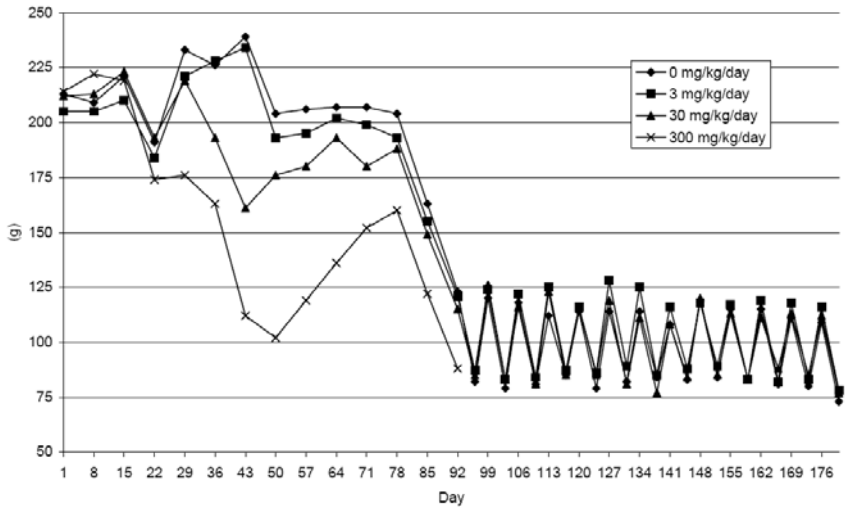
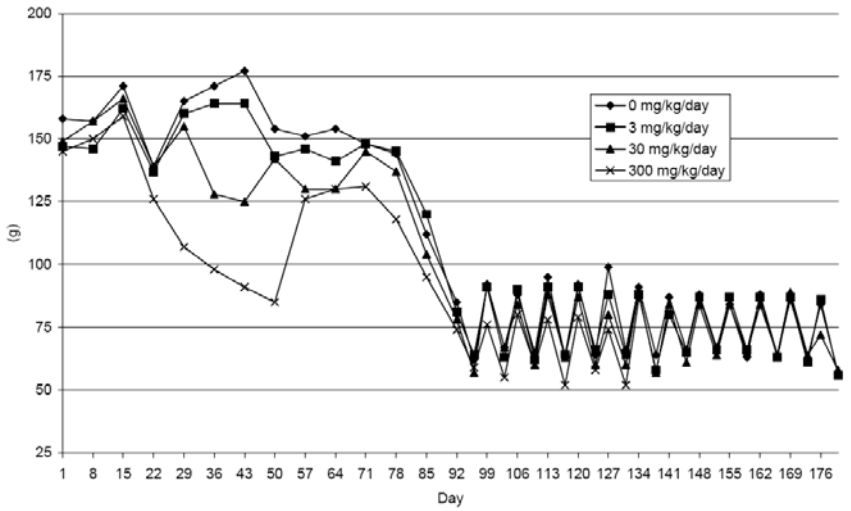


Figure 4 Mean Food Consumption Data (g) - Females



Ophthalmoscopy: Unremarkable.

EKG: Not done

Hematology:

Index	% difference from control group							
Dose (mg/kg/day)	0		3		30		300	
Gender	M	F	M	F	M	F	M	F
RBC								
Week 13					-16	-13	-28	-28
Week 14			--	--	--	--	-28	--
Week 20			--	--	--	--	--	-28
Week 27					-16	-17	--	--
MCV								
Week 13					+18	+12	+31	+26
Week 14			--	--	--	--	+30	--
Week 20			--	--	--	--	--	+25
Week 27					+16	+13	--	--
MCH								
Week 13					+24	+17	+44	+40
Week 14			--	--	--	--	+44	--
Week 20			--	--	--	--	--	+36
Week 27					+21	+18	--	--
PLT								
Week 13					-19		-20	-31
Week 14			--	--	--	--	-10	--
Week 20			--	--	--	--	--	+35
Week 27					-18	+30	--	--
WBC								
Week 13								+31
Week 20			--	--	--	--		+35

Index	% difference from control group							
Dose (mg/kg/day)	0		3		30		300	
Gender	M	F	M	F	M	F	M	F
Week 27						+30	--	--
NEUT								
Week 13							+47	+56
Week 14			--	--	--	--	+108	--
Week 20			--	--	--	--	--	+144
LYMPH								
Week 27						+33	--	--
MONO								
Week 13							+57	+50
Week 20			--	--	--	--	--	+125

Note:
- All values were statistically significant, $p \leq 0.05$
- -- = No animals were available during this time-point.
- Week 13 data include all surviving animals at 300 mg/kg/day and all animals at 30 mg/kg/day and 3 mg/kg/day.
- Week 14 and 20 data include all remaining males (N=9) and females (N=9) at the HD (300 mg/kg/day group) that were euthanized on Week 14 (Study Day 98) for males and Week 20 (Study Day 140) for females.
- Week 27 data include all animals that survived until study termination (Control, 3, and 30 mg/kg/day).

Clinical Chemistry:

Index	% difference from control group							
Dose (mg/kg/day)	0		3		30		300	
Gender	M	F	M	F	M	F	M	F
UREA (nitrogen)								
Week 13								+20
Week 20			--	--	--	--	--	+21
ALB								
Week 13					+18	+12	-13	-16
Week 14			--	--	--	--	-14	--
Week 20			--	--	--	--	--	-26
GLOB								
Week 13			+15		+15	+17		
Week 27			+15		+19		--	--
A/G ratio								
Week 13					-16			-21
Week 20			--	--	--	--	--	-24
Week 27			-12		-18	-15	--	--
CHOL								
Week 13							+45	+42
Week 14			--	--	--	--	+44	--
Week 20			--	--	--	--	--	+31
Week 27					+40	+38	--	--
TRIGLY								

Index	% difference from control group							
Dose (mg/kg/day)	**0**		**3**		**30**		**300**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Week 4							+75	
Week 13								+96
TBILI								
Week 4						+50		+50
Week 13						+50		+50
Week 20			--	--	--	--	--	+50
ALT								
Week 4							+197	+87
Week 13							+149	+90
Week 20							--	+114
PHOS								
Week 13						+17		+17
Week 20			--	--	--	--	--	+18
Week 27						+16	--	--

Note:
- All values were statistically significant, $p \leq 0.05$
- -- = No animals were available during this time-point.
- Week 13 data include all surviving animals at 300 mg/kg/day and all animals at 30 mg/kg/day and 3 mg/kg/day.
- Week 14 and 20 data include all remaining males (N=9) and females (N=9) at the HD (300 mg/kg/day group) that were euthanized on Week 14 (Study Day 98) for males and Week 20 (Study Day 140) for females.
- Week 27 data include all animals that survived until study termination (Control, 3, and 30 mg/kg/day).

Coagulation: Unremarkable

Urinalysis:

Index	% difference from control group							
Dose (mg/kg/day)	**0**		**3**		**30**		**300**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Week 14 and 20 (Interim Deaths)								
No. of animals	**6**	**6**	**0**	**0**	**0**	**0**	**9**	**9**
Urine volume			--	--	--	--		+251
Creatinine excretion			--	--	--	--	-37	
Protein excretion			--	--	--	--	+120	+2416
Protein/creatinine ratio			--	--	--	--	+237	+1708
Week 27 (Study Termination)								
No. of animals	**12**	**12**	**12**	**12**	**12**	**11**	**0**	**0**
Protein excretion			+146	+386	+281	+781	--	--
Protein/creatinine ratio			+109	+427	+200	+802	--	--

Note:
- All values were statistically significant, $p \leq 0.05$
- -- = No animals were available during this timepoint

- Interim deaths refers to the remaining males (N=9) and females (N=9) at the HD (300 mg/kg/day group) that were euthanized on Week 14 (Study Day 98) for males and Week 20 (Study Day 140) for females.
- Study termination refers to all animals that survived until the end of the study or Week 27. These include all animals in the control, 3 and 30 mg/kg/day group.

Gross Pathology:

Organ	Observation	Macroscopic Observations							
		0 mg/kg		3 mg/kg		30 mg/kg		300 mg/kg	
		M	F	M	F	M	F	M	F
	Early/unscheduled Deaths[a]								
	No. of animals	**0**	**0**	**0**	**0**	**0**	**1**	**9**	**9**
Adrenal, cortex	Large	--	--	--	--	--		2	8
	Dark, diffuse	--	--	--	--	--		2	7
	Red, diffuse	--	--	--	--	--		1	2
GI tract	Large luman	--	--	--	--	--		6	8
Teeth, mandible	Missing/broken/worn	--	--	--	--	--		9	9
Teeth, maxilla	Missing/broken/worn	--	--	--	--	--	1	6	7
Testes	Small	--	--	--	--	--		7	
	Interim Deaths[b]								
	No. of animals	**6**	**6**	**0**	**0**	**0**	**0**	**9**	**9**
Adrenal, cortex	Large								9
	Red foci			--	--	--	--		3
GI tract	Large luman			--	--	--	--	5	5
Testes	Small			--	--	--	--	7	
Teeth, mandible	Missing/broken/worn			--	--	--	--	8	9
Teeth, maxilla	Mottled			--	--	--	--	2	
	Missing/broken/worn			--	--	--	--	9	8
	Study Termination[c]								
	No. of animals	**12**	**12**	**12**	**12**	**12**	**11**	**0**	**0**
Teeth, mandible	Mottled						2	--	--
	Missing/broken/worn					2	7	--	--
Teeth, maxilla	Mottled						6	--	--
	Missing/broken/worn					2	1	--	--
	Excessively long					7	7	--	--

Note:
- -- = No animals were available during this timepoint.

[a]= Early/unscheduled deaths refers to 9 males and 9 females at the HD (300 mg/kg/day group) that were euthanized in moribund condition between Study Days 53-96 (males) and Study Days 66-134 (females).
[b]= Interim deaths refers to the remaining males (N=9) and females (N=9) at the HD (300 mg/kg/day group) that were euthanized on Week 14 (Study Day 98) for males and Week 20 (Study Day 140) for females.
[c]= Study termination refers to all remaining animals that survived until the end of the study or Week 27. These include all animals in the control, 3 and 30 mg/kg/day group.

Organ weights:

Index	% difference from control group							
Dose (mg/kg/day)	**0**		**3**		**30**		**300**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Week 14 and 20 (Interim Deaths)								
No. of animals	**6**	**6**	**0**	**0**	**0**	**0**	**9**	**9**
Adrenals			--	--	--	--		
– absolute			--	--	--	--		↑197
– relative to brain weight			--	--	--	--		↑198
Epididymis								
– absolute							↓45	
– relative to brain weight							↓19	
Testes								
– absolute	--		--		--	--	↓53	
– relative to brain weight	--		--		--	--	↓50	
Week 27 (Study Termination)								
No. of animals	**12**	**12**	**12**	**12**	**12**	**11**	**0**	**0**
Testes								
– absolute	--		--		↓24		--	
– relative to brain weight	--		--		↓22		--	

Note:
- All values were statistically significant, $p \leq 0.05$
- -- = No animals were sacrificed during this time period.
- Interim deaths refers to the remaining males (N=9) and females (N=9) at the HD (300 mg/kg/day group) that were euthanized on Week 14 (Study Day 98) for males and Week 20 (Study Day 140) for females.
- Study termination refers to all remaining animals that survived until the end of the study or Week 27. These include all animals in the control, 3 and 30 mg/kg/day group.

Histopathology (Early/unscheduled deaths):

Organ	Observation	Microscopic Observations							
		0 mg/kg		3 mg/kg		30 mg/kg		300 mg/kg	
		M	F	M	F	M	F	M	F
	No. of animals	**0**	**0**	**0**	**0**	**0**	**1**	**9**	**9**
Adrenal, cortex	Angiectasis	--	--	--	--	--	1		
	– *minimal and moderate to severe*							4	6
	Hemorrhage	--	--	--	--	--	1		
	– *minimal and moderate to severe*							9	9
	Necrosis	--	--	--	--	--			
	– *minimal and moderate to severe*							5	3
Bone, femur	Hypertrophy, growth plates	--	--	--	--	--	1		
	– *minimal to severe*							9	9
	Atrophy, tracecular	--	--	--	--	--			
	– *minimal to moderate*							7	3
Bone marrow-	Hypocellular	--	--	--	--	--			
	– *minimal and severe*							8	7

	Observation	Microscopic Observations							
		0 mg/kg		3 mg/kg		30 mg/kg		300 mg/kg	
		M	F	M	F	M	F	M	F
femur									
Bone, sternum	Periosteal chondroid change – *moderate*	--	--	--	--	--		7	
Bone marrow-sternum	Hypocellular – *minimal and marked*	--	--	--	--	--		7	6
Duodenum	Crystalline pigment – *minimal*	--	--	--	--	--			3
	Atrophy, villous – *minimal*	--	--	--	--	--		1	2
Epididymis	Aspermia – *present*	--	--	--	--	--		7	
	Hypospermia – *slight and severe*	--	--	--	--	--		2	
	Cribriform change – *minimal to slight*	--	--	--	--	--		7	
Jejunum	Crystalline pigment – *minimal to slight*	--	--	--	--	--		5	3
Kidney	Nephropathy, chronic, progressive – *minimal to slight*	--	--	--	--	--		8	4
Ovary	Atrophy – *slight to moderate*	--	--	--	--	--			9
Pancreas	Atrophy, acinar – *minimal and moderate*	--	--	--	--	--	1	8	7
Pituitary	Hypertrophy, basophils – *minimal to moderate*	--	--	--	--	--		7	
Teeth, mandible	Fracture – *present*	--	--	--	--	--		8	9
	Inflammation, acute – *minimal to marked*	--	--	--	--	--		6	4
	Reactive bone – *slight to moderate*	--	--	--	--	--		2	5
	Decreased thickness, dentine – *marked to severe*	--	--	--	--	--		9	9
	Decreased thickness, enamel – *severe*	--	--	--	--	--		9	9
	Edema, periodontal – *severe*	--	--	--	--	--		8	9
	Necrosis, ameloblastic – *severe*	--	--	--	--	--		9	9
	Necrosis, dental pulp – *slight to severe*	--	--	--	--	--		9	7
	Necrosis, odontoblastic – *severe*	--	--	--	--	--		9	9
	Degeneration, dentine	--	--	--	--	--		9	9

	Observation	Microscopic Observations							
		0 mg/kg		3 mg/kg		30 mg/kg		300 mg/kg	
		M	F	M	F	M	F	M	F
	– *severe*								
	Degeneration, enamel – *severe*	--	--	--	--	--		9	9
Teeth, maxilla	Fracture – *present*	--	--	--	--	--		8	8
	Inflammation, acute – *minimal to moderate*	--	--	--	--	--		8	9
	Decreased thickness, dentine – *slight to severe*	--	--	--	--	--	1	9	9
	Decreased thickness, enamel – *marked to severe*	--	--	--	--	--		9	9
	Degeneration, dentine – *slight and marked to severe*	--	--	--	--	--		9	9
	Degeneration, enamel – *slight and marked to severe*	--	--	--	--	--		9	9
	Edema, periodontal – *slight to severe*	--	--	--	--	--		9	9
	Necrosis, ameloblastic – *severe*	--	--	--	--	--		9	9
	Necrosis, dental pulp – *marked to severe*	--	--	--	--	--		9	9
	Necrosis, odontoblastic – *severe*	--	--	--	--	--		9	9
Testes	Atrophy, degeneration – *moderate to severe*	--	--	--	--	--		9	
Trachea	Decreased globule leukocytes – *present*	--	--	--	--	--	1	9	9

Note:

– Early/unscheduled deaths refers to 9 males and 9 females at the HD (300 mg/kg/day group) that were euthanized in moribund condition between Study Days 53-96 (males) and Study Days 66-134 (females).

Histopathology (Interim Deaths):

Organ	Observation	Microscopic Observations							
		0 mg/kg		3 mg/kg		30 mg/kg		300 mg/kg	
		M	F	M	F	M	F	M	F
	No. of animals	**6**	**6**	**0**	**0**	**0**	**0**	**9**	**9**
Adrenal, cortex	Angiectasis – *minimal to severe*			--	--	--	--	6	9
	Hemorrhage – *minimal to marked*			--	--	--	--	6	9
Bone, femur	Hypertrophy, growth plates – *minimal to marked*			--	--	--	--	9	5
	Atrophy, tracecular – *minimal to moderate*			--	--	--	--	9	4
Bone marrow-femur	Hypocellular – *minimal to moderate*			--	--	--	--	9	5
Bone, sternum	Periosteal chondroid change – *minimal to moderate*			--	--	--	--	9	3
Bone marrow-sternum	Hypocellular – *minimal to slight*			--	--	--	--	7	5
Epididymis	Aspermia – *present*			--	--	--	--	6	
	Hypospermia – *moderate to marked*			--	--	--	--	3	
	Cribriform change – *minimal to slight*			--	--	--	--	7	
Jejunum	Crystalline pigment – *minimal*			--	--	--	--	7	3
Lymph node, mesenteric	Crystalline pigment – *minimal*			--	--	--	--	1	4
Kidney	Nephropathy, chronic, progressive – *minimal*			--	--	--	--	9	8
Ovary	Atrophy – *moderate*			--	--	--	--		6
Pancreas	Atrophy, acinar – *minimal*			--	--	--	--	6	7
Pituitary	Hypertrophy, basophils – *minimal to moderate*	1		--	--	--	--	9	
Teeth, mandible	Fracture – *present*			--	--	--	--	9	8
	Inflammation, acute – *minimal to marked*			--	--	--	--	4	4
	Reactive bone – *minimal to moderate*			--	--	--	--	2	5
	Decreased thickness, dentine – *marked to severe*			--	--	--	--	9	9
	Decreased thickness, enamel			--	--	--	--	9	9

	Observation	Microscopic Observations							
		0 mg/kg		3 mg/kg		30 mg/kg		300 mg/kg	
		M	F	M	F	M	F	M	F
	– *marked to severe*								
	Degeneration, dentine – *marked to severe*			--	--	--	--	9	9
	Degeneration, enamel – *marked to severe*			--	--	--	--	9	9
	Edema, periodontal – *marked and severe*			--	--	--	--	9	9
	Necrosis, ameloblastic – *severe*			--	--	--	--	9	9
	Necrosis, dental pulp – *severe*			--	--	--	--	9	9
	Necrosis, odontoblastic – *severe*			--	--	--	--	9	9
Teeth, maxilla	Fracture – *present*			--	--	--	--	9	7
	Inflammation, acute – *minimal to moderate*			--	--	--	--	6	9
	Decreased thickness, dentine – *severe*			--	--	--	--	9	7
	Decreased thickness, enamel – *severe*			--	--	--	--	9	9
	Degeneration, dentine – *severe*			--	--	--	--	9	9
	Degeneration, enamel – *severe*			--	--	--	--	9	9
	Edema, periodontal – *moderate to severe*			--	--	--	--	7	9
	Necrosis, ameloblastic – *severe*			--	--	--	--	9	9
	Necrosis, dental pulp – *severe*			--	--	--	--	9	9
	Necrosis, odontoblastic – *severe*			--	--	--	--	9	9
Testes	Atrophy, degeneration – *minimal to severe*			--	--	--	--	9	9
Trachea	Decreased globule leukocytes – *present*			--	--	--	--	8	9

Note:

– Interim deaths refers to the remaining males (N=9) and females (N=9) at the HD (300 mg/kg/day group) that were euthanized on Week 14 (Study Day 98) for males and Week 20 (Study Day 140) for females.

Histopathology (Study Termination):

Organ	Observation	Microscopic Observations							
		0 mg/kg		3 mg/kg		30 mg/kg		300 mg/kg	
		M	F	M	F	M	F	M	F
	No. of animals	**12**	**12**	**12**	**12**	**12**	**11**	**0**	**0**
Adrenal, cortex	Angiectasis – *minimal to marked*		1		6	1	5	--	--
	Hemorrhage – *minimal to slight*		2		3	1	5	--	--
Bone marrow-femur	Hypocellular – *minimal to moderate*					12	2	--	--
Bone marrow-sternum	Hypocellular – *minimal to slight*					10		--	--
Kidney	Nephropathy, chronic, progressive – *minimal to slight*	4	1	7	3	11	5	--	--
Pituitary	Hypertrophy, basophils – *minimal*			2		5		--	--
Teeth, mandible	Decreased thickness, dentine – *slight to moderate*					1	4	--	--
	Decreased thickness, enamel – *slight to marked*					2	2	--	--
	Degeneration, dentine – *moderate*					11	11	--	--
	Degeneration, enamel – *moderate*					5	1	--	--
	Attenuation/atrophy, odontoblastic – *slight to marked*					6	10	--	--
Teeth, maxilla	Degeneration, dentine – *minimal to moderate*					11	9	--	--
	Attenuation/atrophy, odontoblastic – *minimal to marked*					2	5	--	--
Testes	Atrophy, degeneration – *minimal and severe*					4		--	--
Trachea	Decreased globule leukocytes – *present*	4		3	4	9	7	--	--

Note:

– Study termination refers to all remaining animals that survived until the end of the study or Week 27. These include all animals in the control, 3 and 30 mg/kg/day group.

Toxicokinetics:

Toxicokinetic Parameters in Rats Following 26 Weeks of GW786034B Administration						
Dose (mg/kg/day)	**Males**			**Females**		
	3	**30**	**300**	**3**	**30**	**300**
AUC_{0-t} (h*ng/mL)						
Week 4	63480	350860	877076	90014	497764	1308027
Week 13	72232	391888	830010	89736	507120	886825
Week 26	76572	373984	--	100024	543861	--
C_{max} (ng/mL)						
Week 4	6483	37188	72826	11245	66137	114066
Week 13	8402	53813	67511	10299	58105	94133
Week 26	8588	44621	--	10459	60893	--
Tmax (hr)						
Week 4	4.00	1.00	4.00	0.50	1.00	4.00
Week 13	1.00	1.00	4.00	4.00	2.00	4.00
Week 26	4.00	2.00	--	0.50	1.00	--

-- = no sample was taken

- Due to mortality and interim deaths of animals at 300 mg/kg/day, no samples were taken on Week 26.
- GW786034X was detected in the plasma of all drug-treated animals on all sampling days and increased with dose.
- The maximum plasma concentration was reached between approximately 0.50 and 4 hours after dosing.
- AUC increased 12.4-fold to 100-fold increase in doses between 3 and 300 mg/kg/day between Weeks 4 and 13.
- There was no significant change in systemic exposure across all doses between Week 4 and 13.
- There was no significant change in systemic exposure in the 3 and 30 mg/kg/day in Week 26.
- There were no apparent gender-related differences.
- T_{max} values were small and ranged from 1-4.

Other: none

Study Title: GW786034: 1-Month Oral Toxicity Study Cynomolgus Monkeys (Study No. Report CD2002/00103/00)
(Review by Lilliam Rosario, Ph.D; IND 65747) See Attachment.

Study title: GW786034B - 52-Week Oral Gavage Dose Toxicity Study in Cynomolgus Monkeys (Study No. RD2002/01338/02)

Key study findings:

- The high-dose of 500 mg/kg/day was not tolerated resulting in diarrhea and body weight loss. Drug related mortalities occurred at this dose in 1 M during Week 24 (or Study Day 171).
- Non-drug related mortalities occurred in 1 M at 5 mg/kg/day and 1 M at 500 mg/kg/day.
- Due to persistent and severe diarrhea, inappetence, weight loss, and histological evidence of crystalloid material in the GI tract, dosing was discontinued for 1 male and 1 female at 500 mg/kg/day beginning Week 31. These animals were sacrificed at Week 34 to investigate relationship between crystalloid material and diarrhea.
- For all remaining animals at 500 mg/kg/day, dosing was discontinued during Week 34 due and remaining animals (1 M and 3 F) were given a recovery period from Weeks 35 to 52.
- Main target organ of toxicity in animals that were terminated early (Weeks 13 and 25) included the duodenum, jejunum and mesenteric lymph node (specifically microscopic crystalloid material).
- Target organs of toxicity at 5 and 50 mg/kg/day included the ovary and uterus.
- Findings in ovary and uterus were still present in recovery animals at Week 52, however, additional findings were observed in the kidney and salivary gland. These findings were minimal and limited to female at 500 mg/kg/day.

Study no.:	RD2002/01338/02
Volume #, and page #:	Module 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Feb. 12, 2003
GLP compliance:	GLP
QA reports:	yes (X) no ()
Drug, lot #, and % purity:	GW786034B, 786034B-A4-02P-MIC, 91% purity

Dose justification: Dose selection was based on a 1-month oral toxicity and reversibility study in monkeys (Study No. CD2002/00103/00). In this GLP-study, GW786034B (monohydrochloride salt of GW786034X) was administered to monkeys via oral gavage at doses of 0, 5, 50, and 500 mg/kg/day. No significant changes were observed in body weight, food consumption, clinical pathology, gross pathology and histopathology. There was an increase in mean liver weight (~1.5 fold) in 1 M at 500 mg/kg/day. Based on these findings, doses of 0, 50, and 500 mg/kg/day were selected for the 52-week study. These doses were expected to provide separation in exposure and allow assessment of the toxicity.

Methods

Doses:	0, 5, 50, and 500 mg/kg/day
Species/strain:	Monkey/ cynomolgus
Number/sex/group or time point (main study):	4/sex/dose
Route, formulation, volume, and infusion rate:	Oral gavage, 0.5% hydroxpropyl methylcellulose and 0.1%Tween80 in water – pH to 1.3 with HCL, 5 mL/kg

Satellite groups used for toxicokinetics or recovery:	1/sex/dose Cont. and HD (recovery) 1-4/sex/dose (TK)
Age:	4-7 years of age
Weight:	3-3.7 kg M 2-2.8 kg F
Schedule:	once daily x 52 weeks
Unique study design or methodology (if any):	1. Beginning on Week 31, dosing was discontinued for 1 male (Animal I09683) and 1 female (Animal I09828) at 500 mg/kg/day. These animals were sacrificed at Week 34 to investigate drug-related toxicities. 2. Dosing was discontinued on Week 34 for all remaining animals at 500 mg/kg/day (M and F).

Observations and times:

Mortality:	Twice daily
Clinical signs:	At least once daily
Body weights:	Predose, first day of treatment and weekly during treatment.
Food consumption:	Once daily
EKG	Predose and once during Weeks 13, 26, 39, and 52 (1-2 hrs. after each dosing)
Ophthalmoscopy:	Predose, during Weeks 13 and 26
Hematology:	Predose and Weeks 4, 13, 26, 36, 52, and Week 33 (HD only)
Clinical chemistry:	Predose and Weeks 4, 13, 26, 36, 52, and Week 33 (HD only)
Coagulation	Predose and Weeks 4, 13, 26, 36, 52, and Week 33 (HD only)
Urinalysis:	Predose and Weeks 4, 13, 26, 36, 52, and Week 33 (HD only)
Gross pathology:	Conducted on all animals including those found dead, euthanized *in extremis*, and those euthanized on Week 33 (1 M and 1 F at 500 mg/kg/day) and at end of terminal sacrifice, Week 52.
Organ weights:	Week 33 (1 M and 1 F at 500 mg/kg/day) and at end of terminal sacrifice, Week 52.
Histopathology:	Week 33 (1 M and 1 F at 500 mg/kg/day) Week 7 (1 M at 5 mg/kg/day) Week 13 (1 M at 500 mg/kg/day) Week 24 (1 M at 5 mg/kg/day) Week 52 (all remaining animals in Control, LD and MD) Adequate Battery: yes (X), no () Peer review: yes (X), no ( )
Toxicokinetics:	Weeks 4, 13, 26, 39, and 52. An additional collection was done on Week 33 for animals at 500 mg/kg/day. 4 animals/sex/group were bled predose and at approximately 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing.

Results:

Mortality:

Gender	Animal No.	Dose (mg/kg/ day)	Week of death (day)	In-life observations
Males	I09675	5	7(52)	Labored breathing, no food consumption and body weight loss (≤20%). Diaphragmatic hernia contributed to moribund condition. This death was not considered drug-related.
	I09684	500	13 (90)	X-ray of a fractured right front limb near the shoulder region and puncture wound on its right arm (near armpit) that was secondary to possible bite. The left humerus and stifle joint were normal and no other animals at 500 mg/kg/day were observed with lesions in the joints. Therefore, this death was not considered drug-related
	I09669	500	24 (171)	Persistent diarrhea, dehydration, body weight loss (≥20%), and low to no food consumption. A protozoal enteropathy in the colon and rectum may have contributed to the persistent diarrhea, electrolyte imbalance, and deterioration of nutritional status. In addition, drug in the form of crystalloid material was observed in the mesenteric lymph node, and the lamina propria of the villar tips of the duodenum and jejunum. This mortality was considered drug-related.

Note: All animals that died early were euthanized.

Clinical signs:

Index	**No. of animals affected**							
Dose (mg/kg/day)	**0**		**5**		**50**		**500**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
No. of animals	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Excretion								
– green							2	2
– red	2		1		1	1		2
– orange						1	2	3
– white							4	4
– yellow					3	4	1	4
– tan	1	1	2	2	4	3	4	4
Feces								
– few	3	4	3	3	2	4	3	4
– liquid	2		3	1	4	3	4	3
– mucoid		1	1	2	1	3	2	4
– none	3	2	2	4	1	2	1	3
– non-formed	4	3	4	3	4	4	4	4

Body weight:

Gender	Dose	Observations

	(mg/kg/day)	
Males	3	Unremarkable
	30	Unremarkable
	300	Statistically significant ↓ (16%) compared to control between Weeks 28 and 29 in one animal. BW was comparable to control when taken off dosing. Statistically significant ↓ (27%) compared to control between Weeks 21 and 25 in one animal. This animal was euthanized on Week 34.
Females	3	Unremarkable
	30	Unremarkable
	300	Unremarkable

Note: Animals remaining at the 500 mg/kg/day that were allowed to recover (1 M and 3 F) from Weeks 35 to 52 had unremarkable changes in body weight.

Food consumption**:**

Gender	**Dose (mg/kg/day)**	**Observations**
Males	3	Unremarkable
	30	Unremarkable
	300	Statistically significant ↓ (≥20%) compared to control starting on Week 21.
Females	3	Unremarkable
	30	Unremarkable
	300	Unremarkable

Note: Animals remaining at the 500 mg/kg/day that were allowed to recover (1 M and 3 F) from Weeks 35 to 52 had unremarkable changes in food consumption.

Ophthalmoscopy: Unremarkable

EKG: Unremarkable

Hematology: Unremarkable

Clinical Chemistry

Index	**% difference from control group**							
Dose (mg/kg/day)	**0**		**5**		**50**		**500**	
Gender	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Week 4								
ALB								-11
Week 13								
ALB								-11
Week 26								
ALB								-8
TP								-13
Week 39								
ALB								-8

TP								-11
Week 52								
ALB								-11
TP								-8

Note:
- All values were statistically significant, $p \leq 0.05$
- Week 13 and Week 26 animals include 1 M and 3 F at 500 mg/kg/day that were discontinued dosing at 500 mg/kg/day on Week 34.

Coagulation: Unremarkable

Urinalysis: Unremarkable

Gross Pathology: Unremarkable

Organ weights: Unremarkable

Histopathology:

Organ	Observation	Microscopic Observations							
		0 mg/kg		5 mg/kg		50mg/kg		500 mg/kg	
		M	F	M	F	M	F	M	F
	Early/unscheduled Deaths[a]								
	No. of animals	**0**	**0**	**1**	**0**	**0**	**0**	**2**	**0**
Duodenum	Crystalline pigment – *slight to minimal*	--	--	0	--	--	--	2	--
Jejunum	Crystalline pigment – *slight to minimal*	--	--	0	--	--	--	2	--
Mesenteric lymph node	Crystalline pigment – *minimal*	--	--	0	--	--	--	2	--
	Study Termination[b]								
	No. of animals	**4**	**4**	**3**	**4**	**4**	**4**	**0**	**0**
Ovary	Corpora lutea, active/mature – *present*	0	4	0	2	0	4	--	--
Uterus	Ratio endometrial stroma/glands increased – *present*	0	2	0	2	0	1	--	--
	Recovery Sacrifice[c]								
	No. of animals	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**3**
Ovary	Corpora lutea, active/mature – *present*	--	--	--	--	--	--	0	2
Uterus	Ratio endometrial stroma/glands increased – *present*	--	--	--	--	--	--	0	1
Kidney	Infiltrate, lymphohistiocytic – *minimal*	--	--	--	--	--	--	1	2
Salivary gland	Infiltrate, lymphohistiocytic – *minimal*	--	--	--	--	--	--		3

Note:
[a]= Early/unscheduled deaths refers to 1 M at 5 mg/kg/day and 2 M at 500 mg/kg/day that were euthanized in moribund condition Weeks 7-24.

[b]= Study termination refers to all remaining animals that survived until the end of the study, Week 52. These include all animals in the control, 5, and 50 mg/kg/day. This time point does not include animals at 500 mg/kg/day as they were used as recovery animals.
[c]= Recovery sacrifice refers 1 M and 3 F at 500 mg/kg/day that were discontinued dosing at Week 34 and allowed to recover until Week 52.

Toxicokinetics:

Toxicokinetic Parameters in Monkeys Following 52 Weeks of GW786034B Administration						
Dose (mg/kg/day)	**Males**			**Females**		
	5	**50**	**500**	**5**	**50**	**500**
AUC_{0-t} (h*ng/mL)						
Week 4	44091	151207	665016	33059	160056	344769
Week 13	40596	125855	251994	32141	111867	669863
Week 26	49934	128736	172255	30041	171207	456033
Week 34	--	--	--	--	--	449988
Week 39	49213	220916	--	43582	141672	--
Week 52	46015	234967	--	36303	289289	--
C_{max} (ng/mL)						
Week 4	13555	28003	61981	10197	27086	48641
Week 13	12121	23555	44499	10523	21867	57168
Week 26	16868	24100	26836	9203	30051	50072
Week 34	--	--	--	--	--	50597
Week 39	14698	45550	--	181114	29379	--
Week 52	13281	31831	--	12843	44006	--
Tmax (hr)						
Week 4	1.50	1.50	3.00	1.50	1.51	1.25
Week 13	1.02	1.50	2.01	1.00	1.50	1.25
Week 26	1.00	1.00	--	1.54	1.00	3.03
Week 34	--	--	--	--	--	2.00
Week 39	2.00	2.00	--	1.00	1.50	--
Week 52	1.00	2.01	--	0.75	3.95	--

-- =no sample was taken

- Due to mortality and/or toxicity at 500 mg/kg/day, plasma samples were taken on Weeks 4, 13, 26, and 34 for all animals (M and F) at 500 mg/kg/day.
- For all animals (M and F) at 0, 5, and 50 mg/kg/day, plasma samples were taken on Weeks 4, 13, 26, 39, and 52
- AUC ($_{0-t}$) and Cmax values increased 4- and 2-fold, respectively, in doses between 5 and 50 mg/kg/day on Weeks 4, 13, 26, 39 and 52.
- AUC ($_{0-t}$) and Cmax values increased 12- and 4-fold, respectively, in doses between 5 to 500 mg/kg/day on Weeks 4, 13 and 26.
- No gender related differences except mean AUC ($_{0-t}$) was 2.7-fold higher on Week 13 in females compared to males.
- T_{max} values were small and ranged from 1-4.

- After 52-weeks, the dose of 50 mg/kg/day was associated with a Cmax and AUC_{0-t} of 31831.0 ng/mL and 234967.7 ng h/mL, respectively, in males and 44006.0 ng/mL and 289289.2 ng h/mL, respectively, in females.
- Overall, there was no marked (>2-fold) increase in systemic exposure [Cmax and AUC ($_{0-t}$)] with multiple dose administration.

Histopathology inventory

Study	**WD2005/00481/00**	**RD2002/01337/01**	**RD2002/01338/02**
Species	Mice	Rat	Monkey
Adrenals	X	X*	X*
Aorta	X	X	X
Bone Marrow femur	X	X	X
Bone (femur)	X	X	X
Brain	X*	X*	X*
Cecum	X	X	X
Cervix	X	X*	X
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X	X*	X*
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube			X
Feet			X
Gall bladder	X*		X
Gross (abnormal) lesions	X	X	X
Harderian gland		X	
Heart	X*	X*	X*
Ileum	X	X	X
Injection site			
Jejunum	X	X	X
Kidneys	X*	X*	X*
Lachrymal gland		X	
Larynx			
Liver	X*	X*	X*
Lungs	X	X	X*
Lymph nodes, inguinal		X	X
Lymph nodes mandibular		X	X
Lymph nodes, mesenteric		X	X
Mammary Gland	X	X	X
Nasal cavity	X		

Optic nerves	X	X	X
Ovaries	X*	X*	X*
Pancreas	X	X	X
Parathyroid	X	X	X*
Paws/hands	X	X	X
Peripheral nerve			
Pharynx			
Pituitary	X	X	X*
Prostate	X	X*	X*
Rectum		X	X
Salivary gland	X	X	X
Sciatic nerve	X	X	X
Seminal vesicles	X	X*	X*
Skeletal muscle	X	X	X
Skin	X	X	X
Spinal cord		X	X
Spleen	X*	X*	X*
Sternum	X	X	X
Stomach	X	X	X
Teeth		X	X
Testes	X*	X*	X*
Thymus	X*	X*	X*
Thyroid	X	X	X
Tongue	X	X	X
Trachea	X	X	X
Urinary bladder	X	X	X
Uterus	X	X	X
Vagina	X	X	X
Zymbal gland			

X, histopathology performed, *organ weight obtained

2.6.6.4 Genetic toxicology

Study title: Bacterial Mutagenicity Report (Study No. V40962 and Report No. RD2001/01168/00)

This non-GLP study was a preliminary Ames assay to evaluate the mutagenicity of GW786034A (Lot No. U17574-61-1) the dihydrochloride salt of pazopanib. Dose levels of 0, 12, 25, 50, 100, 200, and 400 µg/plate were tested with and without S9 in *Salmonella typhimurium* tester strains TA98 and TA100. Dose levels of 0, 25, 50, 100, 200, 400, and 800 µg/plate were tested in *Salmonella typhimurium* tester strains TA1535 an dTA1537.

Results of this assay reported a significant increase in revertants for TA1535 at 100 and 800 µg/plate without S9. However, there was not a significant increase in revertants for the two intervening dose levels 200 and 400 µg/plate. No effects were observed in

TA1535 in the presence of S9-mix or in any other strain. In addition, the positive controls for TA1535 did not produce a mutagenic response. The Sponsor noted an error during the experiment in that the positive controls were inadvertently omitted for that stain. Due to the equivocal nature of this finding, further screening studies were performed (Study No. RD2002/00279/00 and RD2002/00280/00).

Study title: Bacterial Mutagenicity Report (Study No. V40967 and Report No. RD2002/00279/00)

The non-GLP study was conducted to confirm the results observed in previous preliminary Ames studies (Study No. V40962 and V40938). The first part of this study was conducted to confirm the results obtained in Study No. V40962. The second part of this study wanted to re-test the mutagenicity of GW771127A, a structurally related compound of GW786034A from results obtained from Study No. V40938.

In the first part of this study, the same lot of GW786034A that was used in Study No. V40962 (Lot No. U17574-61-1) was re-tested for mutagenicity but with standard-sized Ames plates. Dose levels of 0, 100, 500, 1000, 2500, and 5000 µg/plate were tested with and without S9 in *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537. Results of this study reported that GW786034A was not mutagenic all tester strains. According to the Sponsor, the difference in data between Study No. V40962 and the current study are not known. The same lot of GW786034A was used in both studies. The data from the standard-sized Ames plates used in the current study are considered more robust than the data from the mini-well assay.

The second part of this study examined a structurally related compound, GW771127A, which only differs from GW786034A by the absence of a methyl group on an aromatic ring. GW771127A was also previously tested in an Ames miniwell study (Study No. V40938). This study was not reviewed and the study reported that GW771127A was not mutagenic when tested in TA98 and TA100. However, since the structurally related compound GW786034A was mutagenic in TA1535 (Study No. V40962), the current study also examined the mutagenicity of GW771127A in TA1535 and TA1537 in standard-sized Ames plates. Results of this study reported that GW771127A (Lot No. U17574/29/1) was mutagenic with and without S9 for both TA100 and TA1535. The reason for the lack of agreement between Study No. V40938 and the current study for TA100 are not known. The same lot of GW771127A was used in both studies. The data from the standard-sized Ames plates used in the current study are considered more robust than the data from the mini-well assay.

Study title: Bacterial Mutagenicity Report (Study No. V40969 and Report No. RD2002/00280/00)

The non-GLP study was conducted to re-confirm results obtained in previous preliminary Ames studies (Study No. V40962 and V40938) using a different batch of GW771127A (Lot No. U17574/52/1) than that used in Study No. V40398 and V40967. Dose levels of 0, 100, 500, 1000, 2500, and 5000 µg/plate were tested with and without S9 in *Salmonella typhimurium* tester strains TA100 and TA1535. Results from the current

study show that GW771127A nor GW786034A are not mutagenic in any of the Salmonella strains using the standard plate Ames protocol. The Sponsor concluded that the batch of GW771127A Lot No. U17574/29/1) used in Study No. V40398 and V40967 contained an impurity that co-eluted with the test article and that this impurity was mutagenic. This impurity is not present in the batch of GW771127A used in the current study and this batch shows no indication of mutagenicity in the current robust screening assay.

Study title: GW786034B: *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay

Key findings:
- This is a definitive GLP standard plate incorporation Ames assay conducted with GW786034B, the monohydrochloride salt of GW786034X.
- GW786034B was not mutagenic in the microbial reverse mutation assay with or without S9 activation at in the dose range-finding and in the initial and confirmatory mutagenicity assays the concentrations up to 5000 µg/mL.

Study no.: RD2002/00887/00
Volume #, and page #: Module 4.2.3.3.1
Conducting laboratory and location: (b) (4)

Date of study initiation: March 5, 2002
GLP compliance: GLP
QA reports: yes (X) no ()
Drug, lot #, and % purity: GW786034B, 786034-A2-01M, 98.5% purity

Methods
Strains/species/cell line:
Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2*uvrA*(pKM101)

Concentrations used in definitive study:

Dose range finding assay using tester strains TA100 and WP2*uvrA*(pKM101)	6.67, 10, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 µg/mL
Initial and confirmatory mutagenicity assay using tester strains TA98, TA100, TA1535 and TA1537 and WP2*uvrA*(pKM101)	33.3, 100, 333, 1000, 3330, and 5000 µg/mL

Basis of concentration selection: Top dose was used.

Negative controls:
DMSO

Positive controls:

Strain	With S9	Without S9
TA98	Benzo(a)pyrene	2-nitrofluorene

TA100	2- aminoanthrace	Sodium azide
TA1535	2- aminoanthrace	Sodium azide
TA1537	2- aminoanthrace	ICR-191
WP2*uvrA*pKM101	2- aminoanthrace	4-nitroquinoline N-oxide (NQO)

Incubation and sampling times:
Incubated for 72 hours

Results
Study validity:
- Three replicate plates used in the confirmatory study
- Methods state that counts for revertant colonies were counted by an automated colony counter or by hand but details about the make or model of the automated counter are not given.
- Criterion for a positive result was as follows:
    - In tester strains TA98, TA100 and WP2*uvrA*(pKM101), a test article to considered positive had to produce at least a 2-fold increase in the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.
    - For tester strains TA1535 and TA1537, a test article to be considered positive had to produce at least a 3-fold increase in the mean revertants per plate of at least on of these tester strains over the mean revertants per plate of at least one of these tester strains over the mean revertants per plate of the appropriate vehicle control. This increase in the mean number of revertants per plate had to be accompanied by a dose response to increasing concentrations of the test article.
- The negative and positive control values were within the historical control data ranges
- Study design is valid.

Study outcome:
- In the dose-range finding assay, revertant frequencies for all doses (6.67 to 5000 µg/ µg/mL) in tester strains TA100 and WP2*uvrA*(pKM101) with and without S9 were less than those observed in the negative control values (See Sponsor's Tables 4 and 5). However, the test article precipitated from solution at doses ≥333 µg/mL.
- In the initial and confirmatory mutagenicity assay, revertant frequencies for all doses (33.3 to 5000 µg/mL) in tester strains TA98, TA100, TA1535 and TA1537 and WP2*uvrA*(pKM101) with and without S9 were less than those observed in the negative control values (See Sponsor's Table 1). Similar to the dose-range assay, the test article precipitated from solution at doses ≥333 µg/mL.

Table 4
Dose Rangefinding Study

Test Article ID: GW786034B

Experiment ID: 23623-A1 Date Plated: 19-Mar-02

Vehicle: DMSO Date Counted: 21-Mar-02

TA100 Revertants Per Plate

µg/Plate		With S9 Revertants Per Plate	With S9 Background Lawn Evaluation[a]	Without S9 Revertants Per Plate	Without S9 Background Lawn Evaluation[a]
0.00 (Vehicle) (100 µL)		103	N	80	N
Test Article	6.67	104	N	89	N
	10.0	95	N	85	N
	33.3	113	N	95	N
	66.7	112	N	86	N
	100	109	N	89	N
	333	101	NP	93	NP
	667	117	NP	82	NP
	1000	111	NP	75	NP
	3330	114	NP	86	NP
	5000	102	NP	62	NP

[a] Background Lawn Evaluation Codes:
N = normal R = reduced A = absent
P = precipitate O = obscured by precipitate

[Table excerpted from Sponsor]

Table 5
Dose Rangefinding Study

Test Article ID: GW786034B

Experiment ID: 23623-A1 Date Plated: 19-Mar-02

Vehicle: DMSO Date Counted: 21-Mar-02

WP2*uvr*A(pKM101) Revertants Per Plate

µg/Plate		With S9		Without S9	
		Revertants Per Plate	Background Lawn Evaluation[a]	Revertants Per Plate	Background Lawn Evaluation[a]
0.00 (Vehicle) (100 µL)		128	N	103	N
Test Article	6.67	151	N	122	N
	10.0	157	N	126	N
	33.3	152	N	107	N
	66.7	136	N	121	N
	100	160	N	134	N
	333	159	NP	107	NP
	667	175	NP	114	NP
	1000	143	NP	87	NP
	3330	165	NP	142	NP
	5000	157	NP	106	NP

[a] Background Lawn Evaluation Codes:
N = normal R = reduced A = absent
P = precipitate O = obscured by precipitate

[Table excerpted from Sponsor]

Table 1: Summary of Microbial Mutagenicity Data for GW786034B (Batch 786034-A2-01M)

Mean Number of Revertant Colonies per Plate [a]											
		Salmonella typhimurium								*Escherichia coli*	
GW786034X Concentration (µg/plate)	S9 metabolic activation	TA98		TA100		TA1535		TA1537		WP2*uvrA* (pKM101)	
		Initial Assay	Confirm-atory Assay	Initial Assay	Confirm-atory Assay	Initial Assay	Confirm-atory Assay	Initial Assay	Confirm-atory Assay	Initial Assay	Confirm-atory Assay
0 [b]	W	28	30	93	87	15	13	15	12	169	163
33.3	W	29	33	93	83	15	16	9	11	184	176
100	W	26	54	106	107	13	18	14	13	186	178
333	W	26	40	103	90	12	16	11	15	153	191
1000	W	21	32	100	97	17	16	11	12	193	193
3330	W	23	31	96	93	14	16	11	9	189	207
5000	W	27	32	92	85	11	12	11	11	180	222
Positive control [c]	W	301+	317+	368+	642+	119+	169+	99+	137+	1470+	967+
0 [b]	N	8	10	60	85	7	13	9	7	132	97
33.3	N	8	16	49	88	9	13	8	6	137	108
100	N	4	14	48	87	6	13	7	4	131	135
333	N	7	13	47	76	13	14	3	3	137	120
1000	N	7	12	44	80	5	16	6	5	125	66
3330	N	5	10	43	58	5	10	5	3	109	89
5000	N	5	8	38	59	7	10	5	4	90	74
Positive control [c]	N	96+	408+	725+	1342+	587+	986+	657+	1160+	2785+	2648+
Experimental work dates: 19 March 2002 – 15 April 2002				Study in compliance with GLP: Yes				Batch No.: 786034-A2-01M			

[Table excerpted from Sponsor]

Study title: GW786034B: *In Vitro* Assay for Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

Key findings:

- GW786034B (monohydrochloride salt of GW786034X) was negative for inducing chromosome aberrations, polyploidy, and endoreduplication in cultured human peripheral blood lymphocytes with and without metabolic activation.
- The highest dose analyzed for each treatment and metabolic activation condition was the first precipitating dose (determined in the initial dose range finding phase of this study) that caused a ≥50% reduction in the mitotic index.

Study no.: RD2002/00238/00
Volume #, and page #: Module 4.2.3.3.1
Conducting laboratory and location: (b) (4) (b) (4)
Date of study initiation: February 21, 2002
GLP compliance: GLP
QA reports: yes (X) no ()
Drug, lot #, and % purity: GW786034B, 786034-A2-01M, 98.5% purity

Methods:

Strains/species/cell line:

Human peripheral lymphocytes from healthy, adult donors (non-smoker without history of radiotherapy, chemotherapy, or drug usage, and lacking current viral infections)

Concentrations used in definitive study:

Initial assay:	
Treatment at 3 hours and harvest at 22 hour without S9 activation	10.1, 25.3, 50.5, 101 µg/mL
Harvest at 22-hours without S9 activation	5.05, 10.1, 25.3, 50.5 µg/mL
Treatment at 3 hours and harvest at 21.9 hour with S9 activation	25.0, 50.0, 100, and 200 µg/mL
Confirmatory assay:	
Treatment at 22 hours and harvest of 45.8 hours without S9 activation	10.0, 25.0, 50.0, and 100 µg/mL
Treatment at 3 hours and harvest at 45.8 hour with S9 activation	10.0, 25.0, 50.0, and 100 µg/mL

Negative controls:

DMSO

Positive controls:

Without S-9	mitomycin C
With S-9	cyclophosphamide (CPA)

Incubation and sampling times:

- In the initial assay without metabolic activation, cells were incubated with the drug for 3 hours and harvested after 22 hours.
- In the initial assay with metabolic activation, cells were incubated with the drug for 3 and harvested after 21.9 hours.
- In the confirmatory assay without metabolic activation, cells were incubated with the drug for 22 hours and harvested after 45.8 hours.
- In the confirmatory assay with metabolic activation, cells incubated with the drug for 3 hours and harvested after 45.8 hours.

Results

Study validity:

- Two replicate plates used in the confirmatory study
- Criterion for a positive result is if there is a significant increase (the difference was considered significant when $p \leq 0.01$) in the number of cells with chromosomal aberrations was observed at one or more concentrations. If a significant increase was seen at one or more concentrations, a dose-response should be observed.
- The negative and positive control values were within the historical control data ranges.
- Study design and results were valid

Study outcome:

- In the initial assay, the high dose analyzed was the first precipitating dose that caused a ≥50% reduction in the mitotic index for the 3.0 hour exposure conditions with metabolic actiavation and in the 22.0 hour exposure condition without metabolic activation.
- In the confirmatory assay, the high dose analyzed was the first precipitating dose that caused a ≥50% reduction in the mitotic index for all exposure conditions.
- However, it was concluded that there was not a statistically significant increase in cells with structural chromosomal aberrations, polyploidy, or endoreduplication at any dose level for cells treated in the absence or presence of metabolic activation in both the initial and confirmatory assay (See Sponsor's Summary Table 1 below).

Table 1. Summary Data of Structural Chromosome Aberrations for GW786034B in Human Lymphocytes

GW786034B Concentration (µg/mL)	% Cells with Aberrations [a]				
	3.0 / 22.0 (–S9)	22.0 / 22.0 (–S9)	22.0 / 45.8 (–S9)	3.0 / 21.9 (+S9)	3.0 / 45.8 (+S9)
Negative Control [b]	1.0	0.5	0.0	0.0	0.5
Solvent Control [c]	0.0	0.0	0.5	0.0	0.0
5.05	ND	0.0	ND	ND	ND
10.0	ND	ND	1.0	ND	0.5
10.1	0.0	0.0	ND	ND	ND
25.0	ND	ND	0.0	0.0	0.0
25.3	0.5	1.0	ND	ND	ND
50.0	ND	ND	0.0	0.5	0.0
50.5	0.5	0.5	ND	ND	ND
100	ND	ND	1.0	1.0	0.0
101	1.0	ND	ND	ND	ND
200	ND	ND	1.0	1.0	ND
Mitomycin C [d]	41.0*	32.0*	.22.5*	ND	ND
Cyclophosphamide [e]	ND	ND	ND	33.6*	68.0*

Study in compliance with GLP: Yes Experimental work dates: 15 March 2002 through 15 May 2002

Assay Results: GW786034B did not cause an increase in structural chromosome aberrations, polyploidy, or endereduplication at any dose level tested in the absence or presence of S9 metabolic activation. The highest dose analyzed for each treatment/ metabolic activation condition was the first precipitating dose or a dose with ≥ 50% reduction in mitotic index. Assays with a ≥ 50% reduction in mitotic index were (i) 22.0 hour treatment/22.0 hour harvest without S9, (ii) 22.0 hour treatment/45.8 hour harvest without S9. GW786034B, batch number 786034-A2-01M, was not clastogenic.

Key for Table

[a] The mean value is given for replicate cultures for the percentage of cells with aberrations (gaps not included). One hundred cells were examined whenever possible from each replicate culture, thus a total of 200 cells were evaluated for aberrations for the negative control, solvent control and test article cultures. Fifty cells were examined for each replicate positive control culture. Table columns give the Treatment / Harvest time in hours.

[b] The negative control was RPMI 1640 tissue culture medium.

[c] The solvent was DMSO which was at a final concentration in tissue culture media of 1% (vol/vol).

[d] Mitomycin C was used as a positive control without metabolic activation at concentrations of 1.00 µg/mL for the initial assay (3.0/22.0) and 0.300 µg/mL for initial assay (22.0/22.0) and 0.100 µg/mL for the confirmatory assay (22.0/45.8).

[e] Cyclophosphamide was used as a positive control with metabolic activation at concentrations of 25.0 µg/mL for the initial assay (3.0/21.9) and 100 µg/mL for the confirmatory assay (3.0, 45.8).

ND Indicates not done.

* Indicates values statistically different from the control values.

[Table excerpted from Sponsor]

Study title: GW786034B: Micronucleus Frequencies in Bone Marrow Polychromatic Erythrocytes from Male Sprague Dawley Rats Following Oral Administration

Key findings:
- GW786034B (monohydrochloride salt of GW786034X) was not clastogenic *in vivo* at doses up to 2000 mg/kg/day, the highest dose tested.

Study no.: RD2002/00227/00
Volume #, and page #: Module 4.2.3.3.2
Conducting laboratory and location: (b) (4)

Date of study initiation: March 15, 2002
GLP compliance: GLP
QA reports: yes (X) no ()
Drug, lot #, and % purity: GW786034B, 786034-A2-01M, 97.5% purity

Methods:

Strains/species/cell line:
Male rats/Sprague Dawley/CRl:CD®(SD)IGS BR strain

Doses used in definitive study:
0, 1250, and 2000 mg/kg/day at a dose volume of 20 mL/kg/day

Basis of dose selection:
An initial dose confirmation study was conducted with two male rats each receiving 2000 mg/kg/day of GW786034B by oral gavage once daily for two consecutive days. Both animals survived approximately 24 hours after the second dose administration without any adverse effects. Since no drug-related effects were observed at this dose, the Sponsor used a top dose of 2000 mg/kg/day in the confirmatory study.

Negative (or vehicle) controls:
0.5% HPMC with 0.1% Tween®80 in reverse osmosis water

Positive controls:
Cyclophosphamide

Incubation and sampling times:
- Bone marrow was obtained from the tibias of each animal approximately 24-hours following the second dose.
- An additional groups of rats (n=3/group) were exposed to the same dosing regimen and blood samples were collected 4 hours following the first dose and analyzed for GW786034B concentration.

Results:
Study validity:
- The micronuclei frequency (expressed as percent micronucleated cells) was determined by analyzing the number of micronucleated polychromatic erythrocytes (PCEs) from at least 2000 PCEs per animal.

- The PCE:NCE ratio was determined by scoring the number of polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) observed while scoring at least the first 1000 erythrocytes per animal.
- Criterion for a positive result is an indication of toxicity, e.g., toxic signs and/or mortality in the test article dosed animal and/or reduction in the PCE:NCE ratio compared to control.
- The negative and positive control values were within the historical control data ranges.
- Study design and findings are valid.

Study outcome:

- No mortality or notable clinical signs occurred in the study.
- No statistically significant decrease in PCE: NCE ratio and produced no significant increase in the frequency of micronucleated PCEs in animals at each dose level.
- The mean plasma concentrations of GW786034B after 4 hours of dosing on Day 1 were 29.3 and 70.9 µg/mL at dose levels of 1250 and 2000 mg/kg/day, respectively.
- Results are summarized in Sponsor's Table below (Table 1).

Table 1:
Micronucleus Data Summary Table

ASSAY NO.: 23623-0-454OECD TEST ARTICLE: GW786034B

TREATMENT	DOSE	HARVEST TIME	% MICRONUCLEATED PCEs MEAN OF 2000 PER ANIMAL ± S.E.	RATIO PCE:NCE MEAN OF AT LEAST 1000 PER ANIMAL ± S.E.
CONTROLS				
VEHICLE	Vehicle	24 hr	0.08 ± 0.01	0.90 ± 0.02
POSITIVE	CP 60 mg/kg	24 hr	3.36 ± 0.18*	0.76 ± 0.03**
TEST ARTICLE	1250 mg/kg/day	24 hr	0.12 ± 0.02	0.90 ± 0.02
	2000 mg/kg/day	24 hr	0.14 ± 0.02	0.95 ± 0.01

* Significantly greater than the corresponding vehicle control, p = 0.01
** Significantly less than the corresponding vehicle control, p = 0.05.
Vehicle = 0.5% hydroxypropyl methylcellulose with 0.1% Tween®80 in reverse osmosis water, adjusted with 1N HCl to approximately pH 1.3 (Density 1 g = 1 mL)
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

[Table excerpted from Sponsor]

2.6.6.5 Reproductive toxicology

Fertility and early embryonic development

Study title: GW786034B: Oral Female Fertility and Early Embryonic Development Study in Rats

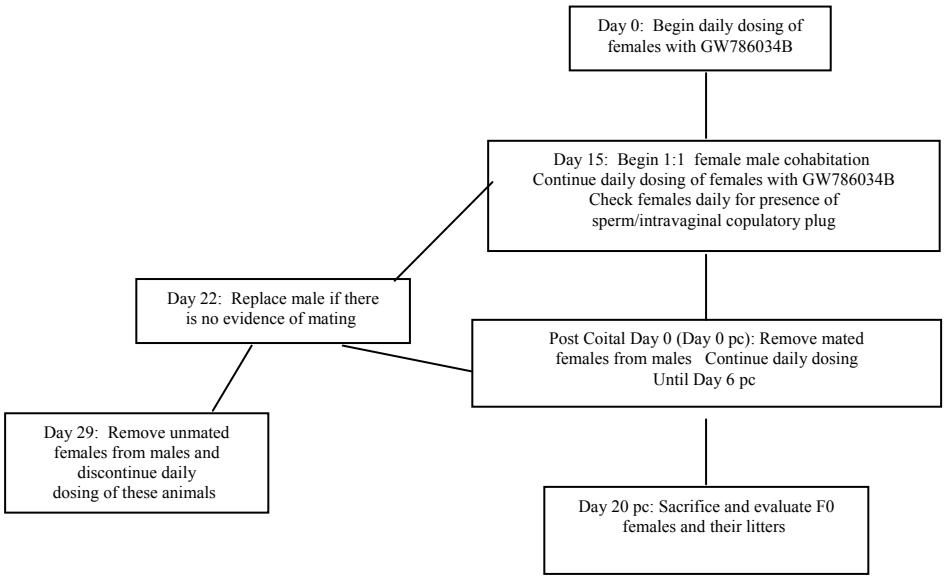
Key study findings:

- Decreased weight gain in 300 mg/kg high dose females after Day 14 due to complete resorption
- Increased resorption in 30 mg/kg mid dose females and 100% resorption of implants seen in 300 mg/kg high dose females
- Decreased male and female fetal weight in 30 mg/kg dose group
- Gross fetal malformation in 3 litters from 30 mg/kg dose group

Study No. G03407
Conducting laboratory and location: GlaxoSmithKline
Safety Assessment
US Research and Development
King of Prussia, PA USA
Date of study initiation: January 27, 2004
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: GW786034B/ 786034B-A4-02P-MIC/ 91%

Methods

Doses: 0, 3, 30, 300 mg/kg
Species/strain: Rats Crl:CD(SD)IGSBR
Number/sex/group or time point: 25 females/group
Route, formulation, volume, and infusion rate: Oral gavage once daily with 10 mL/kg of test article in 0.5% hydroxypropylmethylcellulose with 0.1% Tween
Age: Approximately 10 weeks
Weight: 173-274 g
Sampling times: Day 20 post coital
Study design:



Parameters and Endpoints Evaluated

Clinical Signs	Daily
Body Weight	Twice pretreatment, each dosing day, and Days 7,10, 14, 17, and 20 pc
Food Consumption	Weekly
Estrous Cycle	Daily beginning 19 days predosing until mated

At Necropsy	Number of corpora lutea, live and dead fetuses (number, weight and gender), number and distribution of implantation sites, resorptions

Results

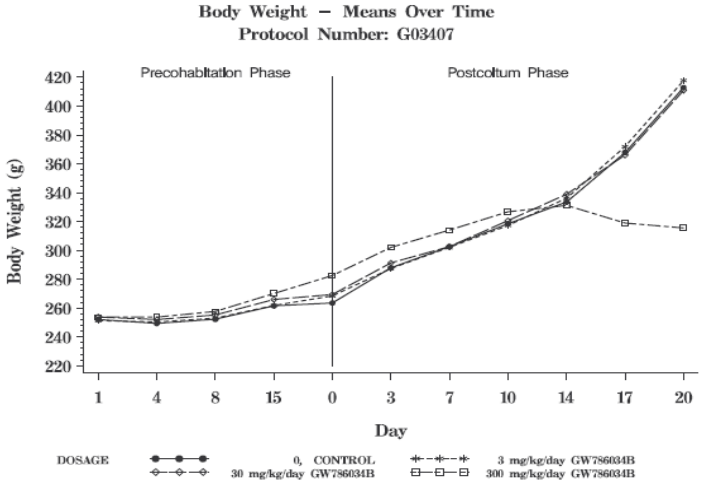
Mortality:

Animal #	Dose (mg/kg)	Sex	Day of Death	Observations		
				Reason for Removal	Cause of Mortality	General
RO47-7326	3	F	2	Found Dead	Perforated Esophagus	Dosing error, not drug-related

Clinical signs: No drug-related clinical observations

Body weight:
Dams treated with 300 mg/kg GW786034B had slightly increased body weights/body weight gain compared to control animals until approximately 2 weeks postcoitum. After this time point, high dose treated dams had significantly decreased body weights compared to controls and lost weight.

(Excerpted from sponsor)



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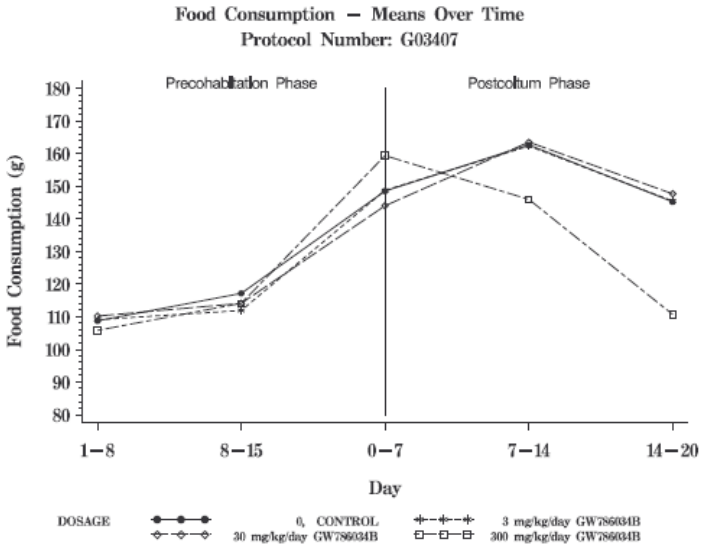
Food consumption:
Food consumption measurements followed a similar trend as body weight with high dose dams consuming more early, but beginning to decrease their food consumption during the first postcoital week.

(Excerpted from sponsor)



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Necropsy:

F0 necropsy results

The F0 females were examined for gross abnormalities. No drug-related changes were observed.

F1 necropsy results

No fetuses survived in the 300 mg/kg high dose treated dams. There were four incidences of fetal malformation. One occurred in the control group. The others occurred in the 30 mg/kg mid-dose group. The mid-dose malformations were seen in the head and jaw and are listed below.

- Agnathia
- Micrognathia, Dome shaped head
- Cleft palate/ cleft lip

Each malformation was seen in only 1 animal. Each of these animals was from a separate litter.

Dose Group mg/kg/day	Control 0	Low 3	Mid 30	High 300
Mean Male Fetal Weight	3.71	3.71	3.22*	na
Mean Female Fetal Weight	3.52	3.54	2.94*	na

*Male and female fetal body weights for the mid dose animals were 16% and 13% lower respectively from the control group weights.

Fertility parameters

The parameters presented below show that while drug exposure had no effect on the ability of the female rat to mate, it had a significant effect on the animal's ability to become pregnant. There was a slight decrease in fertility at the mid-dose group and significant inhibition in fertility in the high dose group.

Dose Group mg/kg/day	Control 0	Low 3	Mid 30	High 300
Mating Index (#mated/#treated)	100%	100%	100%	100%
Fertility Index (# preg/#mated)*	96%	100%	92%	32%
Days to Mating	2	2	2	2
Mean # Estrous Cycles/15 Days Tx	3.3	3.5	3.5	3.6

*uterus stained with ammonium sulfide if apparently not pregnant at necropsy

Pregnancy parameters

The pregnancy parameters are presented as averages for each dose group in the table below. The data show that dosing of females during breeding through the first 7 days of gestation resulted in significant effects in the 300 mg/kg high dose group. Rats in this group had reductions in the number of corpora lutea and implantations. All implantations in this group were resorbed early resulting in 100% loss in this group. Rats treated with 30 mg/kg also showed an increase in percent pre-implantation loss (though the actual numbers of corpora lutea an implantation were higher than control) and early resorption. No other differences were observed.

Dose Group mg/kg/day N	Control 0 24	Low 3 24	Mid 30 23	High 300 8
Number Corpora Lutea	15.6	15.8	19.9	12.6
Number Implantations	14.5	14.8	16	7
Percent Pre-Implantation Loss	6.2%	6.3%	18.9%	46.3%
Number of Resorptions early late	 0.7 0	 0.6 0	 3.9 0	 7 0
Percent Implants Resorbed	5.3%	4.7%	25.6%	100%
Number Live Fetuses-Mean/♀	13.8	14.1	12.6	0
Percent Live Males	47%	47.3%	48.5%	0
Number of Dead Fetuses	0	0	0	0
Gravid Uterus Weight (g)	79.1	80.7	68.6	na

Study title: GW786034B: Oral Male Fertility Study in Rats

Key study findings:

- At doses ≥30 mg/kg male rats showed dose-dependent decreases in sperm concentration and motility as well as decreases in reproductive organ weights.
- Male rats treated with ≥30 mg/kg of GW786034B exhibited some decrease in body weight at late stages of the study period. There were also dose-dependent increases in dental problems.

- There were no changes in clinical signs, body weight, or food consumption by females mated to males from different treatment groups.
- Pups from F1 litters were similar in number, sex, and weight regardless of male treatment group.
- No changes in gross malformations seen in litters.
- No changes in pregnancy parameters.

Study No. (b) -472022
Conducting laboratory and location: (b) (4)

Date of study initiation: December 12, 2003
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: GW786034B/ 786034B-A4-02P-MIC/ 91%

Methods

Doses:	0, 3, 30, 100 mg/kg
Species/strain:	Rats Crl:CD(SD)IGSBR
Number/sex/group or time point:	25 males/dose
Route, formulation, volume, and infusion rate:	Oral gavage once daily with 10 mL/kg of test article in 0.5% hydroxypropylmethylcellulose with 0.1% Tween 80
Age:	Approximately 12 weeks
Weight:	328-437 g
Study design:	F0 males dosed from Day 1 until Day 103-105, mated with females on Day 10 (Phase 1) of dosing for up to 10 days and Day 63-65 (Phase 2) of dosing for up to 10 days. Females were untreated and euthanized on gestation Day 20.

Parameters and endpoints evaluated

Population	Parameters and Endpoints
F0 males	Mortality, clinical observations, body weight, food consumption, mating, fertility, copulation, necropsy—reproductive organ weights, testicular and epididymal sperm counts, sperm production rate, sperm motility
F0 females	Clinical observations, body weight, necropsy—gross examination of uterus, cervix, corpora lutea, and uterine weight
F1 litters	Implantations, resorptions, live and dead fetuses; fetal weight, sex, and morphology

Results

Mortality:

Animal #	Dose (mg/kg)	Sex	Day of Death	Observations		
				Reason for Removal	Cause of Mortality	General
41748	100	M	37	Euthanized	Moribund	3 continuous days of impaired use of hindlimbs; white spots on liver at necropsy

Clinical signs:

Generally there was increased hair loss and dental problems with males in the 100 mg/kg treatment group. In rare instances, these mice also presented with red material around the mouth and nose as well as increased salivation within an hour of dosing. Phase 1 and 2 females had no notable clinical signs.

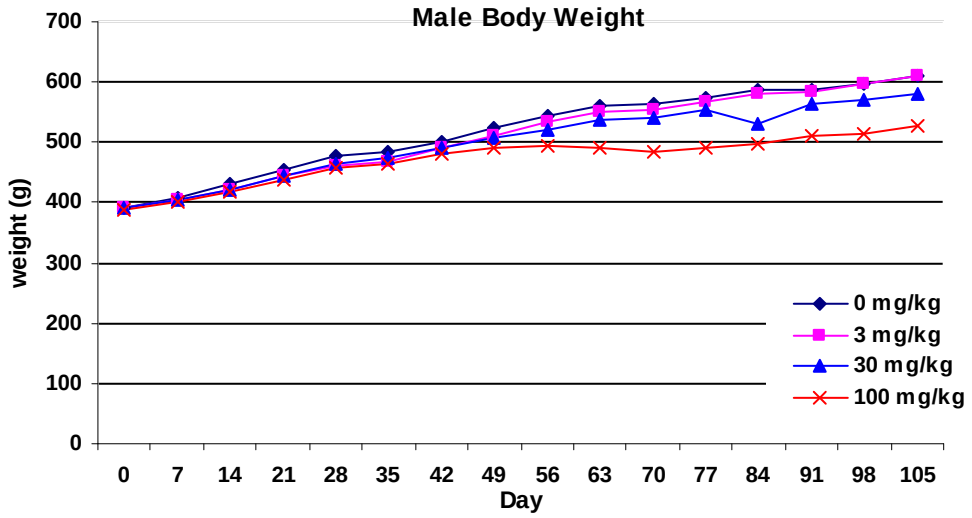
Males

Observation Group Dose	Control (1) 0 mg/kg	Low (2) 3 mg/kg	Mid (3) 30 mg/kg	High (4) 100 mg/kg
Forelimb Hair Loss	6	9	3	19
Abdominal Hair Loss	0	0	0	3
Dried Red Material on Penis	0	0	0	7
Dried Red Material—Urogenital	0	0	0	11
Dried Red Material-Forelimbs	0	0	1	19
Red Staining—Ventral Thoracic/Ab	0	0	1	10
Dried Red Material—Nose	6	7	7	19
Dried Red Material—Eyes	1	3	4	7
Excreta—Soft Stool	6	6	12	16
Upper Incisors—Broken	1	3	13	20
Upper Incisors—Malaligned	1	1	11	9
Lower Incisors—Broken	0	0	25	24*
Lower Incisors—Malaligned	0	0	22	7
Dried Red Material—Mouth	0	0	1	12
Upper Incisors Long, Trimmed	0	0	1	7

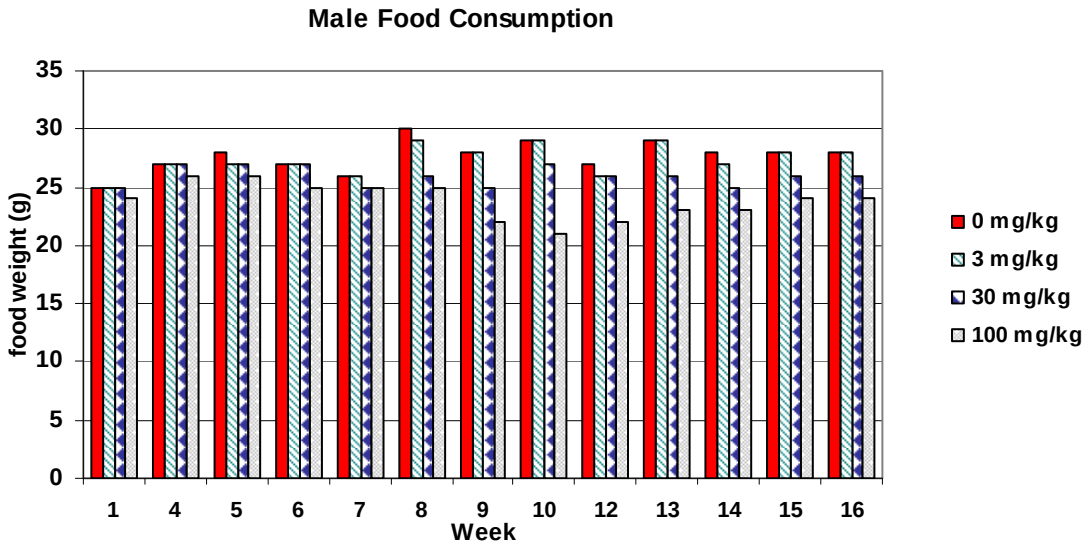
*5-6X Increase in incident number over that seen in the mid-dose group

Body weight:

After Week 8, in males in the 100 mg/kg dose weight gain was decreased such that body weight was approximately 10% lower in the high dose group compared to control. There were no differences in weight gain during the gestation period for Phase 1 or 2 females.



Food consumption:



Necropsy:

F0 males:
Males in the mid-dose and high dose groups ≥30 mg/kg had evidence of toxicity in their reproductive organs. These males had dose-dependent decreases in organ size and weight. Furthermore, there were reductions in sperm production and motility.

Gross Pathology

Observation / Group / Dose		Control (1) 0 mg/kg	Low (2) 3 mg/kg	Mid (3) 30 mg/kg	High (4) 100 mg/kg
Epididymis	Small			2	6
	Soft			1	2
Left Testis	Small			3	10
	Soft			3	9
Right Testis	Small			3	10
	Soft			3	8
Teeth	Fractured	1	1	4	12
	Malaligned				1

Organ Weights

Percent Change from Control: Absolute (Relative to Body Weight)

Observation / Group / Dose	Low (2) 3 mg/kg	Mid (3) 30 mg/kg	High (4) 100 mg/kg
Seminal Vesicle/CG/Fluid		↓13% (↓8%)	↓13% (↑1%)
Left Epididymis		↓12% (↓8%)	↓24% (↓12%)
Right Epididymis		↓18% (↓14%)	↓28% (↓16%)
Left Testis		↓24% (↓20%)	↓39% (↓30%)
Right Testis		↓24% (↓21%)	↓39% (↓29%)
Left w/o Tunica		↓27% (↓24%)	↓44% (↓35%)
Left Cor/Cap Epididymis		↓13% (↓9%)	↓25% (↓12%)
Left Cauda Epididymis		↓14% (↓10%)	↓28% (↓17%)
Right Cauda Epididymis		↓18% (↓13%)	↓30% (↓18%)

Sperm Evaluation
Percent change from control

Observation / Group / Dose	Low (2) 3 mg/kg	Mid (3) 30 mg/kg	High (4) 100 mg/kg
Motility			↓11%
Sperm Concentration—Epididymis (millions/gram)		↓16%	↓43%
Sperm Concentration—Left Testis (millions/gram)	↓12%	↓13%	↓37%
Sperm Production Rate (millions/gram/day)	↓12%	↓12%	↓36%

F0 females:
There were no gross abnormalities observed in females mated to males in any treatment group, including females who failed to become pregnant.

F1 litter:
There was one incident of malformation in a Phase 2 pup from the 30 mg/kg group. This pup was shorter than normal and was missing a tail. No other malformations were observed.

Phase 1

Dose Group mg/kg/day	Control 0	Low 3	Mid 30	High 100
Mean Male Fetal Weight	3.9	3.8	3.9	3.8
Mean Female Fetal Weight	3.7	3.6	3.6	3.6

Phase 2

Dose Group mg/kg/day	Control 0	Low 3	Mid 30	High 100
Mean Male Fetal Weight	3.8	3.9	3.8	3.8
Mean Female Fetal Weight	3.6	3.6	3.5	3.5

Mating Parameters

While high-dose males seemed to have slight decreases in the mating index during Phase 1, this decrease was not evident during Phase 2, indicating that there was no overall effect of the drug on mating.

Male Phase 1

Dose Group mg/kg/day	Control 0	Low 3	Mid 30	High 100
Mating Index (#mated/#treated)	100%	100%	96%	88%
Fertility Index (# preg/#mated)	96%	96%	92%	84%
Days to Mating	2.4	3.7	3.4	3

Male Phase 2

Dose Group mg/kg/day	Control 0	Low 3	Mid 30	High 100
Mating Index (#mated/#treated)	100%	100%	100%	100%
Fertility Index (# preg/#mated)	92%	96%	100%	95.8%
Days to Mating	3	3	3.1	2.6

Fertility Parameters

Phase 1

Dose Group mg/kg/day	Control 0	Low 3	Mid 30	High 100
Number Corpora Lutea-Mean/♀	16	16	16.7	17.2
Number Implantations-Mean/♀	14.7	14.8	15.2	15.9
Percent Pre-Implantation Loss	8%	8%	9%	8%
Number of Resorptions				
early	0.8	0.6	0.8	0.5
late	0	0	0	0
Percent Implants Resorbed	6%	4%	5%	3%

Dose Group mg/kg/day	Control 0	Low 3	Mid 30	High 100
Number Live Fetuses-Mean/♀	13.9	14.2	14.4	15.4
Percent Live Males	49%	56%	50%	49%
Number of Dead Fetuses	0	0	0	0
Gravid Uterus Weight (g)	13.31	17.89	10.12	10.43

Phase 2

Dose Group mg/kg/day	Control 0	Low 3	Mid 30	High 100
Number Corpora Lutea-Mean/♀	16.3	16.6	16.4	16.8
Number Implantations-Mean/♀	15.1	15.3	15.4	14.7
Percent Pre-Implantation Loss	7%	8%	7%	12%
Number of Resorptions				
early	0.8	0.8	0.5	0.9
late	0	0	0	0
Percent Implants Resorbed	5%	6%	3%	6%
Number Live Fetuses-Mean/♀	14.3	14.4	14.8	13.8
Percent Live Males	52%	52%	51%	49%
Number of Dead Fetuses	0	0	0	0
Gravid Uterus Weight (g)	9.19	15.63	9.86	17.6

Embryofetal development

Study title: GW786034B: Oral Dose-Range Embryo/Fetal Development Study in Rats

Key study findings:

- At 10 mg/kg there was increased post-implantation loss.
- At doses ≥30 mg/kg there was 100% post-implantation loss.
- Decreased food consumption was evident at doses ≥30 mg/kg; this decrease was reversed after drug dosing was stopped.
- A decrease in the percentage of male fetuses was observed at 10 mg/kg.

Study No. (b) (4) -472019
Volume #, and page #:
Conducting laboratory and location: (b) (4)

Date of study initiation: November 7,2003

GLP compliance: No
QA report: yes () no (x)
Drug, lot #, and % purity: GW786034B, 786034B-A4-02P-MIC, 91%
Though the sponsor states that the study was not audited by the Quality Assurance Unit, they did generally follow GLP standards.

Methods

Doses:	0, 3, 10, 30, and 300 mg/kg/day
Species/strain:	Rat/ Crl:CD (SD)IGS BR
Number/sex/group or time point:	6 females/group
Route, formulation, volume, and infusion rate:	Oral gavage once daily in 0.5% Hydroxypropyl methylcellulose (HPMC)/ 0.1% Tween 80; 10 mL/kg based on daily body weight
Age:	Approximately 10 weeks
Weight:	225-283 g at Day 0 pc
Study design:	F0 females dosed from Day 6-17 post coital (pc). Females euthanized on Day 21 pc

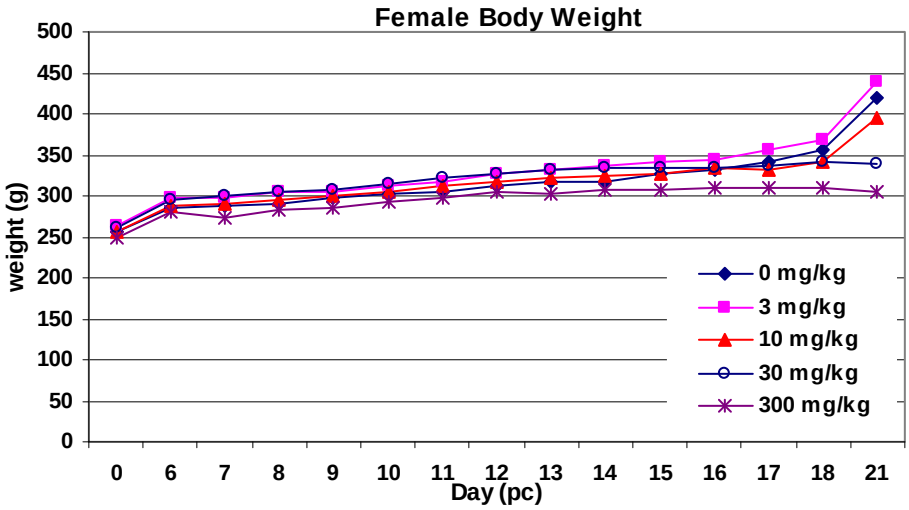
Population	Parameters and Endpoints
F0 females	Clinical observations daily, body weight, food consumption, necropsy—gross examination of uterus, cervix, corpora lutea, and uterine weight
F1 litters	Implantations, resorptions, live and dead fetuses; fetal weight, sex, placental and fetal gross morphology

Results

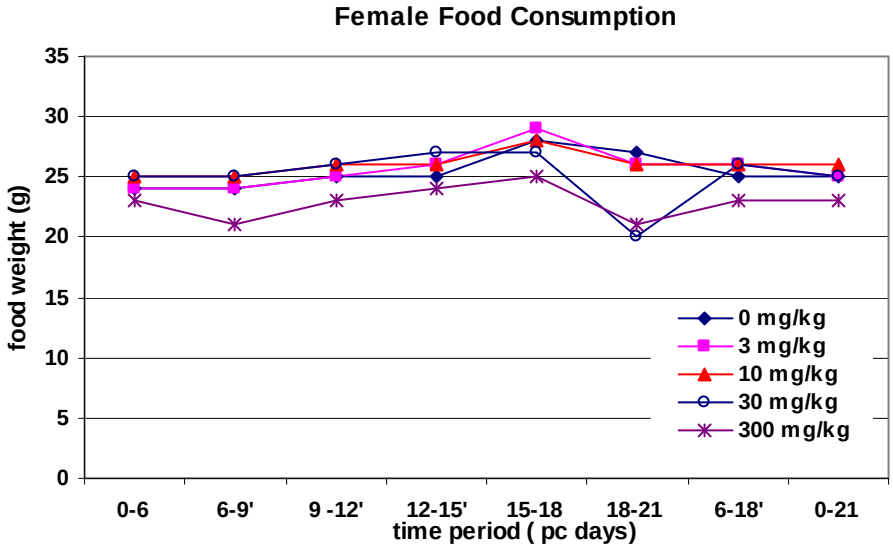
Mortality (dams): No female mortality

Clinical signs (dams): The presence of brown material around the vagina noted was noted in 2 rats from the 30 mg/kg dose group and 1 from the 300 mg/kg dose group. Other observations included hair loss and red material around the mouth and nose. These signs were observed in all dose groups including controls.

Body weight (dams): At doses ≥10 mg/kg there was a dose-dependent decrease in the amount of weight gain. By Day 21 means weight gains were 16%, 54%, and 67% lower than control for the 10, 30, and 300 mg/kg groups respectively.



Food consumption (dams):



Necropsy: No significant maternal findings.

Terminal and necroscopic evaluations:

	Dose Level (mg/kg/day)				
mg/kg/day	0	3	10	30	300
Number Corpora Lutea-Mean/♀	16.8	19	15.8	17.5	18.2
Number Implantations-Mean/♀	14.5	16.6	15	15.7	15.7
Percent Pre-Implantation Loss	11.1	11.7	5.6	9.9	11.9

Post-Implantation Loss- Mean/♀					
Early	0.3	0.6	4.8	15.7	15.7
Late	0.0	0.0	0.0	0.0	0.0
Dead	0.0	0.0	0.0	0.0	0.0
Total	0.3	0.6	4.8	15.7	15.7
Percent Post-Implantation Loss	2.3	3.4	31.2	100	100
Number Live Fetuses-Mean/♀	14.2	16	10.2	0	0
Percent Live Males	56.3	51.3	33.4	0	0
Live Birth Index (%) (live/implants)	97.7	96.6	68.8	0	0
Mean Fetal Weight (g)	5.4	5.4	5.4	0	0
Gravid Uterine Weight (g)	101.1	112.8	72.4	NA	NA

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

Study title: GW786034B: Oral Embryo-Fetal Development Study in Rats

Key study findings:

- At 10 mg/kg there was an increase in post-implantation loss leading to a decreased live birth index.
- A missing innominate was noted at a low but dose-dependent frequency.
- At 10 mg/kg retroesophogeal subclavian arteries were observed.
- There were variations in ossification, particularly in the thoracic vertebrae. Variations occurred at all dose levels and included incomplete ossification of the arch and centrum, variation in shape of the centrum, the centrum being off-center, and incomplete ossification of all parts of the skull. These findings increased in frequency with increasing dose.

Study No. G04040
Volume #, and page #:
Conducting laboratory and location: GlaxoSmithKline Safety Assessment King of Prussia, PA USA
Date of study initiation: March 11, 2004
GLP compliance: Yes
QA report: yes (x) no ()
Drug, lot #, and % purity: GW786034B, 786034B-A4-02P-MIC, 91%

Methods

Doses: 0, 1, 3, 10 mg/kg
Species/strain: Rat/ Crl:CD (SD)IGSBR
Number/sex/group or time point: 22 females/group
Route, formulation, volume, and infusion rate: Oral gavage once daily
Age: Approximately 10 wks.
Weight: 226-286 g

Study design: F0 females dosed from Day 6-17 post coital (pc). Females euthanized on Day 21 pc

Parameters and endpoints evaluated:

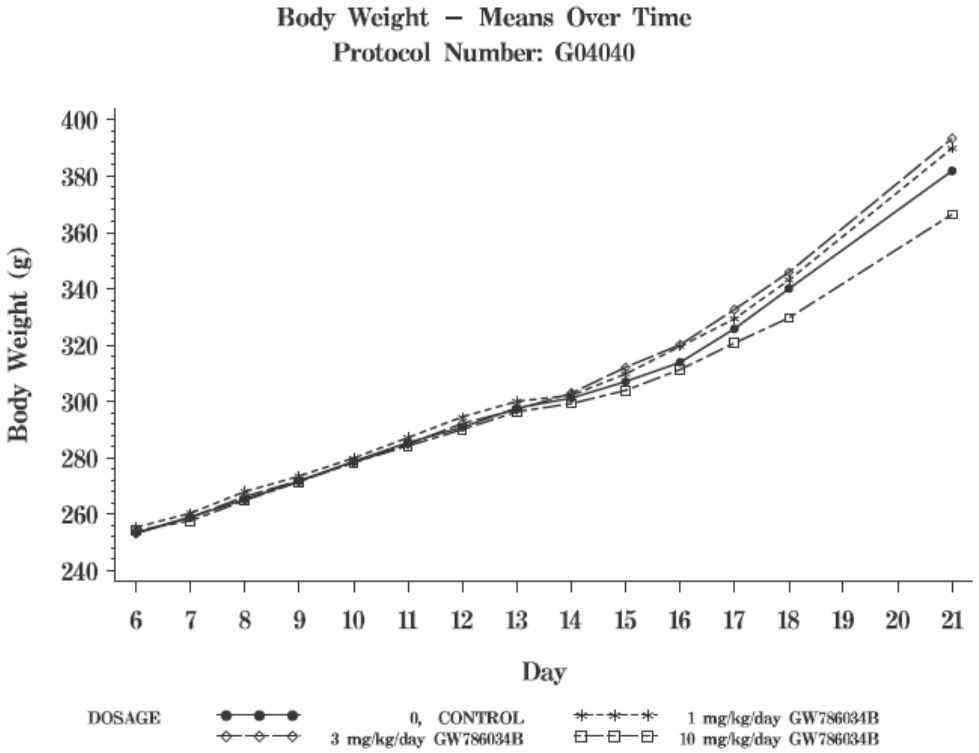
Population	Parameters and Endpoints
F0 females	Clinical observations (daily, detailed—Day 6, 7, 18, and 21 pc), body weight, food consumption, necropsy—gross examination of uterus, cervix, corpora lutea, and uterine weight
F1 litters	Implantations, resorptions, live and dead fetuses; fetal weight, sex, gross morphology, and skeletal alterations

Results

Mortality (dams): No drug-related deaths

Clinical signs (dams): Two females, one in the control group and one in the 1 mg/kg low dose group, had labored breathing. Problems were slight to moderate and transient. The sponsor believed the respiratory difficulty was related to dosing accidents.

Body weight (dams): Female body weights were similar throughout the study regardless of treatment group. The mean weight gain for 10 mg/kg group females was 13% lower between Days 6 and 18 than the weight gain for control; however the largest differences in weight gain occurred after the end of dosing.

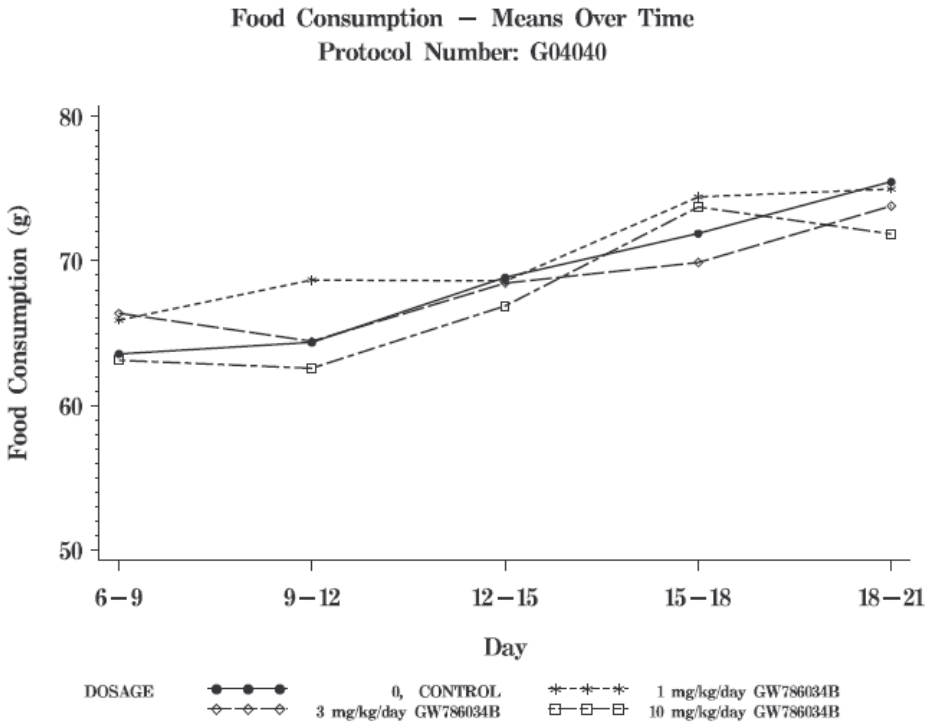


Graph excerpted from sponsor

Change in Body Weight Over Specified Period of Gestation					
Period of Gestation	Control	Low Dose 1 mg/kg	Mid Dose 3 mg/kg	High Dose 10 mg/kg	Percent Change (HD from Control)
6-9 PC	18	18	19	17	↓6%
6-12 PC	38	39	39	36	↓5%
6-15 PC	54	55	59	50	↓7%
6-18 PC	87	88	93	76	↓13%

Overall Average Total Body Weight Gain Adjusted for Uterine Weight			
Control	Low Dose 1 mg/kg	Mid Dose 3 mg/kg	High Dose 10 mg/kg
48.5g	52.7g	53.9g	56.5g

Food consumption (dams):



Graph excerpted from sponsor

Food Consumption Over Specified Period of Gestation				
Period of Gestation	Control	Low Dose 1 mg/kg	Mid Dose 3 mg/kg	High Dose 10 mg/kg
6-9 PC	64	66	66	63
9-12 PC	64	63	64	63
12-15 PC	69	63	68	67
15-18 PC	72	74	70	74

Terminal and necroscopic evaluations:

Dose Group Number of ♀(Litters if Different) mg/kg/day	Control 21 0	Low 21 1	Mid 22 3	High 22 10
Number Corpora Lutea-Mean/♀	13	13.9	14	13.9
Number Implantations-Mean/♀	12.1	13.9	13.3	13
Percent Pre-Implantation Loss	8.2	5.2	4	4.9
Post-Implantation Loss- Mean/♀				
Early	0.5	0.8	0.6	4.3
Late	0	0	0	0.1
Dead	0	0	0	0
Total	0.5	0.8	0.6	4.4
Percent Post-Implantation Loss	3.8	5.6	4.8	34.5
Number Live Fetuses-Mean/♀	11.7	12.2	12.7	8.6
Percent Live Males	43.8%	48%	49.3%	51.4%
Live Birth Index (%) (live/implants)	96.7%	87.8%	95.5%	66.2%
Mean Fetal Weight				
Female	5.36	5.26	5.32	4.96
Male	5.68	5.58	5.62	5.38
Gravid Uterine Weight	87	90	93.4	61.7

Offspring:
Treatment with GW786034 led to malformations in the great vessels that were dose dependent. At the high dose, fetuses displayed defects in the aortic arch and there were several cases of retroesophogeal subclavian arteries. In addition there were rare (though increasingly frequent) cases in which the innominate was missing. Changes in ossification, increasing in a dose dependent manner, were also frequently seen. The most obvious changes in ossification were in the high dose group litters in the thoracic vertebrae. Decreases in ossification in these animals may have contributed to changes in shape and position of the thoracic vertebrae as well.

Number of Pups with Malformations (Number of Litters if >1 incident)

Dose Group Number of ♀(Litters if Different) mg/kg/day	Control 21 (20) 0	Low 21 1	Mid 22 3	High 22 10
External Extremities—Examined	245	256	279	190
Brachyury	0	0	0	1
Great Vessels—Examined	128	132	144	101
Aortic Arch Duplicate	0	0	0	1
Aortic Arch Not Evident	0	0	0	1
Aortic Arch Right Sided	0	0	1	2 (2)
Ascending Aorta Small	0	0	0	1
Descending Aorta Origin from Pulmonary Trunk	0	0	1	0
Left Subclavian Artery Origin from Pulmonary Trunk	0	0	1	0
Rt. Subclavian Artery Origin from Lt Subclavian Artery	0	0	0	1

Dose Group	Control	Low	Mid	High
Number of ♀(Litters if Different)	21 (20)	21	22	22
mg/kg/day	0	1	3	10
Subclavian Artery Retroesophogeal	0	0	0	**4 (4)**
Truncus Communis	0	0	0	1
Innominate Not Evident	**0**	**1**	**3 (2)**	**5 (5)**
Lt Subclavian Artery Origin from Descending Aorta	0	0	0	3 (3)
Heart	128	132	144	101
Ventricular Septal Defect (Membranous)	2 (2)	0	0	2 (2)
Extra Cusp on Heart Valve	0	1	0	0
Extra Lobe	1	0	0	0
Caudal Vertebrae—Examined	117	124	135	89
Less than Expected Number Ossified	0	0	3 (1)	1
Cervical Vertebrae				
Cervical Rib	0	1	0	2 (2)
Hindpaw Phalanges				
Less than Expected Number Ossified	1	7 (4)	6 (3)	8 (4)
Lumbar Vertebrae—Examined	117	124	135	89
Arch Not Ossified	0	0	0	1
Centrum Incompletely Ossified	0	0	0	3 (3)
Centrum Off Center	0	0	0	1
Centrum Variation in Shape	0	0	0	3 (3)
Rib				
Incompletely Ossified	0	0	0	1
Knobby	1	0	0	0
Rudimentary	0	3	0	0
Sacral Vertebrae				
Arch Not Ossified	0	0	0	1
Centrum Incompletely Ossified	0	0	0	1
Centrum Off Center	0	0	0	1
Sternebrae				
Extra point of Ossification	1	0	1	0
Incompletely Ossified	1	2 (2)	1	1
Split	1	0	2 (2)	0
Variation in Shape	3 (3)	3 (2)	3 (3)	2 (2)
Thoracic Vertebrae				
Arch Incompletely Ossified	0	0	0	2 (2)
Centrum Incompletely Ossified	1	2 (1)	7 (7)	59 (21)
Centrum Not Ossified	0	0	0	3 (3)
Centrum Off Center	0	0	0	25 (15)
Centrum Variation in Shape	2 (2)	6 (3)	15 (12)	53 (21)
Skull				
Incompletely Ossified Frontal	0	0	0	1
Incompletely Ossified Hyoid	0	4 (2)	2 (1)	1
Incompletely Ossified Interparietal	1	0	1	1
Incompletely Ossified Jugal	1	3 (2)	2 (2)	3 (1)
Incompletely Ossified Parietal	0	0	2 (2)	2 (2)

Dose Group	Control	Low	Mid	High
Number of ♀(Litters if Different)	21 (20)	21	22	22
mg/kg/day	0	1	3	10
Incompletely Ossified Premaxilla	0	0	0	1
Incompletely Ossified Squamosal	0	0	0	2 (1)
Incompletely Ossified Supraoccipital	0	0	0	1

Study title: GW786034B: Oral Dose Range Study in Nonpregnant Rabbits
Key study findings:

- Doses ≥100 mg/kg/day led to 100% morbidity/mortality.
- Animals were frequently anorexic.

Study No. D03311
Volume #, and page #:
Conducting laboratory and location: GlaxoSmithKline Safety Assessment, King of Prussia, PA USA
Date of study initiation: October 24, 2003
GLP compliance: No
QA report: yes () no (x)
Drug, lot #, and % purity: GW786034B /786034B-A4-02P-MIC, 91%
Though the sponsor states that the study was not audited by the Quality Assurance Unit, they did generally follow GLP standards.

Methods

Doses: 100, 300, 1000 mg/kg
Species/strain: Hra:(NZW)SPF rabbits
Number/sex/group or time point: 4 females/group
Route, formulation, volume, and infusion rate: Oral gavage, GW786034B in0.5% HPMC/0.1% Tween, 5 mL/kg daily
Age: 7-12 weeks
Weight: 3.27-4.14 kg
Study design: Dose Range to determine toxicity and toxicokinetics.
Rabbits were dosed daily on Days 1-13.
Scheduled sacrifice on Day 14.
Toxicokinetics on Day 5

Population	Parameters and Endpoints
F0 females	Clinical signs (daily and detailed at Days 7, 8, 20, and 29), body weight (daily), and food consumption (daily), and toxicokinetics (24 hours postdose on Day 5)

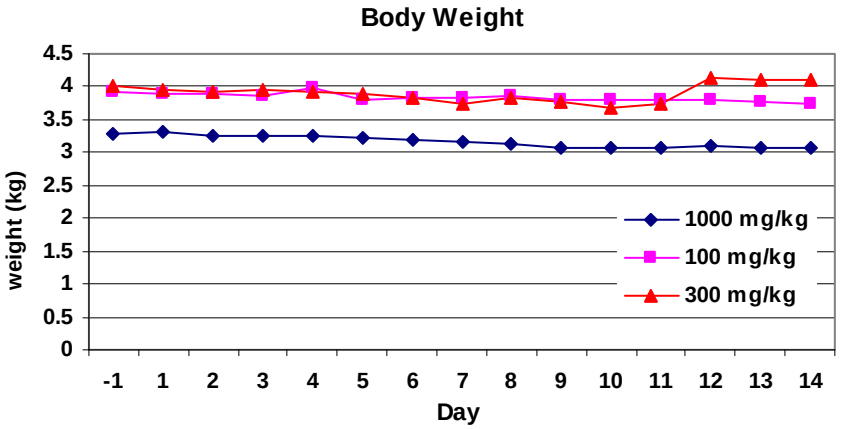
Results

Mortality (dams):

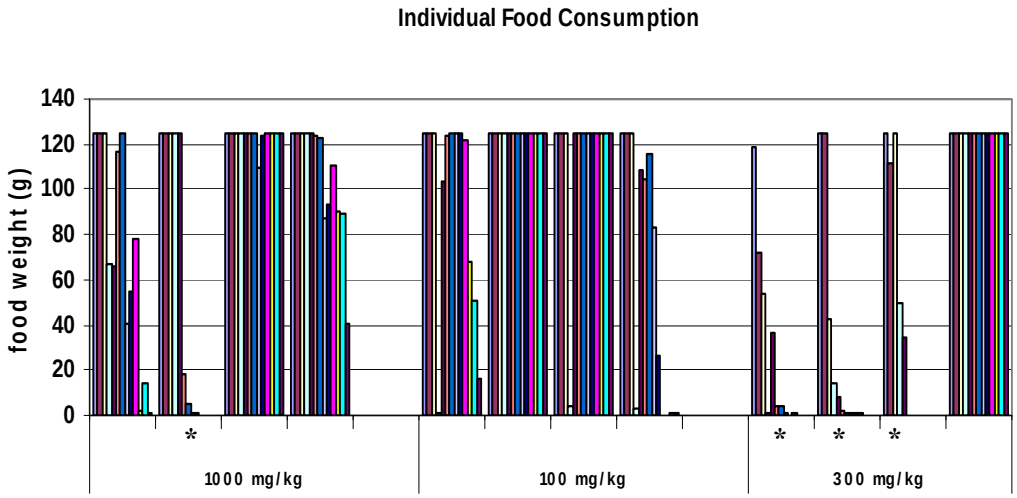
Animal #	Dose (mg/kg)	Day of Death	Observations	
			Reason for Removal	General
H03F8504	300	11	Euthanized	anorexia
H03F8505	300	11	Found Dead	anorexia
H03F8508	300	8	Found Dead	anorexia
H03F9869	1000	11	Euthanized	anorexia

Clinical signs (dams):
The most common in-life observation was a prolonged feces reduction in rabbits from all dose groups. One 1000 mg/kg rabbit also had reddish-orange urine occasionally during the 2 week study, and 1 100 mg/kg rabbit had urine staining on its right flank.

Body weight (dams): Overall changes in body weight were <10% for all dose groups over the 14 day study, but a single rabbit in both dose groups ≥ 300 mg/kg lost ≥15% of initial body weight before being euthanized. Weight loss correlated with reduced food consumption.



Food consumption (dams): Animals that were euthanized or found dead had dramatic decreases in food consumption by the Day 4-5 interval.



* Animal found dead/euthanized early

Toxicokinetics:

(Excerpted from sponsor)

Dose (mg/kg/day)	Mean C_{max} (µg/mL)	Mean $AUC_{(0-24)}$ (µg.h/mL)
100	6.14±1.24	26.1±9.47
300	10.3±3.47	142±59.0
1000	16.0±1.69	189±66.3

No offspring examined.

Study title: GW786034B: Oral Embryo-Fetal Development Dose Range Study in Rabbits

Key study findings:

- All animals in the 100 mg/kg group were euthanized early due to morbidity. No fetuses were examined.
- At 30 mg/kg there was a single mortality. Aborted fetuses were found in the cage.
- In the 30 mg/kg dose group there was a decrease in the number of litters, 2 of 6 rabbits that were found to be pregnant failed to have pups; 1 of 6 had only 2 offspring.
- Decreased fetal birth weight was observed in all treatment groups.
- There was a dose-dependent though not strictly dose-proportional increase in the maternal exposure to pazopanib.
-

Study No. D03409
Volume #, and page #:
Conducting laboratory and location: GlaxoSmithKline Safety Assessment, King of Prussia, PA USA
Date of study initiation: January 15, 2004
GLP compliance: Yes
QA report: yes () no (x)
Drug, lot #, and % purity: GW786034B, 786034B-A4-02P-MIC, 91%

Methods

Doses: 0, 3, 10, 30, 100 mg/kg/day
Species/strain: New Zealand White Rabbits/ Hra: (NZW)SPF
Number/sex/group or time point: 6 females/group
Route, formulation, volume, and infusion rate: Oral gavage once daily in 0.5% Hydroxypropyl methylcellulose (HPMC)/ 0.1% Tween 80
Satellite groups used for toxicokinetics: 3 subgroups of 3 rabbits within each treatment group
Age: Approximately 6 months
Weight: 2.94-3.54 kg
Study design: F0 females dosed from Day 7-19 post coital (pc). Females euthanized on Day 29 pc

Population	Parameters and Endpoints
F0 females	Clinical signs (daily and detailed at Days 7, 8, 20, and 29), body weight (daily), and food consumption (daily), gross necropsy, and toxicokinetics
F1 litters	Implantations, resorptions, live and dead fetuses; fetal weight, sex, gross morphology, and skeletal alterations

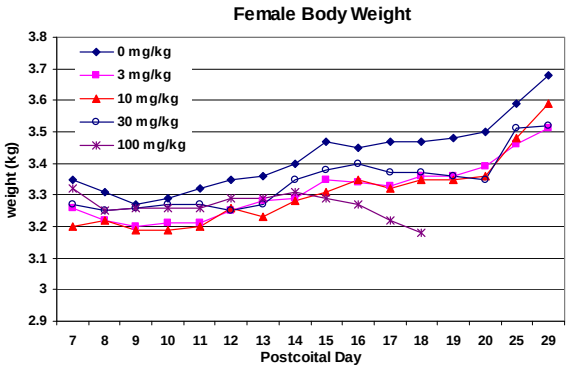
Results

Mortality (dams):

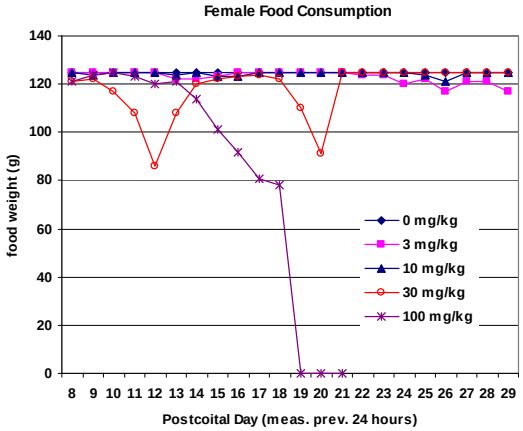
Animal #	Dose (mg/kg)	Day of Death	Observations		
			Reason for Removal	Cause of Mortality	General
H04F7443	30	20pc	Euthanized		Aborted fetuses found
H04F7450	100	18pc	Euthanized	Morbidity	
H04F7454	100	18pc	Euthanized	Morbidity	
H04F7462	100	18pc	Euthanized	Morbidity	
H04F7463	100	18pc	Euthanized	Morbidity	
H04F7467	100	18pc	Euthanized	Morbidity	
H04F7468	100	18pc	Euthanized	Morbidity	

Clinical signs (dams): There was an increase in the incidence of soft feces seen in rabbits at doses ≥ 30 mg/kg.

Body weight (dams): There was a decrease in weight gain for the high dose group rabbits that preceeded euthanization of the group at Day 18. There was no overall difference in the amount of weight gained between control and other test dose groups.



Food consumption (dams): There were significant changes in female food consumption at doses ≥ 30 mg/kg.



Toxicokinetics:
There was a dose-dependent though not dose-proportional increase in the maternal exposure to pazoponib.

(Excerpted from Sponsor)

Parameter	Dose Level (mg/kg/day)				
	0	3	10	30	100
Female					
AUC_{0-t} (ng.h/mL)	N/A	NC	1722.6	7561.3	24711.2
Cmax (ng/mL)	N/A	129.8	1063.4	2253.0	6744.9
Tmax (h)	N/A	0.96	0.99	1.00	2.01

N/A = Not applicable. NC = Not calculated.

Dose Level (mg/kg/day) Comparison	Ratio	Cmax Ratio	AUC Ratio	Dose Level (mg/kg/day) Comparison	Ratio	Cmax Ratio	AUC Ratio
3/3	1	1	NC	NC	NC	NC	NC
10/3	3.333	8.19	NC	10/10	1	1	1
30/3	10	17.36	NC	30/10	3	2.11	4.39
100/3	33.333	51.96	NC	100/10	10	6.34	14.34

Terminal and necroscopic evaluations:C-section data:
Decreases in fetal weight ≥10% compared to control were seen in all treatment groups. In addition, there was an increase in the number of early resorptions at doses ≥10 mg/kg. Though not obvious from the mean data, at 30 mg/kg there was a decrease in the overall number of litters. As noted above, one dam in this dose group was euthanized early after aborted fetuses were found in the cage. In addition, one dam had 100% early resorption at this dose level.

Number of ♀ (Litters if Different)	6	6	6	5 (4)	0
Dose Level mg/kg/day	0	3	10	30	100
Number Corpora Lutea-Mean/♀	9.5	9.2	10.4	9.2	NC
Number Implantations-Mean/♀	6.8	7.7	9.8	9.0	NC
Percent Pre-Implantation Loss	26.7	16.7	5.2	1.8	NC
Post-Implantation Loss					
Early	0.5	0.0	1.2	3.0	
Late	0.2	0.0	0.2	0.2	
Dead	0.0	0.0	0.0	0.0	
Total	0.7	0.0	1.4	3.2	NC
Percent Post-Implantation Loss	8.6	0.0	13.5	45.3	NC
Number Live Fetuses-Mean/♀	6.2	7.7	8.4	7.3	NC
Live Birth Index (%) (live/implants)	65.3%	83.7%	80.8%	79.3%	NC
Mean Fetal Weight (g)	45.9	41.07	37.89	39.01	NC
Gravid Uterine Weight (g)	398	416.2	462.4	390	NC

NC= Not calculated. The 100 mg/kg dose group was euthanized at Day 18 due to morbidity. This group was discontinued from further evaluation.

Offspring (malformations, variations, etc.): There were only 2 recorded malformations. At 30mg/kg/day there was 1 incidence of microtia. At 3 mg/kg/day there was a single incidence of gastroschisis.

	Dose Level (mg/kg/day)			
mg/kg/day	0	3	10	30
Number of pups with any alterations (%)	0 (0)	1 (2.08)	0 (0)	1 (3.13)
Number of litters with at least 1 pup w/alterations (%)	0 (0)	1 (17%)	0 (0)	1 (25%)
Percent of fetuses w/any alterations/litter	0	2.2	0	3.4

The reproductive toxicology program included male and female fertility studies in the Sprague-Dawley rat and embryo-fetal development studies in both the rat and the rabbit. In the male fertility study animals were dosed for 105 days and were mated to untreated females at both early and late timepoints. While overall male fertility was not affected by pazopanib in either phase, there were changes noted in sperm production, concentration and motility. These changes were dose dependent. Lower sperm concentration and production were noted even at the lowest dose of the drug while decreased motility was only seen at the highest dose (600 mg/m^2). In addition, at doses ≥ 180 mg/m^2 animals exhibited decreased organ weights for the testis, epididymis, and seminal vesicles; observations of small, soft testis and epididymis were also seen beginning at this dose. In females dosed daily for 2 weeks prior to mating until Day 6 post-coital (pc) there were changes in fertility. At the highest dose examined (1800 mg/m^2) there were decreases in body weight after Day 14 pc and in food consumption by Day 7 pc. These dams had a fertility index of only 32% and females who became pregnant were unable to maintain the pregnancy: 100% of the implants in this dose group were resorbed. At 180 mg/m^2 females had an increased percentage of resorbed implants as well, 25.6%, approximately 5 times higher than control. Male and female pups from these dams also had lower fetal body weights, (16% and 13%, respectively) and an increase in the number of malformations. There were single cases of agnathia, micrognathia with a dome-shaped head, and a cleft palate/cleft lip. Each case was from a separate litter.

Two embryo-fetal development studies were completed for rats. Pregnant females were dosed from Day 7-16 pc. In the first, a dose range study with only 6 females/dose group, the sponsor reported a 100% post-implantation loss at doses ≥ 180 mg/m^2. There was also an increase in post-implantation loss compared to control at 60 mg/m^2. A decrease in the percentage of live males produced at 60 mg/m^2 was also noted in this study, but the second, more comprehensive embryo-fetal development study in rats did not support this finding and the conclusion was that the drug did not affect the male/female ratio. The second study in rats also confirmed that at 60 mg/m^2 there was an increase in post-implantation loss. At the same dose the average fetal weight was decreased by approximately 6.3%. Incomplete ossification was a frequently seen event, particularly in the thoracic vertebrae. Alterations in the thoracic vertebrae increased in a dose-dependent manner. The sponsor also reported malformations of the great vessels in this study. Increasing numbers of animals had a missing innominate at doses ≥ 6 mg/m^2. At the 60 mg/m^2 dose level 41% of litters had at least 1 animal with 1 or more great-vessel malformations (increased from 14% of litters at the 18 mg/m^2 level). Four of the 60 mg/m^2 animals had retroesophogeal subclavian arteries.

An oral dose range study performed in nonpregnant rabbits showed that doses ≥ 1180 mg/m^2 led to maternal moribidity with frequent anorexia. Mortalities occurred at doses ≥ 3540 mg/m^2. In the pivotal rabbit study females were dosed daily (0, 35.4, 118, 354, and 1180 mg/m^2) from Days 7-19 pc and euthanized on Day 29. All animals in the 1180 mg/m^2 (high dose) group were euthanized early due to morbidity. No fetuses were examined from this group. Decreased fetal birth weight was observed at doses ≥ 35.4 mg/m^2 (all treatment groups). At 354 mg/m^2 there was a single mortality, the mother was euthanized early after aborted fetuses were found in the cage. A second rabbit in this group had 100% pre-implantation loss. A third rabbit from this group produced only 2 offspring. Few malformations were found in this study. At 35.4 mg/m^2 there was a single incidence of gastroschisis and at 354 mg/m^2 there was a single incidence of

microtia. Finally, the sponsor included a toxicokinetic assessment of the female rabbits. At the dose of 118 mg/m^2 the AUC was 1722.6 ng*hr/mL or 1.723 ug*hr/mL with a Cmax of 1063.4 ng/mL or 1.06 ug/mL. This dose in rabbits showed a slight increase in the percentage of post-implantation loss compared to control. At the more frankly toxic dose of 354 mg/m^2 the AUC and Cmax were 7.561 ug*hr/mL and 2.253 ug/mL respectively. Included below is a table excerpted from the sponsor including the exposure of patients given a daily dose of pazopanib at the proposed treatment dose. The AUC and Cmax of pazopanib in these patients is significantly greater than the reported exposure in rabbits (138-fold increase in AUC and 26-fold increase in Cmax) indicating that pazopanib given to patients at a clinically relevant dose is likely to significantly and negatively impact pregnancy.

Summary of Derived Pharmacokinetic Parameters for Pazopanib and Metabolites After a Single Dose in Study VEG10005 and After 16 Days of 800 mg Pazopanib Administered Once Daily in Study VEG10007

Analyte	**AUC(0-t)**		**Cmax**	
	(μg*h/mL)1		**(μg/mL)1**	
	Single Dose	Repeated Dose**,2**	Single Dose	Repeated Dose
	(VEG10005)	(VEG10007)	(VEG10005)	(VEG10007)
Pazopanib	669 (526.3, 851.4)	1,040 (879, 1,230)	20.4 (16.0, 25.9)	58.1 (49.5, 68.3)

*Exceprted from sponsor

2.6.6.6 Carcinogenicity: No studies conducted

2.6.6.7 Local tolerance - No studies reviewed

2.6.6.8 Special toxicology studies

(b) (4) which has a structural alert for genotoxicity, is a starting material of the drug substance (pazopanib monohydrochloride).

(b) (4) was mutagenic in the Ames assay and positive in a mouse lymphoma assay [Study No. CD2005/01104/00 and CD2005/01105/00] but was negative in the mouse micronucleus assay [Study No. CD2006/00012/00].

In study CD2005/01104/00 ((b) (4) Reverse Mutation Assay "Ames Test" using *Salmonella Typhimurium* Single Experiment) *Salmonella typhimurium* strain's TA1535, TA1537, TA102, TA98, and TA100 were treated with impurity, (b) (4) using the Ames plate incorporation at five dose levels in triplicate in the presence and absence of S-9. The doses used in this study were 50, 150, 500, 1500, and 5000 ug/plate. Doses were determined from preliminary toxicity assays. Results of this study showed that (b) (4) caused statistically significant and dose-related increases in revertant colony frequency of all tester strains tested in the presence of S9 only. The increases were observed at all dose levels tested and was in excess of three-fold compared to the controls. The greatest increases occurred at concentrations of 50 and 150 ug/ml. Salmonella strain TA102 showed an increase in revertant colony frequency at concentrations as high as 5000 ug/plate. Therefore based on these observations, (b) (4) was mutagenic in the Ames assay.

In study CD2005/01105/00 ((b) (4) Screening L5178Y TK+/- Mutation Assay), L5178Y TK+/-3.7.2c mouse lymphoma cells (heterozygous at the tymidine kinase locus) were treated with impurity, (b) (4) at doses 123.56, 247.13, 494.25, 988.5, 1482.75, and 1977 ug/mL for 4 hours with and without metabolic activation. No precipitate was observed at any dose levels. Results of this study showed that (b) (4) caused statistically significant and dose-related increase in mutant frequency in both the presence and absence of metabolic activation. The increase in mutant frequency was partly attributed to small colony formation suggesting clastogenic activity resulting in structural chromosome changes. Therefore based on these observations, (b) (4) was considered mutagenic to L5178Y cells.

In nonclinical and clinical batches during development the level of (b) (4) (b) The limit for (b) (4) in (b) (4). Purging studies were conducted to determine the retention of the (b) (4) in the final drug substance. When levels of (b) (4) were artificially spiked to (b) (4) in the intermediate, the level of (b) (4) detected in the drug substance was determined to be (b) (4) Extrapolation from the purging study indicated that when levels of (b) (4) are (b) (4) in the intermediate, carryover of the starting material into the to-be-marketed drug substance would be less than (b) (4) Although (b) (4) is positive in the Ames Assay and mouse lymphoma assay, the maximum level of (b) (4) is significantly lower (b) (4) than the acceptable daily intake of genotoxic impurities defined in the draft Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches for products administered for ≥12 months (1.5 µg/day).

2.6.6.9 Discussion and Conclusions

The general toxicology program has adequately addressed the safety of pazopanib with appropriate animal models and dosing ranges and regimens. In 26-week rat study, microscopic treatment-related findings observed in this study could be categorized into VEGFR-2 mediated (mechanistic/pharmacologic) effects and indirect or secondary effects due to changes in dependent tissues. Treatment-related changes in the femur, sternum, bone marrow and ovary are due to the pharmacologic effects of pazopanib as VEGF and its receptors are required for normal physiological angiogenesis and are involved in endochondral bone formation, hematopoiesis, and angiogenesis in the ovary. Testicular effects are also interpreted to be pharmacologic as VEGF and/or its receptors are expressed in a variety of testicular elements. In contrast to molars, growth of incisors in rodents is continuous and rapid, and the odontogenic changes observed in the incisors resulted from decreased/impaired angiogenesis, a pharmacological effect of pazopanib. Decreased numbers of globule leukocytes in the trachea is due to pharmacologic effect of pazopanib as these cells express VEGF and are involved in the permeability of nearby microvessels.

Treatment-related changes in the adrenal glands (cortex) are adverse, and a dose-response was evident. VEGF receptors (R1 and R2) are expressed in the endothelial cells of the adrenal cortex and proliferation of vascular endothelial cells is a requirement for adrenal cortex development and differentiation supporting a mechanistic basis for the

development of these lesions. In addition, there were treatment-related changes in the kidney. Chronic progressive nephropathy is a spontaneous age-related lesion of rats, however the incidence of lesions was higher than concurrent controls, and renal changes were accompanied by concurrent treatment-related changes in urinalysis and serum chemistry.

The mechanism of treatment-related findings in the gastrointestinal system (duodenum, jejunum and mesenteric lymph node) and pancreas are unknown although VEGF and/or its receptors are expressed in a number of these tissues. Microscopic findings in the epididymis and probably the pituitary were secondary to changes in the dependent tissues (i.e. ovary, testes, and adrenal gland). The toxicological significance of the crystalline material observed in the duodenum, jejunum, and mesenteric lymph node may be considered minimal based on the nature of the finding.

In the battery of genotoxicity studies, pazopanib was not mutagenic or clastogenic *in vitro*. Pazopanib was not clastogenic (induction of micronuclei) in the *in vivo* rat micronucleus study.

Pazopanib did not affect mating or fertility in male rats. In females, however, fertility was effected as evidenced by increased resorptions, reduced number of corpora lutea and increased pre and post-implantation loss. In embryofetal development studies toxicity (resorptions, abortions, decreased fetal body weights) in rats and rabbits occurred at doses below those that caused maternal toxicity. Therefore, administration of pazopanib during pregnancy likely poses a significant risk for fetal toxicity.

2.6.6.10 Tables and Figures

See text of review for pertinent tables and figures

2.6.6 TOXICOLOGY TABULATED SUMMARY

Acute Dose Toxicity Studies						
Study No.	**Species**	**Route/ duration**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2**	**Significant findings**
RD2006/00221/00	Rat	IV	3	1.1 5.4	6.6 32.4	**6.6 mg/m^2**: no toxicity **32.4 mg/m^2**: no toxicity
RD2001/01637/00	Dog	Oral	2	150 450	3000 9000	**3000 mg/m^2**: no toxicity **9000 mg/m^2**: no toxicity

Repeat Dose Toxicity Studies						
Study No.	**Species**	**Route/ duration**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2**	**Significant findings**
WD2005/00481/00	Mouse	Oral Daily x 13 Week No recovery	12	1000 300 100	3000 900 300	**3000 mg/m^2/day**: broken and overgrown teeth (white also) and nails, longer fore and hind paw, and colored feces. ↓ BW and FC (F). ↑ WBC, NEUT, LYMPH, ESO, MONO, ALT (M) and AST (M). Kidney basophilic tubules and inflammation (F), liver reduced hepatocytes, centrilobular aggregates of granular macrophages (M), hepatocyte hypertrophy, eosinophilic foci (F) and hepatocellular adenoma (F), digits distortion and inflammation (M), teeth irregular/thickened predentine, thinning of dentine and irregular ameloblast layer, femur hemosiderosis and growth plate partial fusion (F), small intestine crystalline material (F). **900 mg/m^2/day**: broken and overgrown teeth (white also) and nails, longer fore and hind paw. ↓ BW and FC (F). ↑ WBC, NEUT, LYMPH, ESO, MONO, ALT (M) and AST (M). Liver reduced hepatocytes, centrilobular aggregates of granular macrophages (M), digits distortion and inflammation (M), teeth irregular/thickened predentine, thinning of dentine and irregular ameloblast layer, femur hemosiderosis. **300 mg/m^2/day:** broken and overgrown nails, longer fore and hind paw (M). ↑ WBC, NEUT, LYMPH, MONO. Liver reduced hepatocytes, teeth irregular/thickened predentine, thinning of dentine and irregular ameloblast layer, femur hemosiderosis.
RD2002/00721/01	Rat	Oral Daily x 28 days with recovery (2, 4, 6, and 10 weeks)	5 (M only)	300 30 10	1800 180 60	**1800 mg/m^2/day**: Teeth broken/missing teeth and striations, gums red, swollen, and malocclusion, short nails at Week 5. ↓ BW gain on Days 29-43. ↓ FC on Days 29-40, 57-64, and 92-98. Macroscopic findings in teeth (incisors elongated and pale discoloration) and paw (hindlimb/forelimb nail short). Microscopic findings in teeth (periodontal edema, dentine degeneration, dentine thinning, focal amelobastic/odontoblastic atrophy, mulifocal enamel degeneration, and multifocal/focal dental pulp necrosis). All findings recoverable by week 10.

Repeat Dose Toxicity Studies						
Study No.	**Species**	**Route/ duration**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2**	**Significant findings**
						180 mg/m^2/day: Macroscopic findings in teeth (incisors elongated and pale discoloration) and paw (hindlimb/forelimb nail short). Microscopic findings in teeth (periodontal edema, dentine degeneration, dentine thinning, focal amelobastic/odontoblastic atrophy, mulifocal enamel degeneration, and multifocal/focal dental pulp necrosis). Recoverable by week 6. **60 mg/m^2/day**: Macroscopic findings in teeth (incisors elongated and pale discoloration) and paw (hindlimb/forelimb nail short). Recoverable by week 6.
VD2006/00544/00	Rat	Oral Daily x 13 Week No recovery	12 (M only)	300 30 3	1800 180 18	**1800 mg/m^2/day**: 1 death (TK animal) due to ↓ BW and FC. Overgrown, loose, broken, lost, pale and/or mottled incisor teeth, regrowth of incisor teeth, and long, missing lose, and/or broken nails. Microscopic findings in mandibular and maxillary teeth, femur, sternum, kidneys, pituitary, adrenals, duodenum, trachea, larynx, testes, and epididymides. **180 mg/m^2/day**: Overgrown, loose, broken, lost, pale and/or mottled incisor teeth, regrowth of incisor teeth, and long, missing lose, and/or broken nails. Microscopic findings in mandibular and maxillary teeth, femur, sternum, kidneys, pituitary and adrenals. **18 mg/m^2/day**: no toxicity
RD2002/01337/01	Rat	Oral Daily x 26 Weeks	12	300 30 3	1800 180 18	**1800 mg/m^2/day**: mortality (9 M and 9 F), malocclusion, thin appearance, long/loose/broken/missing teeth, missing nails, few/non-formed liquid feces and rough/yellow haircoat. ↓ BW and FC. ↓ RBC parameters and PLT, ↑ MCV and MCH, ↑ WBC parameters, ↓ A/G ratio, ↑ CHOL, TRIGLY, TBILI, ALT, and PHOS. ↓ urine volume and protein/creat. excreation. ↑ adrenals (F), ↓ epididymis and testes wts. adrenal hemorrhage, femur hypertrophy, bone hypocellularity, ovary atropy, pancreas atropy, mandible teeth decreased thickness/edema/necrosis/degeneration, maxilla teeth fracture/inflammation/decrease thickness/degeneration/edema/necrosis, testes atrophy, trachea decreased leukocytes, kidney nephropathy, and pituitary hypertrophy. **180 mg/m^2/day**: mortality (1 F), thin appearance, long/loose/broken/missing teeth, few/non-formed feces and rough/yellow hair coat (M). ↓ BW and FC (M). ↓ RBC parameters and PLT, ↑ MCV and MCH, ↑ WBC parameters (F), ↑ GLOB, CHOL, TRIGLY, TBILI, and PHOS. ↓ A/G ratio. ↓ protein/creat. excreation and ratio. ↓ testes wts. Adrenal angiectasis/hemorrhage (F), femur hypocellularity (M), sternum hypocellularity (M), kidney nephropathy (M), mandible teeth degeneration/attenuation/atrophy, maxilla teeth degeneration, testes atrophy, and

Repeat Dose Toxicity Studies						
Study No.	**Species**	**Route/ duration**	**N/sex/ dose**	**mg/kg/ day**	**mg/m^2**	**Significant findings**
						trachea decreased globule leukocytes. **18 mg/m^2/day**: long/broken teeth, ↑ GLOB. ↓ protein/creat. excreation and ratio. Adrenals angiectasis/hemorrhage (F), kidney nephrophathy, trachea decreased leukocytes.
RD2002/01338/02	Monkey	Oral Daily x 52 Weeks	4	500 50 5	6000 600 60	**6000 mg/m2/day**: mortality 1 M at Week 24 and early sacrifice 1 M and 1 F at Week 34. Colored feces, few/liquid/mucoid/non-formed feces. ↓ BW and FC (M only). ↓ ALB and TP (F). Duodenum, Jejunum, and mesenteric lymph node crystalline pigment (M). Recovery animals - ovary active/mature corpora lutea, uterus increased ratio endometrial stroma/glands, kidney lymphohistiocytic infiltrate, and salivary gland lymphohistiocytic infiltrate. **600 mg/m2/day**: Colored feces and few/liquid/mucoid/non-formed feces. Ovary active/mature corpora lutea and uterus increased ratio endometrial stroma/glands. **60 mg/m2/day**: Tan feces and few/liquid/mucoid/non-formed feces. Ovary active/mature corpora lutea and uterus increased ratio endometrial stroma/glands.

Note: In toxicology studies, findings occurred in both males and females, unless otherwise stated.

Genetic Toxicology Studies		
Study/Study No.	**Concentration/ Doses**	**Results**
Definitive bacterial mutagenesis (Ames)/ RD2002/00887/00	33.3, 100, 333, 1000, 3330, and 5000 µg/mL in initial and confirmatory assays	Negative in the presence and absence of S9
Human peripheral Lymphocytes/ RD2002/00238/00	5-200 µg/mL in initial and 10-100 µg/mL in confirmatory assay	Negative in the presence and absence of S9
In vivo rat micronucleus/ RD2002/00227/00	0, 1250, and 2000 mg/kg/day	Negative

Reproductive Toxicology Studies								
Phase	Study #, Title, Conducting Lab	Methods	Key Findings	Species	Doses	Mortality and Clinical Signs	Body Weight/ Food Consumption	Necropsy
Fertility and Early Embryonic Development	G03407 GW786034B: Oral Female Fertility and Early Embryonic Development Study in Rats GlaxoSmithKline	Daily oral dosing of females from 2 weeks prior to cohabitation until D6 postcoital	*NOAEL: 3 mg/kg/day *Decreased weight gain in 300 mg/kg high dose females after Day 14 due to complete resorption *Increased resorption in 30 mg/kg * 100% resorption of implants at 300 mg/kg *Decreased male and female fetal weight in 30 mg/kg dose group *Gross fetal malformation in 3 litters from 30 mg/kg dose group	Rat, SD	0, 3, 30, 300 mg/kg (0, 18, 180, 1800 mg/m2)	Obtained Daily 1 mortality at 3 mg/kg; not drug-related No clinical signs	300 mg/kg: ↑ in body weight and food consumption followed by ↓after D14 due to 100% resorption at this dose	↓ in fertility at ≥ 30 mg/kg; ↑ in pre and post-implantation loss at ≥ 30 mg/kg Malformations of the head and jaw seen in litters from the 30 mg/kg dose group; 13-16% ↓ in fetal weight at 30 mg/kg
	G03372 GW786034B: Oral Male Fertility Study in Rats (b) (4)	Daily oral dosing of males from Day 1 to Day 105. Mated with Phase 1 females on Day 10, Phase 2 females on Day 63-65 for up to 10 days	*NOAEL: 3 mg/kg (?) * dose-dependent ↓ in sperm concentration, motility, reproductive organ weights at ≥30 mg/kg. *↓ body weight at late stages of the study period at ≥30 mg/kg *dose-dependent ↑ in dental problems ≥ 30 mg/kg. * no changes in female clinical signs, body weight, or food consumption *No changes in pregnancy parameters or F1 litters	Rat, SD	0, 3, 30, 100 mg/kg (0,18, 180, 600 mg/m2)	Obtained Daily 1 male mortality at 100 mg/kg. Euthanized on D37 after 3 continuous days of impaired use of hindlimbs. White spots on liver at necropsy **Males**: ↑ Dental problems ≥ 30 mg/kg. ↑ Hair loss at 100 mg/kg **Females**: No signs or mortalities	↓ Weight gain in males at 100 mg/kg after 8 wks resulting with a smaller ↓ for males at 30 mg/kg; corresponding ↓ in food consumption	Males: ↓ reproductive organ size and weight at ≥ 30 mg/kg. ↓sperm production and motility ≥ 30 mg/kg; no obvious differences in fertility. Females: No significant findings **F1:** No significant findings

Reproductive Toxicology Studies								
Phase	Study #, Title, Conducting Lab	Methods	Key Findings	Species	Doses	Mortality and Clinical Signs	Body Weight/ Food Consumption	Necropsy
Embryofetal Development	D03349 GW786034B: Oral Dose-Range Embryo/Fetal Development Study in Rats (b) (4) .	Daily oral dosing of females from Day 6-17 postcoital (pc) Scheduled sacrifice Day 21	*NOAEL: 3 mg/kg *At 10 mg/kg there was increased post-implantation loss. *Doses ≥30 mg/kg led to 100% post-implantation loss. *reversible ↓ food consumption at doses ≥30 mg/kg *↓ in the percentage of male fetuses was observed at 10 mg/kg.	Rat, SD	0, 3, 10, 30, 300 mg/kg (0, 18, 60, 180, 1800 mg/m2	Obtained Daily No mortalities Brown material around the vagina in 2 rats from the 30 mg/kg group and 1 from the 300 mg/kg group	Dose dependent ↓ in body weight gain at doses ≥ 10 mg/kg (↓ 16%, 54%, 67% for 10, 30, 300 mg/kg) Slight ↓ in food consumption for females at 300 mg/kg throughout the study, ↓ at 30mg/kg Days 15-21	100% post-implantation loss at ≥ 30 mg/kg. ≥ 10X increase in post-implantation loss at 10 mg/kg Females: No significant maternal findings. **F1:** No findings noted
	G04040 GW786034B: Oral Embryo-Fetal Development Study in Rats GlaxoSmithKline	Daily oral dosing of females from Day 6-17 pc Scheduled sacrifice Day 21	*NOAEL: 1 mg/kg (?) *At 10 mg/kg there was an increase in post-implantation loss *A missing innominate was noted at a low but dose-dependent frequency. *At 10 mg/kg retroesophogeal subclavian arteries were observed. *There were dose-dependent variations in ossification, particularly in the thoracic vertebrae. Variations occurred at all dose levels and included incomplete ossification of the arch and centrum, variation in shape of the centrum, the centrum being off-center, and incomplete ossification of all parts of the skull.	Rat, SD	0, 1, 3, 10 mg/kg (0, 6, 18, 60 mg/m2)	Obtained Daily No mortatlities No drug-related clinical signs	↓ weight gain for 10 mg/kg dose group (13%) D6-18; no ↓ when adjusted for uterine weight No significant differences in food consumption	↑ in post-implantation loss at 10 mg/kg Females: No significant maternal findings **F1:** 1 mg/kg: 1 case of missing inominate; slight (below significance) ↑ in incomplete ossification/ variation in shape of skeleton

Reproductive Toxicology Studies								
Phase	Study #, Title, Conducting Lab	Methods	Key Findings	Species	Doses	Mortality and Clinical Signs	Body Weight/ Food Consumption	Necropsy
Embryofetal Development	D03311 GW786034B: Oral Dose Range Study in Nonpregnant Rabbits GlaxoSmithKline	Daily oral dosing Day 1-13; Scheduled sacrifice Day 14	*NOAEL: not determined *Doses ≥100 mg/kg/day led to 100% morbidity/mortality. *Animals were frequently anorexic.	Rabbit, NZW	100, 300, 1000 mg/kg (1180, 3540, 11800 mg/m2)	Obtained Daily 300 mg/kg: 2 animals found dead, 1 euthanized-anorexia 1000 mg/kg: 1 animal euthanized-anorexia	Mean weight loss of < 10% for surviving animals at all doses. Single animals at ≥ 300 mg/kg lost ≥ 15% of initial body weight before euthanization Frequent dramatic ↓ in food consumption by Day 4-5	Not done
Embryofetal Development	D03409 GW786034B: Oral Embryo-Fetal Development Study in Rabbits GlaxoSmithKline	Daily oral dosing Day 7-19 pc Scheduled sacrifice Day 29	*NOAEL: not determined * 100 mg/kg group euthanized early due to morbidity. *1 mortality at 30 mg/kg. Aborted fetuses were found in the cage. *At 30 mg/kg dose group there was a decrease in the number of litters, 2 of 6 rabbits that were found to be pregnant failed to have pups; 1 of 6 had only 2 offspring. *Decreased fetal birth weight was observed in all treatment groups. *There was a dose-dependent though not strictly dose-proportional increase in the maternal exposure to pazopanib.	Rabbit, NZW	0, 3, 10, 30, 100 mg/kg (0, 35.4, 118, 354, 1180 mg/m2)	Obtained Daily 30 mg/kg: 1 animal euthanized early due to morbidity. Aborted fetuses found in cage 100 mg/kg: Whole group euthanized early due to morbidity	100 mg/kg: ↓ weight gain and food consumption after Day 14. 30 mg/kg: changes in food consumption, not consistent	↑ in post-implantation loss at ≥10 mg/kg; ↓ (≥10%) in fetal weight at all doses. Females: No significant findings noted. F1: 3mg/kg: one incident of gastroschisis 30 mg/kg; one incidence of microtia

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The non-clinical program of pazopanib identified the target areas of toxicity to be the, teeth, bone marrow, gastrointestinal and reproductive system. Dental findings were a significant cause of mortality in rodents. Pazopanib was not genotoxic in either *in vitro* or *in vivo* assays. There was a significant amount of embryo-fetal toxicity in rats and rabbits at doses below those causing maternal toxicity. Administration of the drug, therefore, poses a likely risk for fetal toxicity in humans.

Unresolved toxicology issues (if any): None

Recommendations: None

Suggested labeling: Presented in a separate labeling review

ATTACHMENT

Review of IND 65747 by Lilliam Rosario, Ph.D (10/3/2002)

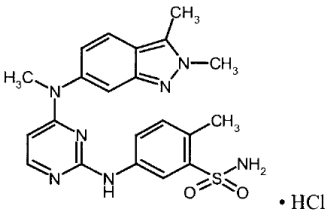
PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number:	IND 65,747
Review number:	1
Sequence number/date/type of submission:	000/09Sept2002/IND
Information to sponsor:	Yes (x) No ( )
Sponsor and/or agent:	SmithKline Beecham Corporation d/b/a GlaxoSmithKline One Franklin Plaza, P.O. Box 7929, Philadelphia PA 19101
Manufacturer for drug substance :	SmithKline Beecham Corp d/b/a GlaxoSmith Kline Research and Development 801 River Road Conshohocken, PA 19428
Reviewer name:	Lilliam A. Rosario, Ph.D.
Division name:	Oncology Drug Products
HFD #:	HFD-150
Review completion date:	10/3/02

Drug:

Trade name:	n/a
Generic name (list alphabetically):	-
Code name:	GW786034
Chemical name:	5-[[4-[(2,3-Dimethyl-2H-Indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methylbenzenesulfonamidemonohydrochloride
CAS registry number:	Not provided
Mole file number:	None
Molecular formula/molecular weight:	$C_{21}H_{23}N_7O_2S \bullet HCl$

Structure:



Relevant INDs/NDAs/DMFs: None

Drug class: VEGFR Tyrosine Kinase Inhibitor

Indication: Treatment of Cancer

Clinical formulation:

Table 11 Composition of GW786034B Tablets (b) (4)

Component	Quantity mg/tablet	Function	Reference to Standard
	(b) (4)		
GW786034B			
(b) (4)			
		(b) (4)	NC[2]
			NF or PhEur
			NF or PhEur
			USP or PhEur
			USP or PhEur
Microcrystalline cellulose			NF or PhEur
Sodium starch glycolate			NF or PhEur
Magnesium stearate			NF or PhEur
(b) (4)			NC
			USP or PhEur
Total (mg/tablet)		-	-

Note:

(b) (4)

Route of administration: PO

Proposed clinical protocol: Multicenter, Phase I, open label, non-randomized, multiple dose-finding study of GW786034B in adult cancer pateints with solid tumors who are refractory to standard therapy or for which no standard therapy exists.

Dose Group	Total Daily Dose GW786034 (mg)
-1	100
1	200
2	600
3	1400
4	2000
5	2600

- Patients will receive a single dose of GW786034B on Day 1 followed by 24 hours of PK sampling. Twice daily dosing, in Cohort 1, will start after the PK analysis for plasma GW786034B concentration-time date is available. Twice daily dosing in all subsequent cohorts will begin after the Day PK sampling is completed. PK sampling will be collected over the first 22 days of treatment (Day 1, 8, 15, 22).
- A minimum of 2 patients will be entered at each dose and monitored for toxicity.

- Dose escalation to subsequent levels will not occur until all patients in the previous cohort reach at least Day 22 of doing. The decision to dose escalate will be based on the safety profile observed during the first 22 days of treatment. The dose will be escalated according to predetermined dose escalation, until the maximum dose level is reached, or a grade 2 or greater toxicity is observed.
- If a grade 2 toxicity is observed in only 1 of 2 subjects, dose escalation continues in cohorts of at least 2 subjects with either a 100% dose increment or an increase to the next dose level, whichever is less. If grade 2 toxicity is observed in 2 of 2 subjects in a dosing cohort, escalation continues in cohorts of at least 3 subjects with either a 50% dose increment or an increase to the next dose, level whittler is less, until grade 3 or 3 non-dose-limiting or dose-limiting toxicity is observed. Dose escalation continues at a minimum of 3 subjects per level with 33% dose increments (rounded down to the nearest 100 mg) between level until DLT is observed. If 1 subject in a cohort experiences a DLT, the cohort is expanded to 6 subjects. If only 1 of 6 subjects ahs a DLT, subsequent dose escalation continues at 3 subjects per cohort with 25 % dose increment between levels. If 2 or more subjects in a dosing cohort experience DLT, the MTD has been exceeded.
- Alterations may be made to the dosing levels or schedule of administration based on safety and the results of the PK analysis.
- Subjects will remain on study until occurrence of unacceptable toxicities, disease progression, withdrawal of consent, or a treatment delay of more than 2 weeks.
- Adult subjects 18 years or older with solid tumors who have progressed on standard therapy or for whom no standard therapy exists will be enrolled in this study. Patients must have a Karnofsky Perfomance Status (KPS) of ≥70%, with a life expectancy of at least 12 weeks, be able to swallow oral medicatiosn, have adequate laboratory parameters, and have no uncontrolled medical conditions. .
- Subjects may receive escalated doses of GW786034 subsequent to the first 22 days of dosing if they have not experienced clinically significant toxicity (grade 3 or 4 non-hematological toxicity, their disease has not progressed and eligibility criteria continue to be fulfilled. Intra-subject dose escalations must receive prior approval by the GSK Medical Monitor.

Previous clinical experience: None

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history:
Most tumors secrete vascular endothelial growth factor (VEGF). In many tumor types VEGF expression correlates with increased tumor vascular density and poor patient outcome. VEGF acts through 3 high affinity receptors: VEGFR1, VEGR2, and VEGR3. GW786034 is an inhibitor of VEGFR2 and VEGFR3 tyrosine-kinase (TK). GW786034 nhibited substrate phosphorylation catalyzed by both VEGFR2 (IC_{50} = 24 nM) and VEGFR3 (IC_{50}= 48 nM). In preclinical models the inhibition of VEGFR2 TK leads to the inhibition of VEFG-dependent tumor angiogenesis.

Studies reviewed within this submission:

Report RD2001/01061/00 - GW786034A: Nonaudited 4- Day Oral Toxicity Study in Female Sprague Dawley Rats	4 210
Report CD2002/00063/00 - GW786034: 1-Month Oral Toxicity and Reversibility Study in Rats (DRAFT REPORT)	5 1
Report CD2002/00103/00 - GW786034: 1-Month Oral Toxicity Study in Cynomolgus Monkeys (DRAFT REPORT)	7 1

Studies not reviewed within this submission:

(b) (4)

(b) (4)

Toxicology Section

(b) (4)

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY: The following information has been excerpted from the Investigator Brochure.

Primary pharmacodynamics:

Studies In Vitro

Enzyme assays:

In studies measuring the effects of GW786034 on the tyrosine kinase activity of Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), other members of the VEGF receptor family, and other protein kinases, GW786034 was shown to be a potent and selective inhibitor of the VEGF receptors. GW786034 inhibited substrate phosphorylation catalyzed by both VEGFR2 (IC_{50} = 24 nM) and VEGFR3 (IC_{50}= 48 nM). GW786034 was 10- to 1000-fold more potent towards the VEGF receptors in comparison to the 15 other kinases tested.

Growth inhibition of human cell lines:

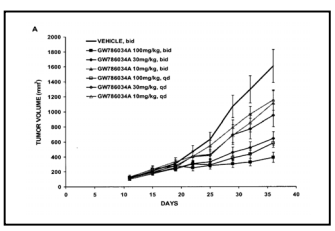
The effects of GW786034 on cell growth were examined in human umbilical vein endothelial cells (HUVEC), normal human foreskin fibroblasts (HFF), and a variety of human tumor cell lines (HT-29 [colon], MDA-MB- 468 [breast], PC3 [prostate], and A375P [melanoma]). GW786034 selectively inhibits proliferation of HUVEC stimulated with VEGF (IC_{50} = 21 nM) compared to bFGF- stimulated proliferation (IC_{50} = 721 nM). VEGF-induced tyrosine phosphorylation of VEGFR2 in HUVEC is inhibited by GW786034 in a dose-dependent manner (IC_{50} = 7 nM). GW786034 did not inhibit the proliferation of human tumor cell lines (HT-29, MDA-MB-468, PC3, and A375P) growing logarithmically in serum containing medium. GW786034 is selective for its effect on angiogenesis as compared to it's effects on tumor cell lines or tumor cell proliferation. GW786034 was >1400-fold selective for VEGF- induced HUVEC proliferation (angiogenesis) relative to all four tumor cells and 48-fold selective relative to HFF proliferation (direct tumor cell effect and tumor cell proliferation).

Studies In Vivo

Xenograft tumor models:

The antitumor activity of oral GW786034 has been investigated in mouse xenograft models for four different human tumors (HT29, colon carcinoma; A375P, melanoma; PC3 prostate carcinoma; HN5, head and neck carcinoma). Following 21 days of 100 mg/kg bid, HT29 and HN5 tumor growth was inhibited by 82% and 101%, respectively, with GW786034A, and by 66% and 90%, respectively, with GW786034B (see Figures 3.1 and 3.2). A375P and PC3 xenografts were less sensitive to GW786034. Inhibition of tumor growth was slightly lower with GW786034B than with GW786034A in all four tumor models.

Figure 3.1 Inhibition of HT29 (Human Colon Carcinoma) Xenograft Growth in Mice Treated with GW786034A or GW786034B for 21 days



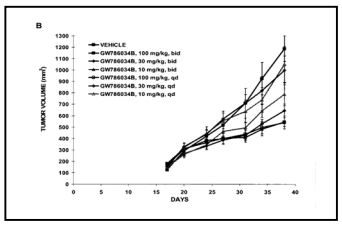


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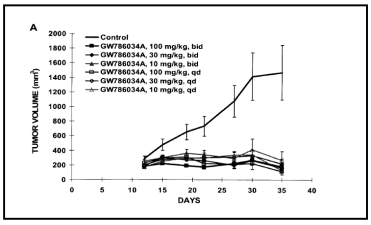
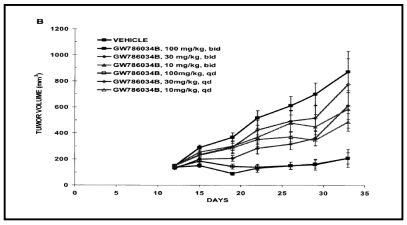
Figure 3.2 Inhibition of HN5 (Human Head and Neck Tumor) Xenograft Growth in Mice Treated with GW786034A or GW786034B for 21 days





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Matrigel angiogenesis model:
In female Swiss Nu/Nu mice, subcutaneous implantation of bFGF containing Matrigel results in new blood vessel growth in the plug which is highly dependent on VEGFR2 signalling. Administration of GW786034A for 5 days either twice daily (bid) or once daily (qd) by oral gavage resulted in inhibition of angiogenesis (bid/qd) by 86/82% at 100 mg/kg, 58/57% at 30 mg/kg, and 32/0% at 10 mg/kg. ED_{50} values were 29.4 mg/kg for once-a-day dosing and 20.3 mg/kg for twice-a- day dosing with GW786034A. In this model, GW786034B had a similar, but less marked effect; inhibition of angiogenesis (bid/qd) was 59/56% at 100 mg/kg, 42/45% at 30 mg/kg, 31/26% at 10 mg/kg, and 9/24% at 3 mg/kg. ED_{50} values were 68.3 mg/kg for once-a-day dosing and 53.1 mg/kg for twice-a-day dosing with GW786034B. These results are consistent with the finding that in the mouse, plasma GW786034 concentrations following oral administration of the GW786034A are approximately 2- fold higher than those observed after an equivalent oral dose of GW786034B.

Cornea micropocket angiogenesis model:
In female Swiss Nu/Nu mice, sucralfate micropellets are formulated with angiogenic growth factors (VEGF or bFGF) and implanted in the normally avascular cornea and angiogenic endpoints are quantified by measuring the degree of vascularization at the cornea-limbus interface (clock hours) and maximum blood vessel length. Administration of oral GW786034A at 100 mg/kg, bid for 5 days inhibited angiogenesis (clock hours/maximum vessel length) by 71/97% against bFGF and 100/135% against VEGF.

Secondary pharmacodynamics:
Enzyme and radioligand binding assays:
When GW786034A (10 μM) was tested in vitro in a panel of 49 enzyme, receptor, ion channel, and transporter targets, significant activity (> 50% inhibition or stimulation) was noted on only the following targets: adenosine A_3, adrenoceptors $\alpha_2\beta_1$, histamine H_2, muscarinic M_1, serotonin 5-HT1A, 5- HTSA, and 5-HT7.

Isolated tissue assays:
In atria isolated from male Sprague Dawley rats, GW786034A (30 µM) increased contractile force, an effect that was not blocked by propranolol (1 µM), without affecting atrial rate. Pre-incubation with GW786034A (30 µM) reduced the maximum contractile force response to isoproterenol (1 to 10 nM), a known beta- adrenoreceptor agonist. These data indicate that GW786034A can exhibit a non-beta adrenoreceptor mediated inotropic effect. However, inhibition of isoproterenol-induced maximal force of contraction does not preclude a weak inhibition of atrial beta- adrenoreceptors at high dose.

II. SAFETY PHARMACOLOGY:

The following data has been excerpted from the Investigator's Brochure.

Neurological effects:
Single oral doses of GW786034B up to 300 mg/kg in female Sprague Dawley rats (n=6/group) did not have effects on neurobehavioral functional assessments reflecting normal central and peripheral nervous system activity.

Cardiovascular effects:
In anesthetized male Sprague Dawley rats (n=3/group), intravenous infusion of GW786034A up to 10 mg/kg was not associated with changes in left ventricular contractility (+dP/dt), heart rate (HR) or mean arterial blood pressure (MAP). Systemic exposure was demonstrated with plasma GW786034A concentrations ranging from 45075 to 60822 ng/mL following a 10 mg/kg intravenous infusion. In contrast, infusions of isoproterenol (30, 100 or 300 ng/kg) were associated with dose-related increases in HR (60, 80, 140 bpm), an increase in +dP/dt (40, 45 and 80%), and a decrease in MAP (-3, - 25, -25 mmHg). Therefore, it is unlikely that GW786034A exerts either an agonist or antagonist effect on cardiac or vascular beta-adrenergic receptors at intravenous doses up to 10 mg/kg.

Cardiovascular studies in monkeys
In male cynomolgus monkeys (n=4/group), single oral doses of GW786034B up to 500 mg/kg had no statistically significant or drug-related effect on arterial blood pressures (systolic, diastolic and mean), pulse pressure, heart rate, electrocardiograms (intervals, rhythm and morphology) or body temperature during the 48 hours period after dosing. In addition, following repeat dosing for 28 days in monkeys at doses up to 500 mg/kg, there were no effects on electrocardiogram (ECG) tracings.

Effect on QT interval
In isolated dog Purkinje fibers, exposure up to 80 nM GW786034B (limit of compound solubility in media) had no effect on action potential duration at 60% (APD_{60}), APD_{90}, resting membrane potential (RMP), maximum rate of depolarization (MRD) or upstroke amplitude (UA) at 1 and 0.5 Hz stimulation frequencies. There was no effect of 80 nM GW786034B on MRD when the stimulation frequency was increased to 3 Hz.

Pulmonary effects:
Single oral doses of GW786034B up to 300 mg/kg in male CD® IGS rats (n=6/group) did not produce any adverse effect on respiratory function (respiratory rate, tidal volume, or minute volume).

III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

Study title: 1-Month oral toxicity and reversibility study in rats (draft report)

Study no: G01200 Volume #5 and page # 1

Cmax (µg/ml)			Males				Females				Ratio Female : Male	
Dose mg/kg/d	Dose mg/m^2/d	Ratio Dose 2 :Dose 1	Day 1	Dose 2 :Dose 1 ratio	Day 28	Ratio Day 28: Day 1	Day 1	Dose 2 :Dose 1 ratio	Day 28	Ratio Day 28: Day 1	Day 1	Day 28
3	18	1	4.28	1	9.58	2	7.08	1	10.4	1	2	1
10	60	3	17.5	4	21.7	1	28	4	28.7	1	2	1
30	180	10	33.6	8	29.9	1	41.3	6	51	1	1	2
100	600	33	33.3	8	26.3	1	43.3	6	38.6	1	1	1
300	1800	100	73.1	17	38.8	1	90.8	13	56	1	1	1

AUC (µg*h/l)			Males				Females				Ratio Female : Male	
Dose mg/kg/d	Dose mg/m^2/d	Ratio Dose 2 :Dose 1	Day 1	Dose 2 :Dose 1 ratio	Day 28	Ratio Day 28: Day 1	Day 1	Dose 2 :Dose 1 ratio	Day 28	Ratio Day 28: Day 1	Day 1	Day 28
3	18	1	44.2	1	54.9	1	71.3	1	83	1	2	2
10	60	3	148	3	116	1	259	4	232	1	2	2
30	180	10	261	6	221	1	388	5	365	1	1	2
100	600	33	253	6	174	1	288	4	257	1	1	1
300	1800	100	779	18	258	0	1090	15	472	0	1	2

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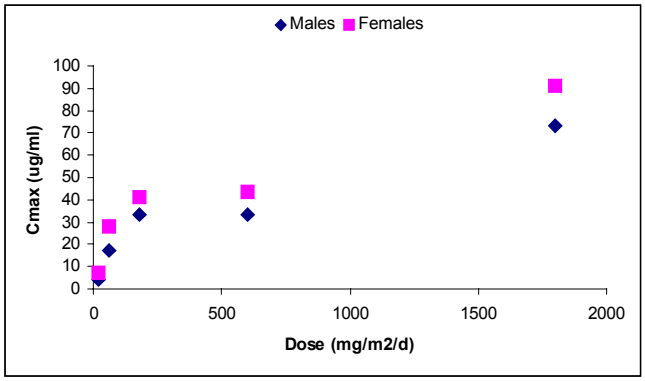
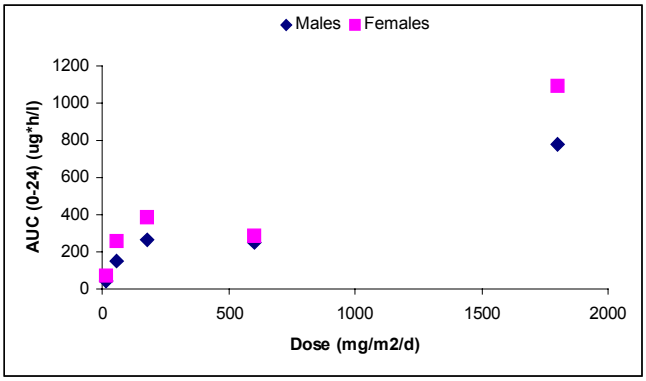
- GW786034X was detected in the plasma of all drug-treated rats and was quantifiable for the entire 24 hour sampling period on both sampling days. Generally, the maximum plasma concentration was attained by approximately 0.50 to 2.00 hours after dosing.
- On both sampling days, there was a trend for higher (~ 2-fold) C_{max} and mean systemic exposure (AUC) for female rats compared to male rats.
- There were no marked differences in systemic exposure between Days 1 and 28 except at 300 mg/kg/day where a 67% and 57% decrease was observed for males and females, respectively. Cmax values were 2-fold higher on day 28 compared to day 1 in males receiving the LD.
- At doses ≥100 mg/kg/d, there was a less than proportional increase in C_{max} whereas at doses ≥30 mg/kg/d, there was a less than proportional increase in systemic exposure suggesting saturation of absorption.
- There were no marked differences in systemic exposure between Days 1 and 28 except for the 300 mg/kg/day group in which systemic exposure on Day 28 was decreased, on average, 61%.

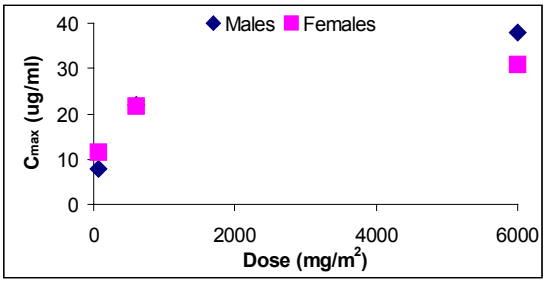


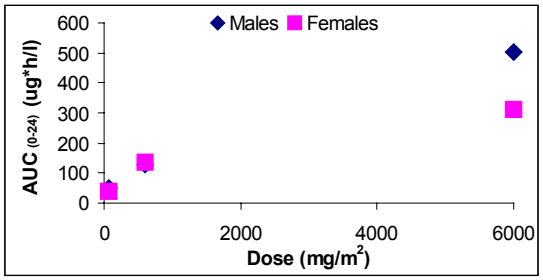


Study title: 1-Month Oral Toxicity Study in Cynomolgus Monkeys (DRAFT)
Study no: G02013 Volume # 7 and page #: 1

Cmax (µg/ml)			Males				Females				Ratio Female : Male	
Dose mg/kg/d	Dose mg/m^2/d	Ratio Dose 2 :Dose 1	Day 1	Dose 2 :Dose 1 ratio	Day 28	Ratio Day 28: Day 1	Day 1	Dose 2 :Dose 1 ratio	Day 28	Ratio Day 28: Day 1	Day 1	Day 28
5	60	1	7.8	1	11.7	2	11.6	1	12.1	1	1	1
50	600	10	21.9	3	20.6	1	21.6	2	19.6	1	1	1
500	6000	100	37.8	5	40.9	1	30.9	3	30.6	1	1	1
AUC (µg*h/l)			Males				Females				Ratio Female : Male	
Dose mg/kg/d	Dose mg/m^2/d	Ratio Dose 2 :Dose 1	Day 1	Dose 2 :Dose 1 ratio	Day 28	Ratio Day 28: Day 1	Day 1	Dose 2 :Dose 1 ratio	Day 28	Ratio Day 28: Day 1	Day 1	Day 28
5	60	1	50.6	6	46.6	1	37.9	3	36	1	1	1
50	600	10	129	17	120	1	135	12	99.5	1	1	1
500	6000	100	503	64	296	1	313	27	335	1	1	1

- GW786034X was detected in the plasma of all drug-treated monkeys and was quantifiable for the entire 24-hour-sampling period on both Days 1 and 28.
- Maximum plasma concentrations increased with dose and the maximum plasma concentration was attained between 1 to 4 hours after dosing.
- There was no marked difference in systemic exposure (Cmax and AUC) between males and females.
- There was no appreciable change in systemic exposure from Days 1 and 28.
- In male and female monkeys, mean systemic exposure (AUC) in the monkey increased less than proportionately with dose on Days 1 and 28 suggesting saturation of absorption.





*The following information has been excepted from the Investigator's brochure.

The pharmacokinetics, metabolism, and elimination of GW786034 have been investigated through a series of oral, intravenous and in vitro studies in the mouse, rat, dog, and monkey using non-radiolabelled and ^{14}C-labelled drug. The toxicokinetics of GW786034 following repeat oral administration to rat, dogs, and monkeys were investigated during the toxicity studies in these species. The studies were conducted using either the GW786034A, the dihydrochloride salt, or GW786034B, the monohydrochloride salt. All doses in this section are expressed in terms of the free base, GW786034.

In female CD-1 mice administered a single oral dose of GW786034A at 10, 30, and 100 mg/kg, the plasma GW786034 Cmax values were 41, 122, and 270 µM. Absorption of GW786034A at the two higher dose levels (30 and 100 mg/kg) was prolonged with Tmax observed at 0.5 to 6 hours. Plasma concentrations were quantifiable through 24 hours after each dose level.

In male Han Wistar rats administered GW786034A intravenously (10 mg/kg), clearance was 1.7 mL/min/kg, the volume of distribution at steady state (Vdss) was 478 mL/kg, and the apparent elimination half-life was 3.6 hour. Oral bioavailability was variable. Following oral administration of GW786034B (10 mg/kg) to fasted rats, bioavailability ranged from 14.6 to 193% whereas after GW786034A (10 mg/kg), oral bioavailability ranged from 49 to 93% (mean 72%).

In male beagle dogs administered GW786034A intravenously (1.0 mg/kg), clearance was 1.4 mL/min/kg, the volume of distribution was 297 mL/kg, and the apparent elimination half-life was 2.2 hours. Plasma GW786034 concentrations showed a slow and steady decline until the animals were fed when plasma concentrations dropped rapidly.

Feeding affected the plasma GW786034 concentrations achieved following oral administration of GW786034B to male dogs (1 mg/kg). Feeding 1 hour prior to oral dose administration resulted in a 4 to 5-fold decrease in plasma GW786034 C max and AUC_{0-t} values compared to dosing in the fasted state. Additionally, plasma concentrations in fasted dogs were observed to decrease quickly (15-fold decline) between 8 and 12 hours, corresponding to the time of feeding, while no change in plasma concentrations was observed in the fed animals during the same timeframe.

In male cynomolgus monkeys administered GW786034A intravenously (5 mg/kg), clearance was 2.0 mL/min/kg, the volume of distribution was 280 mL/kg, and the estimated apparent elimination half-life was 5 hours. The time of feeding after intravenous dose administration (4 hours versus 8 hours) did not markedly affect the mean plasma GW786034 $AUC_{0-\infty}$ value (46.7 and 58.9 µg.h/mL, respectively). Following intravenous administration of GW786034B (2 mg/kg), similar pharmacokinetic parameters were determined; clearance was 1.35 mL/min/kg, the volume of distribution was 268 mL/kg, and the apparent elimination half-life was 2.9 hours.

The oral bioavailability of GW786034 in male cynomolgus monkeys ranged from 30%- 65%, depending on the salt form and formulation administered. Male monkeys were orally administered GW786034A (vehicle 0.5% HPMC/0.1% Tween 80 [pH 2.3 to 2.4]) as a solution

(5 mg/kg) or suspension (50 mg/kg), and the mean plasma GW786034 Cmax and $AUC_{0-\infty}$ values were 8 µg/mL and 28.4 µg.h/mL and 33.7 µg/mL and 174 µg.h/mL, respectively. For both dose levels, Tmax ranged from 0.5 to 3 hours. No substantial changes in GW786034 pharmacokinetic parameter were noted when GW786034A (5 mg/kg) was orally administered to fed monkeys. As GW786034 concentrations in plasma and whole blood were nearly the same at selected timepoints, this finding suggests there is equal partitioning of the compound in monkey blood and plasma.

Following a single oral dose of GW786034B (10 mg/kg; vehicle, 30% Encapsin in water [pH 2.3]) to fasted monkeys, the plasma GW786034 Cmax was 1.17 µg/mL. An oral administration of GW786034B at 50 mg/kg (suspended in 0.5% HPMC/0.1% Tween 80 [pH 1.3]) resulted in mean plasma GW786034 Cmax and $AUC_{0-\infty}$ values of 30.3 µg/mL and 141 µg.h/mL, respectively. These values for GW786034B orally at 50 mg/kg are comparable to those obtained with GW786034A orally at 50 mg/kg.

Distribution:
In a preliminary in vitro assessment, GW786034 (5 pM) displayed very high plasma protein binding in mouse, rat, dog, monkey and human (> 99.9%); GW786034 was not detectable by LC/MS/MS in all ultrafiltrate samples.

In hMDR1/MDCK cells measuring P glycoprotein (Pgp)-mediated efflux and passive permeability of GW786034, it was demonstrated that GW786034 has a high rate of passive permeability across the cell monolayer. GW786034 inhibited calcein-AM efflux, an indicator that GW786034 interacted with Pgp. These data are evidence that GW786034 is a Pgp substrate with high passive permeability.

Metabolism:
Biotransformation
Preliminary analyses for potential metabolic products were conducted using liquid chromatography/mass spectroscopy/ mass spectroscopy (LC/MS/MS) with samples from in vitro microsomal incubations and in vivo plasma samples of several species. Following incubation of non-radiolabelled GW786034A (10 pM) with liver microsomal preparations from the mouse, rat, dog, monkey and human, two mono-oxygenated metabolites were present in all species. The mass spectrometric fragmentation pattern of one of the mono-oxygenated metabolites suggested that oxidation to the alcohol occurred on one of the methyl groups. The location of the oxygen of the second mono-oxygenated metabolite was not assigned. A di-oxygenated metabolite was also identified in all species. A single metabolite that appeared unique to the human microsomal samples was tentatively observed; the mass spectrometric fragmentation pattern suggests that one of the methyl groups was oxidized to the corresponding carboxylic acid.

In plasma samples collected from mouse, rat, dog and monkey following intravenous or oral administration of GW786034A, two mono-oxygenated metabolites were identified in each species. An N-demethylated metabolite was observed in mouse and monkey plasma samples but was absent in the other species. A di-oxygenated metabolite found in the microsomal incubations was not present in any of the plasma samples.

Inhibition/Induction
In vitro inhibition screen: Using preliminary screening assays, the inhibitory effects of GW786034A on selected human cytochrome P450 isozymes were determined in recombinant enzymes expressed in lymphoblastic cells. Potential inhibition by GW786034A (IC_{50} value) was observed for CYP2C9 (1.7±0.1 μM), CYP1A2 (11.7±+4.6 μM), CYP2C19 (12.5±2.1 μM), CYP3A4 (substrate DEF, 12.0±2.7 μM); effects were weak toward CYP2D6 (85±9.9 μM) and CYP3A4 (substrate PPR, 50±4.6 μM).

In vitro CYP3A4 induction potential in a human PXR assay: In a human Pregnane X Receptor (PXR) transient transfection assay, CYP3A4 induction potential was measured in HuH7 cells. Efficacy was measured by the maximum response observed at 10 pM compared to the fold induction (as 100%) of the positive control rifampicin. The maximum induction response at 10 pM for GW786034A was equally potent to rifampicin (107+ 23.2%), suggesting some potential for human CYP3A4 induction at high concentration.

Effects on CYP450 enzyme activities after in vivo treatment: Treatment of male and female rats with GW786034B at oral doses of 3, 10, 30, 100, and 300 mg/kg/day for 1 month caused no notable changes in the mean hepatic microsomal protein yield or generally in total cytochrome P450 content. In males in the 300 mg/kg/day group, the total cytochrome P450 content was marginally higher but this difference is considered unlikely to be of any biological significance. There were no GW786034B-related effects on the activities of CYP1A, CYP2B, CYP2E, CYP3A, and CYP4A.

Oral administration of GW786034B to male and female monkeys at doses of 5, 50, and 500 mg/kg/day for 1 month caused no notable changes in the mean hepatic microsomal total cytochrome P450 levels or in the measured cytochrome P450 enzyme activities of EROD, testosterone 6β-hydroxylase, testosterone 16β-hydroxylase, lauric acid 11- hydroxylase and lauric acid 12-hydroxylase.

Excretion:
Following a single oral administration of ^{14}C-labelled GW786034 (10 mg/kg) to intact male and female rats as a solution, excretion of radioactivity was rapid and occurred largely within 48 hours of dosing. The major route of elimination was via the feces (approximately 52-61%). Urinary excretion accounted for approximately 15-17% of the administered dose for the intact male and female rats, respectively. No major gender differences were apparent in either the routes or rates of elimination. In male bile duct- cannulated rats (BDC) (10 mg/kg as a solution), the major route of elimination of radioactivity was via the feces (approximately 43% of administered dose) with urinary and biliary excretion accounting for approximately 25% and 8% of the dose, respectively. Absorption of drug-related material in the male BDC rat was estimated to be at least 35% of the dose based on biliary secretion, urinary excretion, liver and residual carcass radioactivity. The total recovery of radioactivity was excellent, approximately 90% and 94% from intact males and females, respectively, and 92% from BDC animals. There was no significant retention of radioactivity in the animals. In intact animals, mean plasma concentrations of radioactivity at 2, 8 and 24 hours post-dose were 936, 876, and 121 ng equiv/g, respectively, for male and 1689, 1487 and 384 ng equiv/g, respectively, for females.

Excretion of [^{14}C]GW786034 following an oral dose to monkeys (5 mg/kg as solution) also showed rapid and complete excretion of the radioactivity, and that the excretion of the radioactivity occurred mainly via feces.

Interactions
In preliminary in vitro studies, GW786034A was shown to have some inhibitory potential towards human CYP1A2, CYP2C9, CYP2C19, and CYP3A4 as well as the potential to induce human CYP3A4 (see PXR results above). There appeared to be minimal potential for inductive effects on CYP450 enzyme activities in rats or monkeys after oral doses of GW786034B for 1 month. At this time, no clinical studies have been performed to determine the effect of known inhibitors of CYP450 enzymes on GW786034 metabolism or the effect of GW786034 on drugs metabolized by CYP450 enzymes. Therefore, co- administration of GW786034B with known inhibitors or inducers of CYP450 enzymes and/or compounds metabolized by CYP450 enzymes should proceed with caution.

I. GENERAL TOXICOLOGY:

Report RD2001/01061/00 - GW786034A: Nonaudited 4- Day Oral Toxicity Study in Female Sprague Dawley Rats Volume 4 Page 210

GW786034A: Nonaudited 4-Day Oral Toxicity Study in Female Sprague Dawley Rats (Glaxo Wellcome Study No. R40961)

Glaxo Wellcome Report Number: RD2001/01061/00	**Glaxo Wellcome Study Number**: R40961	**Duration of Treatment**: 4 Days
Species/Strain: Rat/Sprague Dawley®	**Route**: Oral gavage	**Terminal Necropsy Date**: 20 July 2001
Weight Range on Day 1: Female: 201 to 228 grams	**Test Material**: GW786034A **Batch Number**: U17574-61-1	**Dosing Period Dates**: **First dose**: 16 July 2001 **Final dose**: 19 July 2001
Age on Day 1: Approximately 62 Days	**Vehicle**: 0.5% Hydroxypropyl methylcellulose (HPMC) with 0.1% Tween® 80 in reverse-osmosis water	
Study in Compliance with GLP: No	**Dose Volume**: 10 mL/kg **Dosing Frequency**: Once daily	**Testing Facility**: Glaxo Wellcome Inc. Medicines Safety Evaluation Five Moore Drive Research Triangle Park, NC USA
Data Collected: Toxicokinetics (Day 1 and 4), clinical observations, body weight, hematology, clinical chemistry, organ weights, and macroscopic and microscopic pathology		

		Female			
Dose (mg/kg/day)		**0**	**30**	**100**	**300**
Number of Animals:	Main	6	6	6	6
AUC (µg*h/mL)[1]	Day 1	NR	406.1	1162.5	1739.1
	Day 4	NR	419.3	781.7	1487.3
C_{max} (µg/mL)	Day 1	NR	33.5	109.3	112.6
	Day 4	NR	43.3	86.5	107.0

Key:
[1]$AUC_{0\text{-last}}$ for Day 1; $AUC_{0\text{-}\infty}$ for Day 4
NR = Below 10 ng/mL or not quantifiable

Noteworthy findings: Noteworthy findings are detailed below. No treatment-related findings were noted in food consumption or macroscopic pathology.

Dose (mg/kg/day)		**0**	**30**	**100**	**300**
Number of Unscheduled Deaths		0	0	0	0
		Group Mean			
Body Weight (g)	Day 1	212.15	212.98	208.00	212.33
	Day 5	216.58	210.98	210.12	214.12
Hematology Day 5					
Number examined		5	4	5	4
Red blood cell count (x 10^6/µL)		4.876	5.363	4.912	4.865
Hematocrit (%)		32.06	33.93	30.50	30.40
Hemoglobin (g/dL)		9.92	10.75	10.00	9.95
MCHC (g/dL)		30.96	31.73	32.76	32.68
MCV (fL)		65.84	63.84	62.10	62.58
Reticulocytes (%)		10.32	4.93	5.00	3.15
Reticulocytes (x 10^6/µL)		0.5026	0.2615	0.2456	0.1518
Clinical Chemistry Day 5					

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Pathology Findings	Incidence of Findings			
Dose (mg/kg/day)	**0**	**30**	**100**	**300**
Number of Animals Examined	**6**	**6**	**6**	**6**
Microscopic, Terminal				
Femur (distal) and Tibia (proximal)				
Growth plate hypertrophic chondrocytes expansion				
slight	0	0	3	0
moderate	0	0	3	6
Growth plate capillary decreased				
very slight	0	0	2	3
slight	0	0	4	3
Sternum				
Growth plate hypertrophic chondrocytes expansion				
very slight	0	0	1	3
Ovaries				
Corpora lutea necrosis				
very slight	0	0	4	1
slight	0	0	0	2
moderate	0	0	0	3

- Rats tolerated daily oral administration of GW786034A at doses up to 300 mg/kg/day for 4 days.
- Microscopic findings characterized by expansion of the hypertrophic chondrocytes of the growth plates (physis) and decreased physeal capillaries of long bones (femur and tibia), and coagulative necrosis of copora lutea in the ovaries were attributable to the pharmacological properties of GW786034A and were limited to the 100 and 300 mg/kg/day treatment groups.
- Changes in liver enzymes and bile acids were generally mild and were not accompanied by morphologic correlates.
- There were no microscopic findings to account for decreased thymus weights at doses >30 mg/kg/day.

Study title: 1-Month oral toxicity and reversibility study in rats (draft report)

Key study findings:

- GW786034X-related microscopic findings occurred in sternebrae, femur/stifle, and testis of rats given >100 mg/kg/day. After 6-week recovery period, testicular and marrow changes had reversed and 1/4 treated male rats had resolving hypertrophy in the epiphyseal growth plate in the femur/stifle.
- Excessive growth and apparent brittleness of teeth and/or nails was observed for rats in the 300 mg/kg/day group approximately 2-4 weeks off-treatment.
- Increases in serum liver enzymes occurred at 300 mg/kg/day.
- The NOAEL in this study is 30 mg/kg/day based on compound-related microscopic findings in the sternebrae, femur/stifle, and testes at 100 and 300 mg/kg/day.

Study no: G01200
Volume #5 and page # 1
Conducting laboratory and location: Department of Safety Assessment and the Drug Metabolism and Pharacokinetics Area, GlaxoSmithKline, King of Prussia, PA
Date of study initiation: 30-Jan-2002
GLP compliance: No (draft)
QA report: yes ( ) no (x)
Drug, lot #, and % purity: GW786034B (Batch/Lot No. 786034-A2-01M); 90.1% (free base). Certificate of analysis was not included. Drug concentration analysis was submitted.
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose (HPMC)/0.1% Tween 80 (pH adjusted to 1.3 using HC1)

Methods :
Dosing:

Species/strain: ♂/♀ Sprague-Dawley rats (b) (4)

#/sex/group or time point (main study): Five groups of rats (10 or 15/sex/group)
Satellite groups used for toxicokinetics: 3 rats/sex/group
Satellite groups used for recovery: 3 rats/sex at 0 and 300 mg/kg/d after 6-week period off treatment

Age: 12-13 weeks old
Weight: ♂:364-437 g; ♀:228-304 g
Doses: 3, 10, 30, 100 or 300 mg/kg/day once daily for 28 days

Route, form, volume, and infusion rate: orally by gavage; dosing volume of 10 mL/kg

Group	Dose (mg/kg/day)*	Number of Animals	Animal Identification Numbers†
1	0	15M/15F	R02M-531 to R02M-545 R02F-546 to R02F-560
2	3	10M/10F	R02M-561 to R02M-570 R02F-571 to R02F-580
3	10	10M/10F	R02M-581 to R02M-590 R02F-591 to R02F-600
4	30	10M/10F	R02M-601 to R02M-610 R02F-611 to R02F-620
5	300	15M/15F	R02M-621 to R02M-635 R02F-636 to R02F-650
6	0	3M/3F	R02M-651 to R02M-653 R02F-654 to R02F-656
7	3	3M/3F	R02M-657 to R02M-659 R02F-660 to R02F-662
8	10	3M/3F	R02M-663 to R02M-665 R02F-666 to R02F-668
9	30	3M/3F	R02M-669 to R02M-671 R02F-672 to R02F-674
10	300	3M/3F	R02M-675 to R02M-677 R02F-678 to R02F-680
11‡	100	10M/10F	R02M-681 to R02M-690 R02F-691 to R02F-700
12‡	100	3M/3F	R02M-824 to R02M-826 R02F-827 to R02F-829

* Doses represent the parent compound.
† Each animal has a unique identification number, but variations of the animal identification numbers are used for different data collection systems. For example:
Animal Number: R02M-531
In-Life and Pathology tables: 531
‡ A dose of 100 mg/kg/day was included to aid in evaluation of a dose response.

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Observations and times:

Clinical signs:	Daily observations were recorded for all rats prior to randomization and for all rats in Groups 1-5 and 11 following randomization. Detailed clinical examinations were completed prior to initiation of dosing (all rats), on approximately Day 28 (Groups 1-5 and 11) and on Days 56 and 70 (Groups 1 and 5, recovery rats).
Body weights:	Body weights were measured for all rats once prior to Day 1, daily during the dosing period and terminally (Day 29 or 30). Body weights were collected for the recovery rats (Groups 1 and 5) weekly from Week 5 through Week 8, daily from Day 57 through Day 70 and terminally (Day 71). Body weights collected for toxicokinetic rats (Groups 6-10 and 12) were used for the purpose of dose calculation only and were not reported.
Food consumption:	Food consumption was measured over a 6- to 7-day period once prior to Day 1 and weekly during the dosing and recovery periods.
Ophthalmoscopy:	Prior to initiation of dosing (all rats) and on approximately Day 27 (Groups 1-5 and 11).
EKG:	Not conducted
Hematology:	For rats in Groups 1-5 and 11, blood was collected on approximately Day 27 or 28 and at necropsy on Day 29 or 30 for serum total bile acid analysis. For the recovery rats (Groups 1 and 5), blood was collected on Day 56 and at necropsy on Day 71 for serum total bile acid analysis.
Clinical chemistry:	For rats in Groups 1-5 and 11, blood was collected on approximately Day 27 or 28 and at necropsy on Day 29 or 30 for serum total bile acid analysis. For the recovery rats (Groups 1 and 5), blood was collected on Day 56 and at necropsy on Day 71 for serum total bile acid analysis.
Urinalysis:	On Day 29 or 30, following an overnight fast, urine samples were collected prior to necropsy from all rats in Groups 1-5 and 11. On Day 7l, following an overnight fast, urine samples were collected prior to necropsy from recovery rats in Groups 1 and 5.
Gross pathology:	On Day 29 or 30, the first 10 rats/sex in Groups 1 and 5 and all rats in Groups 2-4 and 11 were killed and necropsied. The remaining rats in Groups 1 and 5 were killed and necropsied on Day 71 (approximately 6 weeks following cessation of drug treatment). All rats in Groups 6-10 and 12 were killed and discarded following liver collection for cytochrome P450 evaluation (Day 29).
Organs weighed:	The following organs from all rats in Groups 1-5 and 11 were weighed at necropsy (Days 29 or 30, Day 71): brain, liver, heart, kidney (paired), adrenal gland (paired), thymus, testis (paired), epididymis (paired), seminal vesicle

(paired), ventral prostate and ovary (paired). The liver from each rat in Groups 6-10 and 12 was weighed at termination (Day 29).

Histopathology: On Day 29 or 30, the first 10 rats/sex in Groups 1 and 5 and all rats in Groups 2-4 and 11 were killed and necropsied. The remaining rats in Groups 1 and 5 were killed and necropsied on Day 71 (approximately 6 weeks following cessation of drug treatment). All rats in Groups 6-10 and 12 were killed and discarded following liver collection for cytochrome P450 evaluation (Day 29).

A peer review of the microscopic findings for this study was performed in the following animals from the control and high dose group:

Male		Females	
Control	HD	Control	HD
531	621	546	636
532	622	547	637
533	623	548	638

Potential target organs from animals in all dose groups were reviewed"

	Group No.	
Organ	Males	Females
Sternebrae	1,2,3,4,5,11,	1,2,3,4,5,11,
femus/stifle	1,2,3,4,5,11,	1,2,3,4,5,11,
testes	1,2,3,4,5,11,	

Toxicokinetics: On Days 1 and 28, blood samples were collected from each rat in Groups 6-10 and Group 12 (2-3/sex/group). Samples were collected prior to dosing and at the following nominal times after dosing: 0.5, 1, 2, 4, 8, 12, and 24 hours.

Rat plasma samples, excluding those from control animals, were assayed for GW786034X by LC/MS/MS. The lower limit of quantification (LLQ) was 10.0 ng/mL for a 50 µL aliquot of rat plasma. PK analysis of GW786034X plasma concentration-time data was performed using the standard algorithms of the non-compartmental pharmacokinetic analysis program.

Other: Hepatic cytochrome P450 (n=3/sex/group)

On Day 29, the liver from each rat in Groups 6-10 and 12 was removed. The P450 evaluations were conducted under a separate protocol and will be reported separately.

Results:

Mortality: One rat given 300 mg/kg/day was sacrificed and necropsied on Day 21 because of his deteriorating clinical condition (subdued activity, urine staining, red urine, unkempt haircoat, body weight loss). White, patchy mottling on the cortical surface of the kidney, renal pelvis dilation, enlarged, thickened, reddish discolored urinary bladder and marked distension of ureters were observeded macroscopically. Bilateral pyelonephritis and cystitis were observed microscopically. The Sponsor attributed this animal's death to spontaneous disease and did not considered it drug-related.

Clinical signs: There were no drug-related clinical signs during the treatment period.

Between Days 41 and 71, abnormal teeth (upper and/or lower incisors excessively long, broken, missing and apparently more friable than those of controls) were observed for all rats in the 300 mg/kg/day group. These animals were unable to eat the dry pelleted food and were offered moistened pellets. Additionally, most of these rats had missing/broken nails on one or more digits beginning on approximately Day 59.

Body weights: There were no drug-related effects on body weight during the treatment period.

During the recovery period (Days 35-70), mean body weight for males and females in the 300 mg/kg/day group was lower (~13-28%) compared to control values. The Sponsor considered the reduced weight gain to be attributed to an inability to eat the dry pelleted food due to teeth abnormalities.

Food consumption: There were no drug-related effects on food consumption during the treatment period.

During the recovery period (Days 35, 42 and/or 49), mean food consumption for males and females in the 300 mg/kg/day group was lower (~12-71%) then control values. The reduced food consumption was attributed to an inability to eat the dry pelleted food due to teeth abnormalities; the rats were given moistened pellets and food consumption was not measured after Day 49 (males) and Day 42 (females).

Ophthalmoscopy: There were no drug-relatcd ophthalmologic effects.

Electrocardiography: Not measured

Hematology:

		Reticulocyte (x10e9/L				RDW (%)			
	Dose (mg/kg/d)	Male	% change	Female	% change	Male	% change	Female	% change
Treatment	0	231.4		187.2		12.4		11.7	
	3	223.2	↓4	188.2	1	12.4	0	12	3
	10	203	↓12	193.6	3	12.6	2	12.3	5
	30	196.3	↓15	191.6	2	13.5	↑9	13.1	↑12
	100	207.7	↓10	177.2	-5	14.6	↑18	14.1	↑21
	300	184.6	↓20	184.6	-1	16.3	↑31	15.2	↑30
Recovery	0	191.9		140.7		13.1		11.8	
	300	320.4	↑1.7X	299.2	↑2X	15	↑15	13.8	↑17

By the end of treatment,

- Mean reticulocyte count for males given 300 mg/kg/day was decreased (~20%) compared to controls). There were no significant changes in reticulocyte counts for females. Note: Bone marrow hypocellularity was observed microscopically.
- Red cell distribution width (RDW) was increased at ≥100 mg/kg/d in both sexes.

By the end of recovery,

- Reticulocyte count increased (~2-fold) in HD animals.
- Total red cell count was decreased (~12-16%) in HD animals. The Sponsor associated these changes with the weight loss, reduction in food intake, and potential blood loss due to incisor abnormalities and/or broken nails.
- Red cell distribution width (RDW) remained increased in both sexes.

Clinical chemistry: Table shows mean hematological values and percent change from controls.

e		ALT (U/L)				AST (U/L)				ALP (U/L)				Bile acids µmol/L			
	Dose mg/kg/d	♂	% change	♀	% change	♂	% change	♀	% change	♂	% change	♀	% change	♂	% change	♀	% change
Treat-ment	0	54		52		149		114		214		134		12.8		11.8	
	3	56	4	50	-4	131	-12	106	-7	236	10	157	↑17	14.9	↑16	9.8	-17
	10	69	↑28	56	8	143	-4	106	-7	224	5	181	↑35	14.5	↑13	11.8	0
	30	59	↑9	64	↑23	128	-14	118	4	213	0	168	↑25	8.3	-35	10.7	-9
	100	67	↑24	69	↑33	127	-15	107	-6	221	3	166	↑24	28.7	↑124	17.5	↑48
	300	78	↑44	101	↑94	140	-6	148	30	230	7	167	↑25	14.4	↑13	16.9	↑43
Reco-very	0	52		153		145		117		190		153		22.5		10.4	
	300	54	4	133	-13	106	-27	111	-5	157	-17	133	-13	14.4	-36	9.4	-10

- ALT levels were increased in HD males (2-fold in 5/15 rats) and HD females (2-fold in 6/15 and 4-6-fold in 2/15 rats) compared to controls.
- AST levels were increased in HD males (3-fold in 1/15 rats) and HD females (3-fold in 2/15 rats) compared to controls.
- Serum ALP levels were increased in HD males (2-fold in 1/15 rats) and females (2-3-fold in 3/15 rats) compared to controls.
- Serum total bile acids were increased in HD males (3-fold in 1/10 rats) and females (2-fold in 6/10 rats) compared to controls.

Urinalysis:

		Urine Volume				Urine Creatinine				Urine Total Protein				Urine Protein-Creatinine			
	Dose mg/kg/d	♂	% change	♀	% change	♂	% change	♀	% change	♂	% change	♀	% change	♂	% change	♀	% change
Treat-ment	0	15.1		8.1		7.88		7.81		0.73		0.19		0.87		0.22	
	3	13.6	↓10	11.4	41	7.41	-6	5.87	-25	0.67	-8	0.12	-37	0.8	-8	0.19	-14
	10	11.7	↓23	6.4	↓21	9.27	↑18	8.15	4	1.2	↑64	0.18	-5	1.03	18	0.19	-14
	30	9.7	↓36	13.1	62	10.77	↑37	5.2	-33	0.92	↑26	0.12	-37	0.76	-13	0.18	-18
	100	10.8	↓28	8.6	6	9.65	↑22	7.28	-7	1.55	↑112	0.19	0	1.82	↑109	0.21	-5
	300	11	↓27	8.5	5	8.66	↑10	8.2	5	1.1	↑51	0.24	↑26	1.12	↑29	0.27	↑23
Reco-very	0	16.2		9.9		7.89		6.35		0.84		0.17		1.03		0.21	
	300	17.6	9	10.2	3	6.56	-17	6.09	-4	0.87	4	0.15	-12	1.12	9	0.22	5

After treatment, urine protein to creatinine (P:C) ratios were increased in HD males (2-fold in 5/14 rats) and HD females (2-fold in 3/15 rats) compared to controls.
At 100 mg/kg/d, urine protein to creatinine (P:C) ratios were increased in males (2-3 fold in 2/10 rats and 8-fold in1/10 rats) and females (2-fold in 1/10 rats) compared to controls.
The Sponsor attributed the moderate increase in P:C ratio for one animals to moderate pyelonephritis with calculi and hypertrophy of the urinary bladder with calculi observed microscopically; the slight increase for the other animals was not accompanied by other urinalysis changes or histologic changes in the kidney or urinary bladder. The Sponsor did not consider this change to be drug-related.

After treatment, there was a higher incidence of increased (>1.00 g/L) urine protein concentrations in males given 100 or 300 mg/kg/day (9/10 and 9/14, respectively) compared to control (2/10). The increased urine protein concentrations were often associated with reduced urine volume and/or increased specific gravity values relative to controls. The Sponsor proposes that in the absence of remarkable changes in urine P:C ratios, other renal function parameters (i.e., decreased specific gravity, increased serum urea, creatinine) and histologic

changes in kidney or accessory sex gland, the urine protein findings in males were most likely related to urine concentration or contamination.

Organ weights:

Treatment	mg/kg/d	Liver/BW	% change	Testes/BW	% change
Males	0	3.07	3.07	0.75	0.75
	3	2.97	↓3	0.74	
	10	2.95	↓4	0.68	
	30	2.88	↓6	0.75	
	100	2.95	↓4	0.74	
	300	2.88	↓6	0.65	↓13*
Females	0	2.99	2.99		
	3	2.88	↓4		
	10	2.85	↓5		
	30	2.75	↓8*		
	100	2.72	↓10*		
	300	2.8	↓7*		

On Day 29/30, mean testis weight (absolute and/or relative to body weight) was decreased ~ 12% for males given 300 mg/kg/day compared to control mean values. Note: This finding may be related to minimal to moderate depletion of round spermatids (Stage I-V) observed microscopically.
Day 29/30, mean liver (absolute and/or relative to body weight) was significantly decreased (~ 10%) for females given ≥30 mg/kg/day compared to control mean values.

Recovery	Females			Males		
Dose (mg/kg/d)	0	300	% change	0	300	% change
Brain/BW	0.59	0.7*	↑19	0.41	0.49*	↑20
Liver/BW	2.61	2.89*	↑11	2.91	2.91	
Heart/BW	0.329	0.396*	↑20	0.303	0.361	↑19
Kidney/BW	0.664	0.78*	↑17	0.69	0.74	↑7
Adrenals/BW	0.018	0.031*	↑72	0.013	0.012	
Ovary/BW	0.026	0.034	↑31			
Testes/BW				0.64	0.73	↑14
Epi/BW				0.319	0.353	↑11
SemVes/BW				0.294	0.346	↑18
Prostate				0.103	0.135	↑31

Gross pathology: There were no drug-related macroscopic observations.

Histopathology:

	Treatment						Recovery			
	Males			Females			Males		Females	
Dose (mg/kg/d)	0	100	300	0	100	300	0	300	0	300
Bone Marrow: Hypocellularity	0 (10)		7 (11)	0 (10)		0 (10)				
Sternebra: Hypertrophy, epiphyseal	0 (10)		7 (11)	0 (10)		3 (10)	(5)	(4)	(5)	(5)
Femur/stifle:Hypertrophy, epiphyseal	0 (10)		11 (11)	0 (10)	1 (8)	8 (10)	(5)	1 (4)	(5)	(5)
Liver: Vacuolation, midzonal	0 (10)		2 (9)	0 (10)		0 (10)				
necrosis, focal	0 (10)		1 (9)	0 (10)		0 (10)				
infiltration, neutrophil, focal	0 (10)		1 (9)	0 (10)		0 (10)				
Kidney: Nephropathy, chronic progressive	0 (10)		1 (11)	0 (10)		0 (10)				
Pyelonephritis, bilateral, chronic, active	0 (10)		1 (11)	0 (10)		1 (10)				
Protein cast, focal	0 (10)		1 (11)	0 (10)						
Ureter: hypertrophy, muscularis	0 (10)		1 (1)							
Dilatation	0 (10)		1 (1)							
Depletion of round spermatids, Stage I-V tubules	0 (10)	5 (10)	8 (11)							

Drug-related microscopic observations were observed in sternebrae, femur/stifle and testes by Day 29/30.
Hypertrophy of the epiphyseal growth plate, characterized by an irregular thickening of the zone of hypertrophic chondrocytes with variable disorganization of chondrocyte columns and degenerative matrix changes, was present in sternebrae and femur/stifle of rats of both sexes. These changes were dose-dependent with greater incidence and severity in males. Minimal changes in sternebrae were observed in 3/10 females and 7/10 males given 300 mg/kg/day. Minimal to moderate changes in femur/stifle were observed in 1/10 and 8/10 females and 5/10 and 11/11 males given 100 and 300 mg/kg/day, respectively. Minimal hypocellularity of the metaphyseal bone marrow was noted in 7/11 male rats given 300 mg/kg/day.

Dose-dependent depletion of round spermatids, Stage I-V tubules, was observed in drug-treated males. Minimal depletion was noted in 5/10 males given 100 mg/kg/day and minimal to moderate depletion was noted in 8/11 males given 300 mg/kg/day.

By the end of the recovery period (Day 71), changes in the epiphyseal growth plates had largely reversed. One of four males treated with 300 mg/kg/day had resolving hypertrophy in the epiphyseal growth plate of the femur/stifle with evidence of vascular invasion. Testicular and metaphyseal marrow changes were reversed by the end of the recovery period.

Toxicokinetics: Refer to PK section

Summary of individual study findings:
Rats were given 0, 3, 10, 30, 100 or 300 mg/kg/day GW786034X for approximately 1 month followed by an approximately 6-week recovery period for control and high-dose animals. After one month of treatment, there was hypertrophy of epiphyseal growth plates and depletion of round spermatids in rats treated with 100 or 300 mg/kg/day and bone marrow hypocellularity in male rats treated with 300 mg/kg/day. After an approximate 6-week recovery period, testicular and marrow changes had reversed and 1/4 treated male rats had resolving hypertrophy in the epiphyseal growth plate in the femur/stifle. Elevations in ALT were clearly treatment-related at 300 mg/kg/day. Two males and 3 females were affected at this dose level. Two of the 3 females also had increased AST and one of the females also had an increased value for ALP. These 5 animals had no microscopic findings in the liver.

Growth plate changes in this study are consistent with those described as expansion of the hypertrophic chondrocyte zone of the growth plate of bones in a previous 4-day study in rats with GW786034A. This change is attributable to the pharmacological activity of GW786034X and is considered secondary to impaired vascular invasion of the growth plate of the sternebrae and femur/stifle. The mechanism of the morphological changes in metaphyseal bone marrow and testes may also be a consequence of the pharmacological activity of GW786034X and inhibition of VEGF activity. In bone marrow, VEGF plays an important role in growth and function of the hematopoietic microenvironment and, in vitro, VEGF receptor antagonism has been demonstrated to effect a reduction in cellular components of marrow microenvironment (fibroblasts, macrophages, endothelial cells), decrease blood cell production and increase fat cells. In the testis, VEGF may function as paracrine mitogenic and angiogenic factor. VEGF and/or its receptors are expressed in a variety of testicular cellular elements including endothelial and perithelial cells, spermatogenic cells, and Sertoli and Leydig cells. The basis for the excessive growth and apparent brittleness of incisors and nails observed in rats given 300 mg/kg/day during the recovery period has not been definitively established but may be a manifestation of rebound growth following pharmacological inhibition of VEGF activity by GW786034X.

Study title: 1-Month Oral Toxicity Study in Cynomolgus Monkeys (DRAFT)

Key study findings:
- The NOAEL was the highest dose tested 500 mg/kg/day.

Study no: G02013
Volume # 7 and page #: 1
Conducting laboratory and location: Department of Safety Assessment and the Drug Metabolism and Pharacokinetics Area, GlaxoSmithKline, King of Prussia, PA
Date of study initiation: 26-Feb-2002
GLP compliance: No (draft)
QA report: yes ( ) no ( )
Drug, lot #, radiolabel, and % purity: GW786034B (Batch/Lot No. 786034-A3-01P); 91.5% (free base)
Formulation/vehicle: 0.5% hydroxypropylmethylcellulose/0.1% Tween80, pH 1.3

Methods:
Dosing:

Species/strain:	Cynomolgus monkeys (Macaca fascicularis; Primate Products, Inc.)
#/sex/group or time point (main study):	3/sex/group
Satellite groups used for toxicokinetics or recovery:	None
Age:	2-7 years of age
Weight:	♂: 3.6-5.2 kg; ♀2-2.8 kg
Doses:	0, 5, 50 or 500 mg/kg/day daily, for at least 28 days
Route, form, volume, and infusion rate:	Oral by gavage; 5 mL/kg

Group	Dosage (mg/kg/day)*	Number of Animals	Animal Numbers
1	0	3M/3F	P02M-961 to P02M-963 P02F-964 to P02F-966
2	5	3M/3F	P02M-967 to P02M-969 P02F-970 to P02F-972
3	50	3M/3F	P02M-973 to P02M-975 P02F-976 to P02F-978
4	500	3M/3F	P02M-979 to P02M-981 P02F-982 to P02F-984

* Doses represent the parent compound.
† Each animal has a unique identification number, but variations of the animal identification numbers are used for different data collection systems. For example
Animal Number: P02M-961
In-Life (Datatox) and Pathology tables: 961

Observations and times:

Clinical signs:	Following animal release, all monkeys were observed daily for viability. A detailed clinical examination was performed for all monkeys once prior to the initiation of dosing and on Day 27.
Body weights:	Body weight measurements were recorded for all monkeys once prior to the initiation of dosing and on Days 1, 8, 15, 22 and 28.
Food consumption:	Not measured

Ophthalmoscopy:	Prior to the initiation of dosing and on Day 26, an indirect ophthalmoscope was used following pupillary dilation.
EKG:	Prior to the initiation of dosing and on Day 23 (approximately 2 hours post-dose), leads I, II, III, aVR, aVL, aVF, mV°, mVp and mVg were monitored using a three-channel electrocardiograph.
Hematology:	Following an overnight fast, blood was collected prior to the initiation of dosing and on Day 28.
Clinical chemistry:	Following an overnight fast, blood was collected prior to the initiation of dosing and on Day 28.
Urinalysis:	Following an overnight fast, urine was collected prior to the initiation of dosing and on Day 28.
Gross pathology:	Day 29 or 30
Organs weighed:	Brain, liver, heart, kidneys, adrenals, thymus, ovaries, testes, epidiymides, seminal vesicle.
Histopathology:	Day 29 or 30
Toxicokinetics:	On Days 1 and 28, serial blood samples were collected in Groups 1-4 (3/sex/group). Samples were collected prior to dosing and at the following nominal times after dosing: 0.5, 1, 2, 4, 8, 12, and 24 hours. Monkey plasma samples were assayed for GW786034X using a method based upon protein precipitation followed by LC/MS/MS analysis. The lower limit of quantification was 10.0 ng/mL for a 50 uL aliquot of monkey plasma. PK analysis of GW786034X plasma concentration-time data was performed using the standard algorithms of the non-compartmental pharmacokinetic analysis program.
Other:	On Day 29 or 30, two sections of liver were taken from each animal in Groups 1-4 and frozen immediately in liquid nitrogen prior to subsequent storage at approximately -60ºC or colder. The liver samples were the subject of a further study to investigate effects on the hepatic cytochrome P450 system. The cytochrome P450 evaluations were conducted under a separate protocoland reported separately. This additional study was the responsibility of Development Drug Metabolism and Pharmacokinetics. Stage-Dependent Evaluation of Spermatogenesis Stage-dependent evaluation of spermatogenesis was performed, when possible, for all male monkeys. PAS-stained sections of testis were examined for cell associations and proportions expected to be present during each of the following stages of spermatogenesis: I-III, IV dk: V, VI, VII, VIII A IX, X R XI and XII.

Results:

Mortality:	There were no drug-related deaths.
Clinical signs:	There were occasional episodes of emesis of similar frequency in both control and drug-treated animals throughout the dosing phase of the study. Discolored feces (tan), observed in most drug-treated animals, was thought to be due to the presence of drug-material and is not considered toxicologically significant.
Body weights:	There were no drug-related body weight changes.
Food consumption:	Not measured
Ophthalmoscopy:	There were no drug-related ophthalmologic changes.
Electrocardiography:	There were no drug-related electrocardiographic changes.
Hematology:	There were no remarkable hematology findings.
Clinical chemistry:	There were no remarkable clinical chemistry findings. Serum creatinine values for one male (P02M-981) and one female (P02F-983) given 500 mg/kg/day were minimally increased (~25%) on Day 28 compared to baseline values.
Urinalysis:	There were no drug-related urinalysis findings.
Organ weights:	There were no drug-related organ weight changes. The absolute and relative liver weight of one male monkey (P02M-979) given 500 mg/kg/day was increased approximately to 1.5-fold compared to the concurrent mean control values. Because there was no associated clinical or histopathological changes in this monkey, the liver weight was not considered by the Sponsor to be an adverse finding.
Gross pathology:	No gross observations were considered drug related or significant. A single animal (P02F-972) given 5 mg/kg/day had pinpoint red depressed areas in the pyloric region of the stomach which were determined to be focal areas of hemorrhage microscopically. Based on a single incidence and no dose response, this was considered by the Sponsor to be an incidental agonal change and was not considered drug- related.

Histopathology:

	Males N=3				Females N=3			
Dose (mg/kg/d)	0	5	50	500	0	5	50	500
Heart: Inflammatory cell infiltrate, mononuclear	0	1	0	0	0	0	0	2
Femus/stifle	0	0	0	0	0	0	0	0
Kidney: mineral deposit, tubular	1			1				
Vacuolation, transitional cell		1						
Inflammatory cell infiltrate, mononuclear		1	2	1	2	1	1	1
Dilatation, tubular	1							
Testis, immature	3	3	3	2				
Atrophy, unilateral, focal			1	1				
Epididymis-inclusion body, intracytoplasmic, epithelial, focal			1	1				
Inflammatory cell infiltrate, mononuclear			1	1				

There were no significant drug-related microscopic lesions identified in this study.

In the male monkeys, all but one animal (P02M-981) had immature testes, although all showed signs of maturing spermatogenesis. For the male monkey which had stage-dependent evaluation of spermatogenesis performed, the testis had expected cell associations and proportions in the various stages of spermatogenesis. There were two males (P02M-973, P02M-981) with focal epididymal epithelium intracytoplasmic eosinophilic round inclusions, in regions containing no luminal spermatids. These inclusions likely represent protein droplets engulfed by the epithelial cells, and were not considered by the Sponsor to be drug-related.

Two animals (P02M-968, P02M-980) had marked autolysis in several organs (adrenal glands, inguinal lymph node, thyroid/parathyroids, pituitary, and thymus). The pattern of individual animals with similar affected organs indicates that for these animals these organs were not immersed in fixative in a timely manner. However, the autolysis did not preclude proper histologic examination.

Toxicokinetics: Refer to PK section

Summary of individual study findings:
GW786034X was given orally by gavage, once daily, for up to 29 days to male and female monkeys at doses of 5, 50 and 500 mg/kg/day. There were no drug-related findings. The no observed adverse effect level (NOAEL) was 500 mg/kg/day.

Toxicology conclusions:

Species	Rat	Monkey
Doses	3, 10, 30, 100 or 300 mg/kg/day once daily for 28 days	0, 5, 50 or 500 mg/kg/day daily for 28 days
Mortality	One rat given 300 ing/kg/day was sacrificed on Day 21. Bilateral pyelonephritis and cystitis were observed microscopically. Sponsor attributed this animal's death to spontaneous disease and did not considered it drug-related.	There were no drug-related deaths.
Clinical Signs	None during the treatment period. During the recovery period, abnormal teeth (upper and/or lower incisors excessively long, broken, missing and apparently more friable than those of controls) and missing/broken nails on one or more digits were observed for all HD rats.	Discolored feces (tan), observed in most drug-treated animals, was thought to be due to the presence of drug-material.
Hematology	• ↓ Reticulocyte count for HD males (~20%). • End of recovery, (~↓12-26%) in red cell mass (hemoglobin concentration, hematocrit and total red cell count) and an ↑reticulocyte count (~2-3 fold) in HD.	• No significant hematological changes.
Clinical chemistry	• ↑ALT (~ 4- to 6-fold), AST (~ ↑3-fold) and ↑ALP (~3-fold) at HD • ↑ serum total bile acids at 100 mg/kg/day (24%; ♂) and by 48% and 56% at 100 and 300 mg/kg/d (♀), respectively.	• There were no remarkable clinical chemistry findings. •
Urinalysis	• ↑ Urine protein, ↑ urine creatinine, and ↓ urine volume ≥10 mg/kg/d ♂. • ↑ Urine protein to creatinine (P:C) ratios in two males given 100 mg/kg/day. • ↑ Urine protein in males given 100 or 300 mg/kg/day	There were no drug-related urinalysis findings.
Organ weights	• ↓ Mean testis weight (~ 12%) for HD ♂. • ↓ Mean liver (~ 10% ♀) at ≥30 mg/kg/day	• ↑ Mean liver weight of 1♂ at 500 mg/kg/day (~1.5-fold.)
Histopathology	• Bone marrow hypocellularity at HD • Minimal to moderate depletion of round spermatids (Stage I-V)at ≥100 mg/kg/d • Hypertrophy of the epiphyseal growth plate present in sternebrae and femur/stifle of rats of both sexes at ≥100 mg/kg/d • By the end of the recovery period, changes in the epiphyseal growth plates, testicular and metaphyseal marrow changes had largely reversed.	

The preclinical safety of GW786034 was evaluated in 4 to 30 day oral repeat dose studies in rats and cynomolgus monkeys. GW786034 was tolerated in a repeat dose oral toxicity study in rats at doses up to 300 mg/kg/day for 28 days, the highest dose tested. Increases in liver function tests (ALP, ALT, AST, and/or bile acids) occurred in individual animals receiving doses > 100 mg/kg/day, however there were no liver histopathological findings. Additional target organ effects were observed in bone, bone marrow, testes and ovary. Hypertrophy of the epiphyseal growth plate was seen after 4 and 29 days in the sternebrae and/or femur of rats given > 100 mg/kg/day while minimal hypocellularity of the metaphyseal bone marrow occurred after 28 days in male rats given 300 mg/kg/day. Both the bone and bone marrow effects were attributed to the pharmacological inhibition of VEGFR2 by GW786034 as VEGF has been shown to be involved in the cartilage remodeling, ossification and angiogenesis during endochondral bone formation [Gerber, 1999] and hematopoiesis [Hamada, 2000]. Male rats given > 100 mg/kg/day for 28 days also exhibited a dose-dependent depletion of round spermatids in Stages I-V. The testes effect may also be attributed to VEGFR2 inhibition as VEGF and/or its receptors are expressed in a variety of testicular elements including endothelial and perithelial cells, spermatogenic cells, and Sertoli and Leydig cells [Ergun, 1997; Korpelainen, 1998]. All target organ effects had reversed or were progressing to recovery by the end of a 6 week recovery period. Coagulative necrosis of the corpora lutea, also believed to be VEGF mediated [Ferrara, 1998], occurred after 4 days in rats given > 100 mg/kg/day, however this finding was not seen after 29 days of dosing. The lack of effect on corporea lutea may have resulted from lower systemic exposures obtained in the 29 day study compared to the 4 day study. Alternatively, this effect could be dependent upon follicular growth that, if suppressed, is not observed in longer exposure studies. This issue will be more fully assessed in reproductive toxicology studies. Approximately 2 weeks into the recovery phase, excessive growth and apparent brittleness of the upper/lower incisors and missing/broken nails were noted in the rats that had been given 300 mg/kg/day. These findings, which may be attributed to rebound growth following pharmacologic activity of GW786034, had not fully resolved by the end of the recovery period. The relevance of the incisor finding to the adult human does not correlate because unlike the adult human where tooth growth is absent, rat incisors are growing continually making the incisor a potential target for vascular disruption due to a VEGFR2 inhibitor like GW786034. Nonetheless, it should be noted that this finding uderscores a pharmacodynamic action suggesting that the rat is an appropriate species for toxicological assessment.

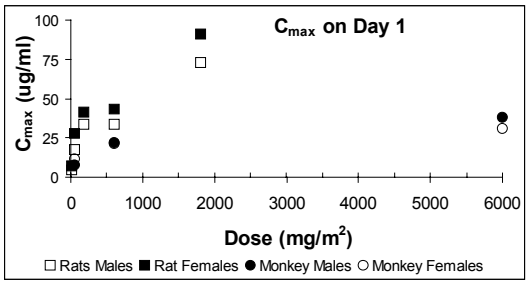
There was a trend for higher mean systemic exposure (AUC) for female rats when compared to male rats, while the increase in exposure in both sexes was less than proportionate to dose suggesting saturation of exposure. There were no marked differences in systemic exposure between Days 1 and 28 except for the 300 mg/kg/day dose group where an average 61% decrease in systemic exposure occurred from Day 1 to Day 28.

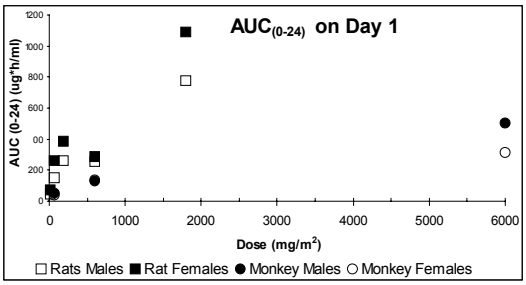
GW786034 was well tolerated with no toxicity observed in a repeat dose oral toxicity study in monkeys given up to 500 mg/kg/day for 28 days. In monkeys, the increase in maximum plasma concentrations of GW786034 was also less then proportionate to the increase in dose also suggesting saturation of exposure, however there were no marked differences in systemic exposure between males and females or between Days 1 and 28.

It is noteworthy that feeding affected the plasma GW786034 concentrations achieved following oral administration of GW786034B to male dogs. Feeding 1 hour prior to oral dose

administration of 1 mg/kg GW786034B resulted in a 4 to 5-fold decrease in plasma GW786034 C max and AUC_{0-t} values compared to dosing in the fasted state. Additionally, plasma concentrations in fasted dogs were observed to decrease quickly (15-fold decline) between 8 and 12 hours, corresponding to the time of feeding, while no change in plasma concentrations was observed in the fed animals during the same timeframe.

		Cmax				**AUC**			
	Dose	Day 1		Day 28		Day 1		Day 28	
Species	mg/m2/d	Male	Female	Male	Female	Male	Female	Male	Female
Rat	18	4.28	7.08	9.58	10.4	44.2	71.3	54.9	83
Rat	60	17.5	28	21.7	28.7	148	259	116	232
Rat	180	33.6	41.3	29.9	51	261	388	221	365
Rat	600	33.3	43.3	26.3	38.6	253	288	174	257
Rat	1800	73.1	90.8	38.8	56	779	1090	258	472
Monkey	60	7.8	11.6	11.7	12.1	50.6	37.9	46.6	36
Monkey	600	21.9	21.6	20.6	19.6	129	135	120	99.5
Monkey	6000	37.8	30.9	40.9	30.6	503	313	296	335





In summary, there were no toxic-effects in monkeys given 28 daily oral doses of up to 500 mg/kg/day (NOAEL) GW786034 resulting in a maximum systemic exposure of 335 µg.h/mL. In the rat, drug-related effects were limited to mild liver enzyme increases at AUC values as low as 258 µg.h/mL (> 100 mg/kg/day), with pharmacologically mediated changes due to VEGFR2 inhibition seen in bone and bone marrow at an AUC > 174 µg.h/mL (> 100 mg/kg/day) and a reduction in the number of Stage I-V round spermatids at an AUC of 258 µg.h/mL (300 mg/kg/day) occurring after 29 days of dosing. Effects on rat nail growth and structure were seen 2 weeks into the recovery period after one month of dosing, however, all drug-related effects had reversed or were progressing to recovery by the end of a 6 week recovery period.

The Sponsor proposes a starting dose of 200 mg GW786034X. Administration of up to 300 mg/kg/d x 28 days of GW786034B (1800 $mg/m^2/d$) in rats and doses of up to 500 mg/kg/d (6000 $mg/m^2/d$) in monkeys did not result in any mortality. Based on the toxicology data, a reasonably safe starting dose can be estimated as 180 mg/m^2 (~306 mg for an average 1.7 m^2 human). Thus, based on preclinical studies in rats and monkey, the dose proposed by the Sponsor appears reasonably safe.

Histopathology Inventory for IND

Study	G01200	G02013			
Species	Rat	Monkey			
Adrenals	X	X			
Aorta	X	X			
Bone Marrow smear					
Bone (femur)	X	X			
Brain	X	X			
Cecum	X	X			
Cervix	X	X			
Colon	X	X			
Duodenum	X	X			
Epididymis	X	X			
Esophagus	X	X			
Eye	X	X			
Fallopian tube					
Gall bladder		X			
Gross lesions	X	X			
Harderian gland	X	X			
Heart	X	X			
Ileum	X	X			
Injection site					
Jejunum	X	X			
Kidneys	X	X			
Lachrymal gland					
Larynx	X	X			
Liver	X	X			
Lungs	X	X			
Lymph nodes, cervical	X	X			
Lymph nodes mandibular	X	X			
Lymph nodes, mesenteric	X	X			
Mammary Gland	X	X			
Nasal cavity					
Optic nerves					
Ovaries	X	X			
Pancreas	X	X			
Parathyroid	X	X			
Peripheral nerve					
Pharynx					
Pituitary	X	X			
Prostate	X	X			
Rectum	X	X			
Salivary gland	X	X			
Sciatic nerve					
Seminal vesicles	X	X			
Skeletal muscle					
Skin	X	X			
Spinal cord	X	X			
Spleen	X	X			
Sternum					
Stomach	X	X			
Testes	X	X			
Thymus	X	X			
Thyroid	X	X			
Tongue	X	X			
Trachea	X	X			
Urinary bladder	X	X			
Uterus	X	X			
Vagina	X	X			
Zymbal gland					
Standard List					

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY:

This information was excerpted from the Investigator's Study Summary.
Report RD2002/00887/00 - Salmonella-Escherichia Coli/mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay Vol 7 Page 339

SUMMARY

Table 1: Summary of Microbial Mutagenicity Data for GW786034B (Batch 786034-A2-01M)

Mean Number of Revertant Colonies per Plate [a]											
		Salmonella typhimurium								*Escherichia coli*	
GW786034X Concentration (µg/plate)	S9 metabolic activation	TA98		TA100		TA1535		TA1537		WP2*uvrA* (pKM101)	
		Initial Assay	Confirm-atory Assay	Initial Assay	Confirm-atory Assay	Initial Assay	Confirm-atory Assay	Initial Assay	Confirm-atory Assay	Initial Assay	Confirm-atory Assay
0 [b]	W	28	30	93	87	15	13	15	12	169	163
33.3	W	29	33	93	83	15	16	9	11	184	176
100	W	26	54	106	107	13	18	14	13	186	178
333	W	26	40	103	90	12	16	11	15	153	191
1000	W	21	32	100	97	17	16	11	12	193	193
3330	W	23	31	96	93	14	16	11	9	189	207
5000	W	27	32	92	85	11	12	11	11	180	222
Positive control [c]	W	301+	317+	368+	642+	119+	169+	99+	137+	1470+	967+
0 [b]	N	8	10	60	85	7	13	9	7	132	97
33.3	N	8	16	49	88	9	13	8	6	137	108
100	N	4	14	48	87	6	13	7	4	131	135
333	N	7	13	47	76	13	14	3	3	137	120
1000	N	7	12	44	80	5	16	6	5	125	66
3330	N	5	10	43	58	5	10	5	3	109	89
5000	N	5	8	38	59	7	10	5	4	90	74
Positive control [c]	N	96+	408+	725+	1342+	587+	986+	657+	1160+	2785+	2648+
Experimental work dates: 19 March 2002 – 15 April 2002				Study in compliance with GLP: Yes				Batch No.: 786034-A2-01M			

- GW786034B, batch number 786034-A2-01M, was not mutagenic in the Salmonella and E. coli / mammalian microsome standard plate incorporation assay (Ames test). No mutagenic effects were detected with any of five strains [TA98, TA100, TA1535, TA1537, and WP2uvrA(pKM101)] in the presence and the absence of rat liver S9 metabolic activation at dose levels ranging up to 5000 pg/plate.

- The standard plate incorporation assay was performed at dose levels of 33.3, 100, 333, 1000, 3330, and 5000 pg/plate; all dose levels are expressed as the free base, GW786034X. Two independent assays were conducted. Positive controls produced an appropriate mutagenic response in all bacterial strains in the presence and absence of S9. All dosing solutions were within+ 10% of the nominal concentration except for one dosing solution in the initial assay, which was about 137% of the nominal concentration.

Thus, GW786034B, batch 786034-A2-01M, was not mutagenic in either the presence or the absence of S9 metabolic activation in the standard plate incorporation assay.

Report RD2002/00238/00 - GW786034B: In vitro Assay for Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes Vol 8 Page 1

Table 1. Summary Data of Structural Chromosome Aberrations for GW786034B in Human Lymphocytes

GW786034B Concentration (µg/mL)	% Cells with Aberrations [a]				
	3.0 / 22.0 (–S9)	22.0 / 22.0 (–S9)	22.0 / 45.8 (–S9)	3.0 / 21.9 (+S9)	3.0 / 45.8 (+S9)
Negative Control [b]	1.0	0.5	0.0	0.0	0.5
Solvent Control [c]	0.0	0.0	0.5	0.0	0.0
5.05	ND	0.0	ND	ND	ND
10.0	ND	ND	1.0	ND	0.5
10.1	0.0	0.0	ND	ND	ND
25.0	ND	ND	0.0	0.0	0.0
25.3	0.5	1.0	ND	ND	ND
50.0	ND	ND	0.0	0.5	0.0
50.5	0.5	0.5	ND	ND	ND
100	ND	ND	1.0	1.0	0.0
101	1.0	ND	ND	ND	ND
200	ND	ND	1.0	1.0	ND
Mitomycin C [d]	41.0*	32.0*	.22.5*	ND	ND
Cyclophosphamide [e]	ND	ND	ND	33.6*	68.0*

Study in compliance with GLP: Yes Experimental work dates: 15 March 2002 through 15 May 2002

Assay Results: GW786034B did not cause an increase in structural chromosome aberrations, polyploidy, or endereduplication at any dose level tested in the absence or presence of S9 metabolic activation. The highest dose analyzed for each treatment/ metabolic activation condition was the first precipitating dose or a dose with ≥ 50% reduction in mitotic index. Assays with a ≥ 50% reduction in mitotic index were (i) 22.0 hour treatment/22.0 hour harvest without S9, (ii) 22.0 hour treatment/45.8 hour harvest without S9. GW786034B, batch number 786034-A2-01M, was not clastogenic.

Key for Table

[a] The mean value is given for replicate cultures for the percentage of cells with aberrations (gaps not included). One hundred cells were examined whenever possible from each replicate culture, thus a total of 200 cells were evaluated for aberrations for the negative control, solvent control and test article cultures. Fifty cells were examined for each replicate positive control culture. Table columns give the Treatment / Harvest time in hours.

[b] The negative control was RPMI 1640 tissue culture medium.

[c] The solvent was DMSO which was at a final concentration in tissue culture media of 1% (vol/vol).

[d] Mitomycin C was used as a positive control without metabolic activation at concentrations of 1.00 µg/mL for the initial assay (3.0/22.0) and 0.300 µg/mL for initial assay (22.0/22.0) and 0.100 µg/mL for the confirmatory assay (22.0/45.8).

[e] Cyclophosphamide was used as a positive control with metabolic activation at concentrations of 25.0 µg/mL for the initial assay (3.0/21.9) and 100 µg/mL for the confirmatory assay (3.0, 45.8).

ND Indicates not done.

* Indicates values statistically different from the control values.

- GW786034B was considered negative for inducing structural chromosome aberrations, polyploidy, and endoreduplication in human peripheral lymphocytes both in the absence and presence of exogenous S9 metabolic activation.
- The high dose for these assays was limited by the (i) presence of test article precipitation or (ii) significant toxicity, as evidenced by a reduction in relative mitotic index to < 50% of the control values.
- The positive and solvent controls fulfilled the requirements for a valid test.

Report RD2002/00227/00 - GW786034B: Micronucleus Frequencies in Bone Marrow Polychromatic Erythrocytes from Male Sprague-Dawley Rats Following Oral Administration Vol 8 Page 89

GW786034B: Micronucleus Frequencies in Bone Marrow Polychromatic Erythrocytes from Male Sprague Dawley Rats Following Oral Administration (Glaxo Wellcome Study No. R41043)

Glaxo Wellcome Report No.: RD2002/00227/00	**Glaxo Wellcome Study No.:** R41043	**Pharmacological Class:** Vascular endothelial growth factor receptor 2 (VEGFR2) inhibitor
Species/Strain: Rat/Sprague Dawley	**Route:** Oral Gavage	**Testing Facility:** (b) (4)
Weight Range on Day 1: 231 – 266 grams	**Drug Reference No.:** GW786034B	
	Drug Batch No.: 786034-A2-01M	
	Vehicle: 0.5% HPMC with 0.1% Tween®80 in reverse osmosis water	
Age on Day 1: Approximately 8 weeks	**Dose Volume:** 20mL/kg/day	**Study in Compliance With GLP:** Yes

Study Design

Group[1] No.	Dose (mg/kg/day)[2]	Dose Concentration (mg/mL)	Number of Male rats
1	0	0	7
2	1250	62.5	7
3	2000	100	7
4 [3]	60	6	7
5 [4]	0	0	3
6 [4]	1250	62.5	3
7 [4]	2000	100	3

[1] GW786034B and vehicle control animals were dosed on Day 1 and Day 2. Animals were sacrificed and the bone marrow was removed approximately 24-hr following the second dose.
[2] All dose levels refer to the free base.
[3] Cyclophosphamide at 60mg/kg was given once on Day 2
[4] Animals used for plasma samples only

Data Collected: Micronucleus frequencies, PCE/NCE ratios, clinical signs, body weight

Results

Treatment	Dose (mg/kg/day)	Mean Ratio PCE:NCE 1000 cells per animal ± S.E.	Mean % MPCEs, ± S.E., 2000 cells per animal examined	Mean Plasma Levels[1] 4hr post dose, Day 2 (ng/mL)
Vehicle Control	0	0.90 ± 0.02	0.08 ± 0.01	ND
GW786034B	1250	0.90 ± 0.02	0.12 ± 0.02	79256.1
	2000	0.95 ± 0.01	0.14 ± 0.02	70945.4
Positive Control	60	0.76 ± 0.03*	3.36 ± 0.18*	ND

Key to table:
1 For TK analysis samples were taken approximately 4-hr following the second dose.
* Values statistically different than the vehicle control values
ND: Not Determined

- GW786034B did not produce an increase in the frequency of micronuclei in bone marrow polychromatic erythrocytes of male Sprague Dawley rats receiving administrations of 1250 and 2000 mg/kg/day by oral gavage.
- Equivalent systemic exposure was seen 4 hours after dosing on day 2 in both doses (79.3 verses 70.9 pg/mL).
- Positive and negative controls gave the appropriate responses.

Thus GW786034B was not clastogenic in an in vivo micronucleus assay.

Genetic Toxicology Summary
GW786034 produced no evidence of mutagenic or clastogenic activity in a range of *in vitro* microbial and mammalian cell test systems or in an *in vivo* micronucleus test.

VI. CARCINOGENICITY:
No carcinogenicity studies were submitted.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:
No studies were submitted.

VIII. SPECIAL TOXICOLOGY STUDIES:
No studies were submitted.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:
The Sponsor proposes a starting dose of (b) (4) GW786034X. Administration of up to 300 mg/kg/d x 28 days of GW786034B (1800 mg/m^2/d) in rats and doses of up to 500 mg/kg/d (6000 mg/m^2/d) x 28 days in monkeys did not result in any mortality. Based on the toxicology data, a reasonably safe starting dose can be estimated as 180 mg/m^2 (~306 mg for an average 1.7 m^2 human). Thus, based on preclinical studies in rats and monkey, the dose proposed by the Sponsor appears reasonably safe.

General Toxicology Issues:

- After one month of treatment with GW786034X, there was hypertrophy of epiphyseal growth plates in rats treated with 100 or 300 mg/kg/day. After an approximate 6-week recovery period, 1/4 treated male rats had resolving hypertrophy in the epiphyseal growth plate in the femur/stifle. This change appears attributable to the pharmacological activity of GW786034X and is considered secondary to impaired vascular invasion of the growth plate of the sternebrae and femur/stifle. In humans, growth plate closure may occur up to approximately 21 years of age, thus GW786034X has the potential to cause a disruption in this process. For first time in humans, the Sponsor should consider the accrual of patients older that 21 years of age.

- Approximately 2 weeks into the recovery phase, excessive growth and apparent brittleness of the upper/lower incisors and missing/broken nails were noted in the rats that had been given 300 mg/kg/day. These findings, which may be attributed to rebound growth following pharmacologic activity of GW786034, had not fully resolved by the end of the recovery period. The relevance of the incisor finding to the adult human is unclear because unlike the adult human, where tooth growth is absent, rat incisors are growing continually making the incisor a potential target for vascular disruption due to a VEGFR2 inhibitor like GW786034.

- Feeding affected the plasma GW786034 concentrations achieved following oral administration of GW786034B to male dogs. Feeding 1 hour prior to oral dose administration of 1 mg/kg GW786034B resulted in a 4 to 5-fold decrease in plasma GW786034 C_{max} and AUC_{0-t} values compared to dosing in the fasted state. Additionally, plasma concentrations in

fasted dogs were observed to decrease quickly (15-fold decline) between 8 and 12 hours, corresponding to the time of feeding, while no change in plasma concentrations was observed in the fed animals during the same timeframe.

Recommendations:
This trial may proceed as proposed by the Sponsor.

Communication to Sponsor:

1. Your IND included draft reports of preclinical toxicology studies. We thus remind you that finalized, quality assured reports for study G01200 "1-Month oral toxicity and reversibility study in rats" and study G02013 "1-Month Oral Toxicity Study in Cynomolgus Monkeys" should be available to us upon request before 1/8/2003 which is 120 days from the date of our receipt of the IND. You should also submit to us a description of any differences between the finalized study reports and the information presented in the initial draft reports. Please refer to the Guidance for Industry document "Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-characterized, Therapeutic, Biotechnology-derived Products" for further details.

2. After one month of treatment with GW786034X, there was hypertrophy of epiphyseal growth plates in rats treated with 100 or 300 mg/kg/day. This change appears attributable to the pharmacological activity of GW786034X and is considered secondary to impaired vascular invasion of the growth plate of the sternebrae and femur/stifle. In humans, growth plate closure may occur up to approximately 21 years of age, thus GW786034X has the potential to cause a disruption in this process. For first time in humans, you should enroll only patients older that 21 years of age.

3. Given your findings showing that feeding prior to oral administration of GW786034 to dogs resulted in a 4 to 5-fold decrease in drug plasma concentrations, please clarify whether GW786034 will be administered to patients with or without food.

4. Please initiate the 6-month toxicology study in rats as soon as practical. If necessary, contact the Division for further discussion of this request.

Labeling with basis for findings: N/A

Reviewer signature: Lilliam A. Rosario, Ph.D.

Supervisor signature: Concurrence - _____

Non-Concurrence - _____
(see memo attached)

cc: list:
rosariol
leightonj
whiter
johnsonj

X. APPENDIX/ATTACHMENTS:

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

/s/

Lilliam Rosario
10/7/02 01:08:07 PM
PHARMACOLOGIST

John Leighton
10/7/02 02:08:18 PM
PHARMACOLOGIST

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22465	ORIG-1	GLAXO WELLCOME MANUFACTURING PTE LTD DBA GLAXOSMITHKLINE	VOTRIENT TABLETS

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/s/

ROBEENA M AZIZ
09/18/2009

WHITNEY S HELMS
09/18/2009

SANDI L VERBOIS
09/18/2009

MEMORANDUM

Votrient (pazopanib)

Date: October 9, 2009
To: File for NDA 22-465
From: John K. Leighton, PhD, DABT
Associate Director for Pharmacology
Office of Oncology Drug Products

I have examined pharmacology/toxicology supporting review and labeling provided by Drs. Aziz and Helms and supervisory concurrence provided by Dr. Verbois. I concur with their conclusions that Votrient may be approved. No additional pharmacology or toxicology studies are needed to support the proposed indication.

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/s/

JOHN K LEIGHTON
10/09/2009

MEMORANDUM

Date: September 18, 2009
From: S. Leigh Verbois, Ph.D.
Supervisory Pharmacologist
Division of Drug Oncology Products
To: File for NDA #22-465
Pazopanib (VOTRIENT)
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies that investigated the pharmacology and toxicology of pazopanib provided to support NDA 22-465 for the treatment of patients with advanced renal cancer were reviewed in detail by Robeena Aziz, Ph.D. and Whitney Helms, Ph.D. The supporting information included studies of intravenously and orally administered pazopanib that investigated the drug's pharmacology, pharmacokinetics, safety pharmacology, general toxicology (rats and dog), genetic toxicity (*in vivo* and *in vitro*), and reproductive toxicity in both rats and rabbits. The studies cited in the review by Drs. Aziz and Helms consist primarily of original research conducted by the applicant.

The general pharmacology studies submitted to the NDA demonstrate that pazopanib is a kinase inhibitor. Toxicities observed after pazopanib administration included toxicity in the bone marrow, gastrointestinal tract, liver, reproductive tract, and teeth and bone (in rats). These toxicities are consistent with the mechanism of action.

Pazopanib was neither mutagenic (Ames assay) nor clastogenic *in vitro (*human peripheral blood lymphocyte assay), nor was pazopanib clastogenic in the *in vivo* (rat micronucleus study). (b) (4), a starting material for the drug substance, has a structural alert for genotoxicity and was found to be positive in both the Ames Assay and the mouse lymphoma assay. However the level of (b) (4) in the drug substance is significantly lower (b) (4) than the acceptable daily intake of genotoxic impurities defined in the draft Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches for products administered for ≥12 months (1.5 µg/day) and is therefore acceptable. Carcinogenicity was not assessed but is not considered necessary for approval of treatment of this patient population.

Pazopanib did not affect mating or fertility in male rats. In females, pazopanib increased resorptions, reduced number of corpora lutea and increased pre and post-implantation loss. In embryofetal development studies toxicity in rats and rabbits (resorptions, abortions, decreased fetal body weights) occurred in the absence of maternal toxicity.

Recommendations: I concur with Dr. Aziz's and Dr. Helms' conclusion that pharmacology and toxicology data support the approval of NDA 22-495, VOTRIENT (pazopanib). There are no outstanding nonclinical issues related to the approval of VOTRIENT (pazopanib) for the proposed indication.

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/s/

SANDI L VERBOIS
09/18/2009