CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202806Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Tafinlar (dabrafenib)

Date: April 25, 2013
To: File for NDA 202806
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Tafinlar conducted by Drs. Putman and Weis and secondary memorandum and labeling provided by Dr. Helms. I concur with Dr. Helms' conclusion Tafinlar may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
04/25/2013

MEMORANDUM

Date: April 17, 2013
From: Whitney S. Helms, Ph.D.
Pharmacology Team Leader
Division of Hematology Oncology Toxicology for Division of Oncology Products 2
To: File for NDA #202806
Dabrafenib (TAFINLAR)
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies examining the pharmacology and toxicology of dabrafenib provided to support NDA 202806 for the treatment of patients with V^{600E} mutation positive metastatic melanoma were reviewed in detail by Alexander H. Putman, Ph.D., and Shawna L. Weis, Ph.D. The submission included studies of orally administered dabrafenib in mice, rats, and dogs that investigated the drug's pharmacology, pharmacokinetics, safety pharmacology, general toxicology, genetic toxicity (*in vivo* and *in vitro*), and reproductive toxicity.

The pharmacology studies submitted to this NDA demonstrate that dabrafenib is an ATP competitive kinase inhibitor. Dabrafenib inhibited several BRAF kinases with mutations resulting in substitutions at the V600 amino acid residue. Specifically dabrafenib was able to inhibit BRAF V600E, V600K, and V600D at IC_{50} values of less than 2 nM *in vitro* in biochemical assays. Dabrafenib was also able to inhibit wild type BRAF and CRAF at similar concentrations (≤ 5 nM) along with LMK1, ALK5, NEK11, CK1 and SIK1 (all ≤50 nM). In cellular assays dabrafenib showed growth inhibition in the majority of the tested cell lines carrying BRAFV600E mutations but low inhibition of most cells with wild type BRAF.

Three major human metabolites were identified during the development of dabrafenib. All three were present in animal models of toxicity and were shown to have inhibitory activity against BRAF and the BRAFV600E, V600K, and V600D mutations. The desmethyl-dabrafenib metabolite had activity similar to that of the parent compound followed by the hydroxyl-dabrafenib and carboxy-dabrafenib metabolites. While levels of desmethyl- and carboxy-dabrafenib were higher in humans than in animals, the presence of these compounds in animal toxicology studies at levels of up to 30% and 50% of the human exposure at the recommended dose, respectively, suggests that their toxicity has been reasonably characterized in animal studies. No dedicated studies examining the safety of these metabolites are required for the use of dabrafenib in patients with unresectable or metastatic melanoma. Several unique human metabolites were identified as well; however, all unique human metabolites were present at levels <1% of the parent compound and, consistent with the ICH S9 guidance for the development of pharmaceuticals to treat advanced cancer, further characterization of these metabolites is not warranted at this time.

Consistent with the effects of other known inhibitors of BRAF and BRAFV600E mutations, treatment with dabrafenib has been associated with the development of cutaneous squamous cell carcinomas. The mechanism for this increase in cutaneous squamous cell carcinomas was not addressed in this application. The Applicant did submit published reports of ongoing

investigations into the apparent pharmacologically-mediated growth enhancement of cell lines containing wild type BRAF or mutations in RAS following exposure to BRAF inhibitors. Reports suggest that treatment of cells with RAF inhibitors in the absence of BRAF activating mutations can lead to paradoxical upregulation of MEK signaling in the RAS/RAF/MEK/ERK pathway. In these published reports, exposure to Raf inhibitors resulted stimulated tumor growth in BRAF wild type xenograft models and led to hyperplasia of gastric and esophageal epithelial cells in mice. In additional models hyperplasia was also observed in transitional epithelium of the kidney, heart, and urinary bladder. While dabrafenib itself was not specifically included as a test-article in the published reports included in this NDA submission describing this phenomenon, similar findings of hyperplasia of the non-glandular stomach were reported in the rat toxicology studies submitted to support the application. Currently, no substantial clinical evidence exists for the development of secondary tumors following treatment with dabrafenib other than the cutaneous squamous cell carcinomas and new primary melanomas described in the label. If, in the future, use of dabrafenib in other patient populations is explored, further pharmacology studies may needed in order to understand the activity of the drug in these populations and in the development of secondary tumors. Genotoxicity tests were negative both *in vivo* and *in vitro*. *In vitro* genotoxicity tests were not submitted for the major active human metabolites; however, as all three metabolites were shown to be present in rat plasma and the *in vivo* micronucleus test performed in rats was negative, no further testing of these metabolites was requested. Carcinogenicity studies were not required to support the marketing application and, with positive findings of carcinogenicity in humans, carcinogenicity studies are neither planned nor expected as a post-marketing requirement.

The major target organs for toxicity in both rats and dogs appeared to be the skin and male reproductive system. Skin toxicities are the most broadly reported adverse events noted clinically as well. Findings of toxicity to the male reproductive tract consisted primarily of degeneration/depletion and aspermia in the testis and epididymis, sometimes accompanied by decreases in organ weights, of all dabrafenib-treated males in both species in 13-week toxicology studies. These findings were observed at all dose levels with dose-dependent increases in severity. In the 13- week rat toxicology study, at doses resulting in dabrafenib exposures (by AUC) that exceeded the exposure at the recommended human dose by approximately two-fold, at least 50% of the animals had histopathological signs of severe aspermia even at the end of the recovery period. In dogs there were no recovery animals at dose levels that survived until the end of the study, thus, it was not possible to assess the reversibility of the findings. The possibility of male infertility is included in the label for TAFINLAR.

Based on the results of *in vitro* hERG testing ($IC_{50} \geq 11 \mu M$) and *in vivo* findings from cardiovascular studies, there appears to be low potential for dabrafenib-mediated QT prolongation; however histopathological findings in both 4- and 13-week dog studies do suggest that the heart is a potential target organ for dabrafenib-mediated toxicity. In the 4-week study, one of six dogs at the high dose level displayed marked hypertrophy of the right atrioventricular valve characterized by focal hemorrhage with fibrin deposition in the valve. Similar findings were noted in male and female dogs in the 13-week study. Though all histopathological findings of toxicity to the atrioventricular valve in animals occurred at doses resulting in exposures more than 10 fold greater than those observed clinically at the recommended dose, this information was included in the label under section 13.2.

Reproductive toxicity studies conducted for dabrafenib consisted of two combined fertility and embryofetal development study conducted in female Sprague Dawley rats. Due to the design of the studies, specific effects of dabrafenib on female fertility and early embryonic exposure cannot be easily distinguished from effects during later stages of pregnancy; however, fertility studies are not required to support a marketing application for a drug indicated for the treatment of patients with advanced cancer and there were clear effects of dabrafenib on embryofetal development. In the main study at doses resulting in exposures approximately equal to the human exposure at the recommended dose, fetal rats presented with delays in skeletal development and reduced body weight. At higher doses resulting in clear decreases in maternal body weight, administration of dabrafenib to female rats resulted in decreases in number of corpora lutea and increases in pre- and post-implantation loss. Increased pre-implantation loss was also observed in the dose range finding study at doses resulting in exposures approximately equivalent to those in humans at the recommended dose. Based on these studies and its mechanism of action, Pregnancy Category D is recommended for dabrafenib.

The phototoxic potential of dabrafenib was assessed in the Neutral Red Uptake assay. Under the conditions of the assay, dabrafenib has clear phototoxic potential; however clear signs of phototoxicity have not been reported in clinical trials to date.

Recommendations: I concur with the conclusion of Drs. Putman and Weis that the pharmacology and toxicology data support the approval of NDA 202806 for TAFINLAR. There are no outstanding nonclinical issues related to the approval of TAFINLAR for the proposed indication in the BRAFV600E positive patient population.

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/s/

WHITNEY S HELMS
04/17/2013

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	202806
Supporting document/s:	3
Applicant's letter date:	July 30, 2012
CDER stamp date:	July 30, 2012
Product:	Dabrafenib (Tafinlar™)
Indication:	Treatment of patients with unresectable or metastatic melanoma with BRAF V600 mutation as detected by an FDA approved test
Applicant:	GlaxoSmithKline
Review Division:	Division of Hematology Oncology Toxicology
Reviewer:	Alexander H. Putman, Ph.D. Shawna L. Weis, Ph.D.
Supervisor/Team Leader:	Whitney S. Helms, Ph.D.
Division Director:	John Leighton, Ph.D.
Project Manager:	Norma S. Griffin

Disclaimer
Except as specifically identified, all data and information discussed below and necessary for approval of 202806 are owned by GlaxoSmithKline or are data for which GlaxoSmithKline has obtained a written right of reference. Any information or data necessary for approval of 202806 that GlaxoSmithKline does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of 202806.

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1 Executive Summary

1.1 Introduction

GlaxoSmithKline submitted a New Drug Application (NDA) for Dabrafenib (Tafinlar™) in patients with unresectable or metastatic melanoma with BRAF V600 mutation as detected by an FDA approved test.

Dabrafenib is an orally administered, RAF kinase inhibitor of the mutated forms BRAF V600E, BRAF V600K, and BRAF V600D as well as wild-type BRAF and CRAF kinases. Other kinases inhibited at clinically achievable concentrations include CK1, SIK1, NEK11, ALK5, and LIMK1.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical pharmacology studies conducted *in vitro* and *in vivo* demonstrated that dabrafenib is an inhibitor of wild-type BRAF (IC_{50} = 3.2 nM), wild-type CRAF (IC_{50} = 5 nM), and some mutant forms of BRAF kinases. Specifically, dabrafenib inhibited BRAFV600E, BRAFV600K, and BRAFV600D kinases with IC_{50} values of 0.65, 0.5 and 1.84 nM, respectively. Dabrafenib-induced inhibition of BRAF kinases appeared to be time-dependent, reversible, and ATP-competitive. *In vitro* incubation with dabrafenib also led to decreased phosphorylation of extracellular signal regulated kinase (ERK) in cell lines and produced tumor growth inhibition in mice bearing BRAFV600E mutant human tumor xenografts.

Dabrafenib exhibited high oral bioavailability in animals (46-82%), moderate- to low-clearance, particularly in dogs (< 12% hepatic blood flow), and strong protein binding (>98% in all species tested, including human). *In vivo* distribution studies indicated that dabrafenib was widely distributed to most major organs. Elimination of dabrafenib in humans occurs predominantly via the fecal route (~71% of administered dose) and urine (~22%). This elimination profile is distinct from both nonclinical species, which exhibited little (~ 1% or less) or no urinary elimination of dabrafenib.

Dabrafenib was a moderate inhibitor of CYP3A4, CYP2C9, and CYP2C19, a weak inhibitor of CYP1A2 and CYP2D6, and a substrate of human P-glycoprotein. The three main dabrafenib metabolites in humans were GSK2285403 (M7; hydroxyl-dabrafenib), GSK2298683 (M4; carboxy-dabrafenib), and GSK2167542 (M8; desmethyl-dabrafenib). Following administration of the recommended twice daily 150 mg dose of dabrafenib, these three metabolites were present at human plasma levels (AUC_{0-24}) of approximately 8, 100, and 6 µg*h/mL, respectively. All three metabolites were also present in rats and dogs during the 13-week repeat-dose toxicity studies. Specifically, animals tolerated GSK2285403 at plasma levels (AUC_{0-24}) ≥ 4 times the level of human exposure following the recommended dose of dabrafenib. At the maximum tolerated dose in animals, GSK2167542 and GSK2298683 plasma exposure levels (AUC_{0-24}) were up to approximately 30% and 50% of human exposure levels, respectively. Based on these data and the indicated advanced cancer patient population, the safety of these metabolites presents a minimal risk.

There are four unique human metabolites denoted M28, M29, M30, and M31. Given that these are minor metabolites by virtue of overall exposure level (generally < 1% of administered dose) and that the proposed indication is for patients with advanced cancer, further evaluation of these metabolites is not required at this time.

The toxicity of repeated daily doses of oral dabrafenib was assessed by conducting 4- and 13-week toxicity studies in rats and dogs. Rats and dogs were considered relevant species for use in toxicity studies since dabrafenib showed similar inhibitory activity against wild-type BRAF in humans, rats, and dogs. The main target organs of toxicity were the skin, male reproductive organs, heart (dog only), and stomach (rats only). Dose-responsive increases in skin lesions and papules were considered clinically relevant since these toxicities occurred in rats and dogs at plasma levels (AUC_{0-24}) equivalent to human exposure at the recommended human dose of dabrafenib. Male reproductive toxicity (degeneration/depletion in the testis and aspermia in the epididymis) is also expected to manifest itself in humans at the recommended dose of dabrafenib and was therefore noted in the Tafinlar™ label. Heart toxicity in dogs consisted of marked hypertrophy and hemorrhage of the right atrioventricular valve at plasma levels (AUC_{0-24}) ≥ 5 times human exposure following the recommended dose of dabrafenib. Stomach toxicity consisted of histopathological findings of hyperplasia, epithelial down-growth, and infiltration at all doses tested in rats. Epithelial hyperplasia in the forestomach of mice and rats and in other tissues, including the esophagus, urinary bladder, and renal pelvis, has been reported with other RAF inhibitors. Development of proliferative skin and epithelial forestomach lesions in animals is considered to be pharmacologically-mediated as RAF inhibition has been shown to enhance cell growth in BRAF wild-type cells with subsequent paradoxical activation of RAS/RAF/MEK/ERK pathway signaling.

Dabrafenib was not mutagenic *in vitro* in the bacterial reverse mutation assay (Ames test) or the mouse lymphoma assay, and was not clastogenic in an *in vivo* rat bone marrow micronucleus test. Carcinogenicity studies were not conducted or required for dabrafenib due to its intended use in patients with advanced cancer. In clinical studies treatment with dabrafenib resulted in an increase in the incidence of cutaneous squamous cell carcinomas

Dabrafenib was evaluated in a combined fertility and embryo-fetal study in Sprague-Dawley rats. Plasma exposure levels (AUC_{0-24}) were up to 3 times the exposure level in humans receiving the recommended dose of dabrafenib. Dabrafenib-induced toxicity included cardiac malformations in developing fetuses (cardiac ventricular septal defects), and a number of visceral and skeletal malformations, including misshapen or split thymuses and decreased skeletal ossification. Dabrafenib also caused a decrease in the number of corpora lutea, implantations, and live fetuses, an increase in pre- and post-implantation loss, and a reduction in fetal body weights.

In vitro, dabrafenib was phototoxic. However, a low incidence of photosensitivity reactions in clinical studies suggests a minimal risk to cancer patients.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, the approval of Dabrafenib (Tafinlar™) is recommended.

1.3.2 Additional Non-Clinical Recommendations

None. From a Pharmacology/Toxicology perspective, no additional post-marketing commitments (PMCs) or post-marketing requirements (PMRs) are recommended for this indication.

1.3.3 Labeling

The recommendations to the Applicant's proposed labeling were discussed internally and communicated to the Applicant. Information in the non-clinical sections of the label reflects findings of studies reviewed within this document.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

1195768-06-9

2.1.2 Generic Name

Dabrafenib

2.1.3 Code Name

GSK2118436B

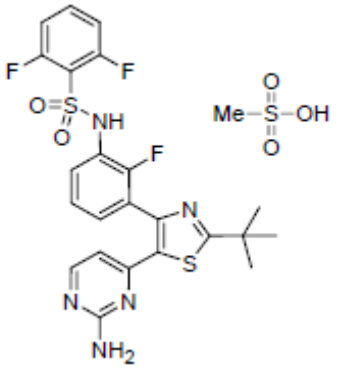
2.1.4 Chemical Name

Benzenesulfonamide, *N*-[3-[5-(2-amino-4-pyrimidinyl)-2-(1,1-dimethylethyl)-4-thiazolyl]-2-fluorophenyl]-2,6-difluoro-, methanesulfonate (1:1)

2.1.5 Molecular Formula/Molecular Weight

$C_{23}H_{20}F_3N_5O_2S_2 \cdot CH_4O_3S$ / 615.68 g/mol

2.1.6 Structure



(figure excerpted from Applicant's NDA)

2.1.7 Pharmacologic class

B-Raf kinase (BRAF) inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND105032 - GSK2118436B (dabrafenib)

2.3 Drug Formulation

Dabrafenib Capsules, 50 mg, are opaque, (b) (4) capsules composed of a dark red body and a dark red cap. Capsule shells will be printed with the identifying codes 'GS TEW', and '50 mg'. Each capsule, intended for oral administration, contains 59.25 mg of dabrafenib mesylate equivalent to 50 mg of dabrafenib free base.

Dabrafenib Capsules, 75 mg, are opaque, (b) (4) capsules composed of a dark pink body and a dark pink cap. Capsule shells will be printed with the identifying codes 'GS LHF', and '75 mg'. Each capsule, intended for oral administration, contains 88.88 mg of dabrafenib mesylate equivalent to 75 mg of dabrafenib free base.
Dabrafenib Capsules, 50 mg and 75 mg are packed with silica gel desiccant into opaque, white HDPE bottles, and closed with (b) (4) (b) (4), with a (b) (4) induction heat seal liner.

The detailed composition of Dabrafenib, 50 mg and 75 mg, capsules is shown in the table below.

Composition of Dabrafenib Capsules, 50 and 75 mg

Component	Quantity [mg/capsule]		Function	Reference to Standard
	50 mg	75 mg		
Dabrafenib Mesylate, Micronized[1]	59.25	88.88	Active	GSK[2]
Microcrystalline Cellulose	(b) (4)		(b) (4)	USP/NF
Magnesium Stearate				USP/NF
Colloidal Silicon Dioxide				USP/NF
Total Unit Dose	(b) (4)		-	-
Hypromellose Capsules[3]	(b) (4)	(b) (4) (b) (4)	Capsule shell	Supplier/GSK[2]

Note: (b) (4)

(table excerpted from Applicant's BLA)

2.3.3 Comments on Impurities/Degradants of Concern

(b) (4)

2.6 Proposed Clinical Population and Dosing Regimen

Dabrafenib (Tafinlar™) is indicated to treat patients with unresectable or metastatic melanoma with BRAF V600 mutation as detected by an FDA approved test. The recommended dose of Dabrafenib (Tafinlar™) is 150 mg orally, twice daily.

2.7 Regulatory Background

On February 14, 2012, GlaxoSmithKline (GSK) requested a pre-NDA meeting for IND 105032 to discuss the planned NDA application for dabrafenib (GSK2118436) in patients with unresectable or metastatic melanoma with BRAF V600 mutation as detected by an FDA approved test. During clinical development of dabrafenib, GSK used a laboratory developed test (LDT) from Response Genetics Inc. (RGI). According to the Briefing Document, the RGI BRAF assay is an allele-specific polymerase chain reaction (PCR) assay, which differentiates the V600E and K mutation forms, and is performed on DNA extracted from fresh frozen paraffin embedded (FFPE) melanoma tumors. Following interactions with the FDA's Office of In Vitro Diagnostics (OIVD) on May 19, 2010, the RGI LDT underwent full analytical validation, rendering the assay as an "investigational use only (IUO)" assay which has been used to screen subjects for eligibility onto GSK-sponsored clinical studies with dabrafenib. GSK stated that it partnered with bioMerieux (bMx) in the co-development of a companion diagnostic (cDx) assay to be available at the time of dabrafenib and trametinib registration. Clinical validation in support of licensure of the cDx would come from the Phase 3 trial (MEK114267). GSK and partners have worked with the OIVD throughout development with regard to the data needed to demonstrate the comparability of the RGI IUO to the intended commercial cDx; they agreed that concordance and equivalency would be demonstrated using the bMx THxIDTM BRAF assay retrospectively, with the banked samples from the clinical studies. All data were to be submitted by bioMerieux as part of a PMA application at the time of the NDA submission.

This application qualifies for an Orphan Drug exception of the FDA User Fee under section 736(a)(1)(F) of the Federal Food, Drug and Cosmetic Act.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology:

Study #	Study title
UH2008-00147	*In Vitro* Biochemical Characterization of GSK2118436
UH2010-00028	*In Vitro* Biochemical Activity of GSK2118436 against Multiple BRAF Mutant Proteins at Amino Acid V600
UH2010-00045	*In Vitro* Characterizations of GSK2285403, GSK2298683, and GSK2167542: Active Metabolites of B-Raf Inhibitor GSK2118436
UH2008-00132	Characterization of Cellular Activity of GSK2118436A
UH2008-00145	Efficacy of the Oral B-Raf Inhibitor GSK2118436 in Various Mouse Xenograft Models of Human Cancer

Safety Pharmacology:

Study #	Study title
VD2008-00869	GSK2118436A: Acute Neurobehavioral Effects Following Oral

	Administration in the Conscious Crl:CD(SD) Rat
CD2008-01279	GSK2118436A: Acute Effects on Respiratory Function Following Oral Administration in the Conscious Crl:CD(SD) Rat
FD2008-01280	GSK2118436A: Acute Effects on Cardiovascular Function Following Oral Administration of in the Conscious Beagle Dog
FD2008-00376	GSK2118436A: Effect on hERG Tail Current Recorded from Stably Transfected HEK-293 Cells

Pharmacokinetics:

Study #	**Study title**
UH2008/00115/02	Preliminary Drug Metabolism and Pharmacokinetics of GSK2118436
CD2009-0041	An Evaluation of the Systemic Exposure of GSK2118436 Following Oral Gavage Administration of Micronized GSK211843A (free Base, (b) (4) in Suspension at 5 or 10 mg/kg to Male Crl:CD(SD) Rats
2011N126179	The Assessment of the Systemic Exposure of GSK2118436 and its Metabolites GSK229683 (M4), GSK2285403 (M7) and GSK2167542 (M8) Following a Single Oral Administration of GSK2118436B Suspension at 20 mg/kg to Male Crl:CD(SD) Rats
2011N119114	The Assessment of the Systemic Exposure of GSK2118436 and its Metabolites GSK229683 (M4), GSK2285403 (M7) and GSK2167542 (M8) Following a Single Oral Administration of GSK2118436B Suspension at 20 mg/kg to Male Crl:CD(SD) Rats
RD2009-0091	Quantitative Tissue Distribution of Radioactivity Using Whole-Body Autoradiography Following a Single Oral Administration of [^{14}C]GSK2118436 (10 mg/kg) to Partially Pigmented Male Rats
2010N108408	Quantitative Tissue Distribution of Radioactivity Using Whole-Body Autoradiography Following a Single Oral Administration of [^{14}C]GSK2118436 (10 mg/kg) to Partially Pigmented Female Rats
2011N111703	Metabolism of GSK2118436 Following a Single Oral Administration (10 mg/kg) of [^{14}C]GSK2118436 to Intact Male and Female and Bile Duct-Cannulated Male Rats
2011B111704	Metabolism of GSK2118436 in Male and Female Intact Dogs Following a Single Oral Administration of [^{14}C]GSK2118436 at a Dose Level of 10 mg/kg
CD2009-00079	An *in vitro* Investigation into the Human oxidative Enzymology of GSK2115436
RD2008-01676	Elimination of Radioactivity Following a Single Oral Dose Administration of [^{14}C]GSK2118436 to Male and Female Intact and Male Bile Duct-Cannulated CRL:CD(SD)^{TM} Rats at a Target Dose of 10 mg/kg

RD2009-00137	Elimination of Radioactivity Following a Single Oral (10 mg/kg) Administration of [^{14}C]GSK2118436 to Male and Female Dogs

Repeat-dose Toxicology:

Study #	Study title
CD2008-01511	GSK2118436A: 4-Week Oral Toxicity in Rats Followed by a 2-Week Recovery Period
CD2008-01503	GSK2118436A: 4-Week Oral Toxicity in Dogs Followed by a 2-Week Recovery Period
CD2010-00052	GSK2118436A: A 13-Week Oral Gavage Toxicity Study in the Rat Followed by a 4-Week Recovery
CD2010-00051	GSK2118436B: A 13-Week Twice Daily Oral Capsule Toxicity Study in the Beagle Dog Followed by a 4-Week Recovery

Genetic Toxicology:

Study #	Study title
WD2008-01655	GSK2118436A: Bacterial Mutation Test (AMES Test) with *Salmonella typhimurium* and *Escherichia coli*
WD2008-01806	GSK2118436A: *In Vitro* Mutation Assay with L5178Y/TK +/- Mouse Lymphoma Cells at the TK Locus
WD2009-00233	GSK2118436A: Oral Bone Marrow Micronucleus Assay in Rats
2010N105217	GI-147517 (N-Bromosuccinimide): Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli* (screening study)

Reproductive and Developmental Toxicology:

Study #	Study title
2010N107959	GSK2118436A: Oral Female Fertility, Early Embryonic and Embryo-Fetal Development Dose Range Study in Rats
2011N113146	GSK2118436A: Oral Female Fertility, Early Embryonic and Embryo-Fetal Development Study in Rats

Special Toxicology:

Study #	Study title
2012N141617	GSK2118436B: Evaluation of Phototoxicity *in vitro* on Balb/c 3T3 Fibroblasts using the Neutral Red Uptake Assay

3.2 Studies Not Reviewed

Primary Pharmacology:

Study #	Study title
2011n111729	Enzyme Inhibition Profiling for GSK2285403A and GSK2167542A: Two Active Metabolites of BRAF Inhibitor, GSK2118436A against 292 Kinases from Millipore

UH2009-00016	*In Vitro* and Cell Based Activity of GSK2285403A, a Metabolite of GSK2118436A
2012N139510	Kinase Profiling for GSK2118436A and its Active Metabolites, GSK2298683A, GSK2285403A, and GSK2167542, as well as GSK2794360A
2011N116394	Cellular Assays with GSK2118436, GSK1120212, and GSK2126458, as a Single Agent and in Combination with each other, in BRAFV600E Melanoma Cell Lines that Acquired Resistance to GSK2118436
2001N116395	Cellular Assays with GSK2118436, GSK1120212, and GSK2126458, as a Single Agent and in Combination with each other, in BRAFV600E/K/D Mutant Melanoma Cell Lines
2010N109380	GSK2118436: A Repeat Dose Pharmacodynamic Study in Mice Bearing A375pF11 Tumor Xenografts
UH2010-00041	Discovery of Candidate Circulating Metabolic PD Biomarkers for Raf/MEK Inhibition in Melanoma
2011N120928	Evaluation of GSK2118436A (KN59) and GSK1120212B (KN45) in the A375P F11S Human Melanoma Xenograft Model
2001N111685	Anti-tumor Effect of BRAF and MEK Inhibitors in Combination with Pazopanib (GW786034B) in BRAF Mutant Melanoma Xenograft in Mice
2012N139280	Efficacy of MEK Inhibitor GSK1120212B and BRAF Inhibitor GSK2118436A, Administered Separately or in Combination, in Mouse Tumor Xenograft Models of Human Melanoma
2012N132871	Evaluation of GSK2118436A (KN59) Combined with GSK1120212B (KN45) in the A375P F11S Human Melanoma Xenograft Model

Safety Pharmacology:

Study #	**Study title**
UD2009-00043	Preclinical Test of GSK2118436A for QT Prolongation and TdP Risk Using the Rabbit Left Ventricular Wedge Preparation
CD2008-01717	GSK2118436A: Investigative Study of the Acute Effects on Cardiovascular Function Following Single Oral Administration in the Crl:CD(SD) Rat

Pharmacokinetics:

Study #	**Study title**
2011N125779	A Comparison of Systemic Exposure of GSK2118436 and its Metabolites GSK2298683 (M4), GSK2285403 (M7), and GSK2167542 (M8) Following 22 Days of Repeat Oral Administration of Two Different Batches of GSK2118436A to CD-1 Female Nude Mice Bearing a A375P F11s-Human Melanoma Tumor Xenograft
UH2009-00035	Investigation of the Systemic Exposure of GSK2118436A

	(micronized [redacted] (b) (4) in the Conscious Rat
CD2008-01638	An Evaluation of the Systemic Exposure of GSK2118436 following Oral Adminstration of GSK2118436A (free base, micronized in capsule), GSK2118436B (mesylate salt in capsule or suspension) at 10 mg/kg in Fasted Male Beagle Dogs
CD2009-00595	An Evaluation of the Systemic Exposure of GSK2118436 and its Metabolite GSK228503 (M7) following Oral Administration of Micronized GSK2118436B (mesylate salt) in Capsules at 10 mg/kg in Fasted Male Beagle Dogs
UH2009-00038	Investigation of the Systemic Exposure of GSK2118436A (micronized [redacted] (b) (4) in the Conscious Dog
CD2009-00039	An Evaluation of the Systemic Exposure of GSK2118436 Following Oral Administration of GSK2118436A (micronized free base, [redacted] (b) (4) at 10 mg/kg to Fast Male Beagle Dogs
CD2009-00040	An Evaluation of the Systemic Exposure of GSK2118436 Following Oral Administration of GSK2118436A (micronized free base, [redacted] (b) (4) and GSK2118436H (micronized free base monohydrate) in Capsules at 10 mg/kg to Fasted Male Beagle Dogs
2011N117006	Determination of Systemic Exposure of GSK2118436 Following a Single Oral Administration of Non-micronized GSK2118436B at a Nominal Dose of 10 mg/kg to Fasted Male Beagle Dogs
2012N139932	Investigation of the Intravenous Pharmacokinetics and Oral Bioavailability of GSK228503 in the Conscious Rat
2012N139933	Investigation of the Intravenous Pharmacokinetics and Oral Bioavailability of GSK228503 in the Conscious Dog
2012N139011	Investigation of the Intravenous Pharmacokinetics and Oral Bioavailability of GSK2167542 in the Conscious Rat
UH2009-00043	GSK2118436A: Preliminary Investigation of the *In Vitro* Plasma Protein Binding in Mouse, Rat, Dog, Monkey, and Human Using Equilibrium Dialysis
UH2009-00085	Investigation of the *In Vitro* Plasma Protein Binding of GSK2298683B and GSK2167542A (M4 and M8 Metabolites of GSK2118436) in Fresh Mouse, Rat, Dog, and Human Plasma
2011N129517	An Investigation of the *In Vitro* Protein Binding of GSK2118436 and its Circulating Metabolites GSK2298683 (M4), GSK228503 (M7), and GSK2167542 (M8) in Male Human Plasma
UH2009-00042	Preliminary Investigation of the *In Vitro* Blood to Plasma Partitioning of GSK2285403A in Human, Monkey, Dog, Rat, and Mouse
UH2009-00084	Preliminary Investigation of the *In Vitro* Blood to Plasma Partitioning of GSK2298683B and GSK2167542A (M4 and M8 Metabolites of GSK2118436) in Human, Monkey, Dog, Rat, and Mouse
UD2010-00042	Assessment of Possible P-glycoprotein-mediated Efflux and

	Intrinsic Permeability of GSK2285403A, GSK2167542A, and GSK2298683B, 3 Known Metabolites of GSK2118436, across MDR1-MDCKII Cell Monolayers
CD2009-00143	An *In Vitro* Investigation of the Inhibition by GSK2118436B of Xenobiotic Transport via Human P-glycoprotein, Heterologously Expressed in MDCKII Cells
2011N119324	An *In Vitro* Investigation of the Inhibition by GSK2285403, GSK2298683, and GSK2167542 of Xenobiotic Transport via Human P-glycoprotein, Heterologously Expressed in MDCKII Cells
2011N112849	An *In Vitro* Investigation of the Inhibition by GSK2118436 of Xenobiotic Transport via Human Breast Cancer Resistance Protein Heterologously Expressed in MDCKII Cells
2011N119323	An *In Vitro* Investigation of the Inhibition by GSK2285403, GSK2298683, and GSK2167542 of Xenobiotic Transport via Human Breast Cancer Resistance Protein Heterologously Expressed in MDCKII Cells
CD2009-00116	An *In Vitro* Investigation into the Inhibition by GSK2118436 of Xenobiotic Transport via Human OATP1B1 and OATP1B3
2010N110986	An *In Vitro* Investigation into the Inhibition by GSK2285403 of Xenobiotic Transport via Human OATP1B1 and OATP1B3
2010N110987	An *In Vitro* Investigation into the Inhibition by GSK2298683 of Xenobiotic Transport via Human OATP1B1 and OATP1B3
CD2010-00386	An *In Vitro* Investigation into the Inhibition by GSK2167542 of Xenobiotic Transport via Human OATP1B1 and OATP1B3
2012N131808	An *In Vitro* Investigation into the Inhibition by GSK2118436 and its Circulating Metabolites, GSK2298683, GSK2285403, and GSK2167542 of Xenobiotic Transport via Human OAT1 and OAT3
2011N113981	*In Vitro* Permeability Categorization of GSK2118436 According to the Biopharmaceutics Classification System
2011N112641	An Evaluation of Tissue and Systemic Exposure of GSK2118436 and its Metabolites GSK2298683 (M4), GSK2285403 (M7), and GSK2167542 (M8) Following 5 Days Repeat Oral Administration of GSK2118436
RD2010-00439	An Evaluation of the Tissue Exposure and Spatial Distribution of GSK2118436 and its Metabolites GSK2298683 (M4), GSK2285403 (M7), and GSK2167542 (M8) Following 5 Days Repeat Oral Administration of GSK2118436
2011N113766	A Positron Emission Tomography Study to Evaluate the CNS Penetrancy of [18F]GSK2118436 in the Pig Brain
CD2010-00233	An *In Vitro* Investigation into the Human Oxidative Enzymology of [14C]GSK2118436
2011N129873	A Preliminary *In Vitro* Investigation into the Human Oxidative Enzymology of GSK2167542, GSK2285403, and GSK2298683, the Metabolites of GSK2118436

CD2009-00061	An *In Vitro* Investigation of the Potential for GSK2118436 Bioactivation Following Incubation of [14C]GSK2118436 with Human Liver Microsomes
CD2009-00471	An *In Vitro* Investigation of the Hepatic Metabolism of [14C]GSK2118436 in the Mouse, Rat, Female Rabbit, Dog, Cynomolgous Monkey, and Human
CD2009-00153	Investigation of Biliary Metabolites of GSK2118436 Following Administration of [14C]GSK2118436 to the Isolated Perfused Livers of Male Sprague-Dawley Rats
2010N110340	An *In Vitro* Investigation into the Inhibition of Human Cytochrome P450 Enzymes: CYP1A2, CYP2C9, CYP2C19, and CYP2D6 by GSK2118436
CD2009-00012	An *In Vitro* Investigation into the Inhibition of Human Cytochrome P450 Enzymes by GSK2118436
2010N111279	An *In Vitro* Investigation into the Inhibition of Human Cytochrome P450 Enzymes by GSK2285403
2010N110991	An *In Vitro* Investigation into the Inhibition of Human Cytochrome P450 Enzymes by GSK2298683
2010N111291	An In Vitro Investigation into the Inhibition of Human Cytochrome P450 Enzymes by GSK2167542
CD2008-01428	An *In Vitro* Evaluation of the Effect of GSK2118436 on the mRNA Levels of Cytochrome P450 Enzymes in Cultured Human Hepatocytes
CD2009-00144	A Preliminary Biotransformation Study to Investigate the Circulating Metabolites Following a Single Oral Administration of 30 mg/kg [14C]GSK2118436 to Female Mice

Repeat-dose Toxicology:

Study #	**Study title**
2010N109898	GSK2118436A: 14-Day Oral Dose Range Toxicity Study in Crl:CD1 (ICR) Mice
RD2008-00505	GSK2118436A: 10-Day Oral Toxicity Study in Rats
CD2008-00951	GSK2118436A: 10-Day Oral Dose Range Toxicity Study in Rats
RD2008-01229	GSK2118436A: 7-Day Oral Dose-Range Toxicity Study in Beagle Dogs
2011N112335	GSK1120212B and GSK2118436B: 4-Week Twice Daily Oral Toxicity Study in Dogs

Genetic Toxicology:

Study #	**Study title**
2011N112667	GSK2487755A: Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli* (screening study)
2011N115905	GSK2198155A: Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli* (screening study)

Reproductive and Developmental Toxicology:

Study #	Study title
2011N121500	Oral Juvenile Tolerability Study in Rats

Local Tolerance:

Study #	Study title
2012N133066	GSK2118436A: Determination of Skin Irritation Potential using the Skinethic Reconstructed Human Epidermal Model
2012N133069	GSK2118436A: Determination of Eye Irritation Potential using the Skinethic Reconstructed Human Corneal Epithelial Model
2012N133070	GSK2118436A: Local Lymph Node Assay in the Mouse

3.3 Previous Reviews Referenced

The Pharmacology/Toxicology and other discipline reviews for IND 105032 (GlaxoSmithKline, LLC. for treatment of patients with BRAF mutation-positive tumors)

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology studies submitted by the Applicant are adequate, confirming the mechanism of action as described in the label and demonstrating the anti-tumor activity of dabrafenib under the non-clinical conditions tested.

UH2008-00147: *In Vitro* Biochemical Characterization of GSK2118436.

The potency (IC_{50}) of GSK2118436 was determined by the BRAF/CRAF activated MEK ATPase (BRAMA/CRAMA) coupled assays. In these assays, RAF enzyme activity was indirectly monitored through the acceleration of the intrinsic ATPase activity of MEK1, which is dependent upon its activation by RAF enzyme. The production of ADP was followed by coupling to pyruvate kinase and lactate dehydrogenase (PK/LDH), and monitoring the decrease in NADH absorbance at 340 nm.

As summarized in the following table, GSK2118436A inhibited wild-type BRAF, BRAFV600E, and CRAF kinases in the BRAMA/CRAMA assay, with respective IC_{50} values of 3.2, 0.8, and 5.0 nM.

Inhibition of Wild-type (WT) BRAF, BRAFV600E and CRAF by GSK2118436A

	WT BRAF	$BRAF^{V600E}$	CRAF
pIC_{50}	8.5 ± 0.1	9.1 ± 0.04	8.3 ± 0.05
IC_{50} (nM)	3.2	0.8	5.0

All pIC_{50} values represent the mean ± standard deviation
(table excerpted from Applicant's NDA)

Furthermore, GSK2118436A (N3320-93-8) displayed similar *in vitro* activity against purified human, cynomolgus monkey, dog and rat wild-type BRAF kinases (see table below).

Activity of GSK2118436A against Full-length Human, Cynomolgus Monkey (Cyno), Dog, and Rat Wild-type BRAF Enzymes

	Human BRAF	Cyno BRAF	Rat BRAF	Dog BRAF
pIC_{50}	8.3 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.1
IC_{50} (nM)	4.8	4.1	4.3	4.0

All pIC50 values represent the mean ± standard deviation
(table excerpted from Applicant's NDA)

To evaluate the selectivity of GSK2118436A, the Applicant tested the activity of GSK2118436A against a panel of protein/lipid kinases both at GlaxoSmithKline and through Upstate/Millipore. As summarized in the following table, GSK2118436A showed potent inhibition (IC_{50} values < 100 nM) against the following kinases, not including RAF kinases.

Kinases other than RAF Enzymes Inhibited by GSK2118436A with IC_{50} < 100 nM

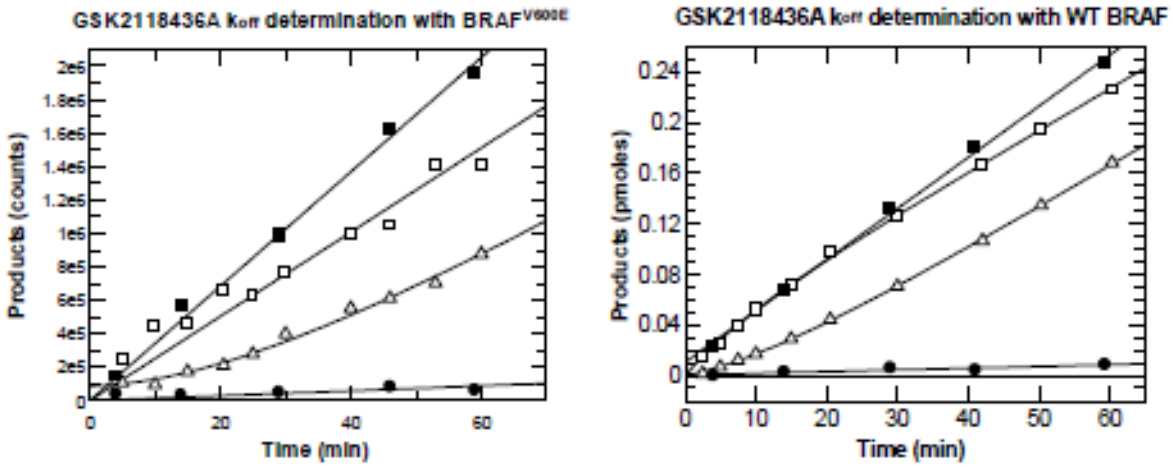
Kinase	IC_{50} (nM)
BRK(H)	79
CK1(Y)	41
LIMK1(H)	15
ALK5	17
NEK11(H)	20
PKD2(H)	57
SIK(H)	27
SIK2	76

(table excerpted from Applicant's NDA)

To characterize time-dependent inhibition, the intrinsic dissociation rate of GSK2118436A from BRAFV600E and wild-type BRAF was determined in a dissociation kinetics assay. The recovery of enzymatic activity was measured following rapid

dilution of a preformed GSK2118436A and BRAFV600E (or wild-type BRAF) complex into substrate mix (open triangles). In the figure below, closed squares represent the DMSO control (reaction in the absence of GSK2118436A); open squares represent the control reaction where GSK2118436A and enzyme at the final diluted concentrations were mixed together and the reaction progress curve was monitored immediately without enzyme/inhibitor pre-incubation; closed circles represent the control where the pre-incubation mix containing 100x enzyme and inhibitor was diluted into reaction mix containing 200 nM GSK2118436A; lines represent the fitting of data to Equation 1 (also shown below). Based on these data, GSK2118436A dissociates from wild-type BRAF and BRAFV600E enzymes with $t_{1/2}$ values of 12 ± 2 and 17 ± 3 min, respectively. The $t_{1/2}$ values represent the mean ± standard deviation (n=2).

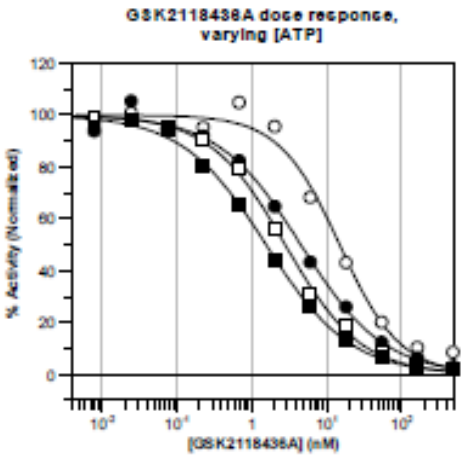
Off-rate of GSK2118436A from Human BRAFV600E and Wild-type (WT) BRAF Determined in the Dissociation assay

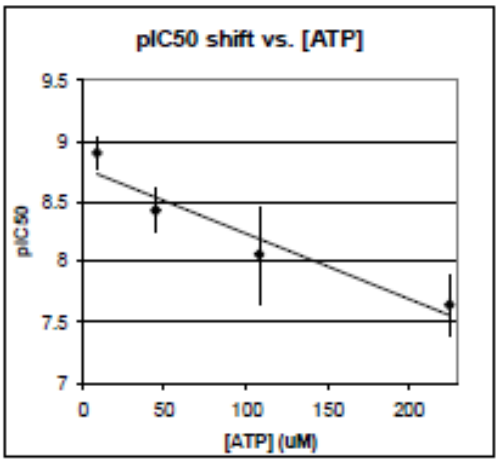


(figures excerpted from Applicant's NDA)

For an ATP competitive inhibitor, the inhibitory potency decreases with increasing ATP concentrations. When GSK2118436A was tested against CRAF at varying ATP concentrations, a linear decrease in pIC_{50} (increase in IC_{50}) was observed at increasing ATP concentrations (see figure below). Using a SDS-PAGE assay, reactions were run at 225 µM (25xKm, open circle), 108 µM (12xKm, closed circle), 45 µM (5xKm, open square), or 9 µM (1xKm, closed square) ATP. A representative dose response curve is shown in the following figure. The curves were fit to Equation 2 (also shown below) to generate pIC_{50} values. Three-parameter fits with y_{min} fixed to 0 were used. These data suggest that GSK2118436A is an ATP competitive inhibitor against CRAF.

IC50 Shift of GSK2118436A against CRAF at Increasing ATP concentrations

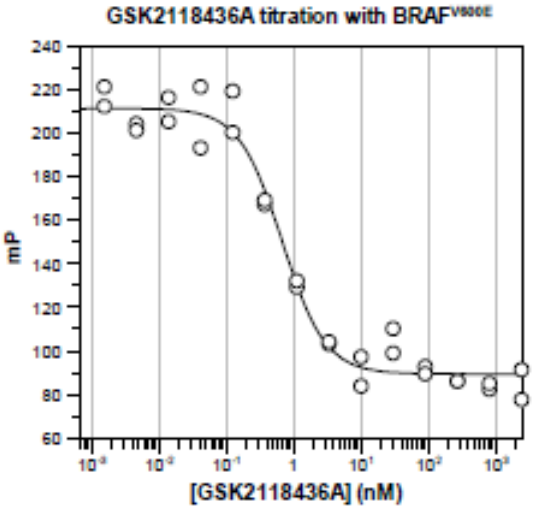


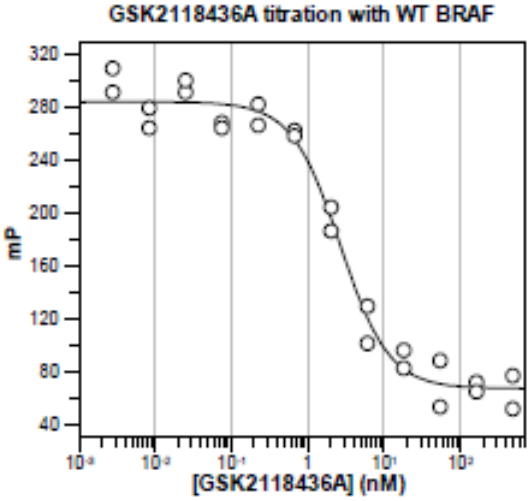


(figures excerpted from Applicant's NDA)

To further support that GSK2118436A is an ATP competitive inhibitor, GSK2118436A was shown to be able to compete with a ligand for the binding of the ATP pocket on both wild-type BRAF and BRAFV600E. Using a fluorescence polarization assay, a rhodamine green labeled ligand that binds to the ATP pocket of BRAF was used and the ability of GSK2118436A to compete with the ligand for binding to BRAF was measured. Displacement of the ligand from the ATP pocket induces a decrease in fluorescence anisotropy and the dose response data can be fitted to generate the binding IC_{50} for GSK2118436A. As shown in the following figures, GSK2118436A was able to compete for ligand binding with both BRAFV600E and wild-type BRAF. The IC_{50} values determined from this analysis (0.7 and 2.7 nM for BRAFV600E and wild-type BRAF, respectively) are consistent with those obtained from the BRAMA activity assay. These data support that the mode of action of GSK2118436A is consistent with ATP competitive inhibition.

Fluoresence Polarization Competition Assay with GSK2118436A against Human BRAFV600E and Wild-type (WT) BRAF





mP values taken at 120 min were used for fitting to Equation 2 to generate IC_{50} values (0.7 and 2.7 nM for BRAFV600E and wild-type BRAF, respectively). Four-parameter fits (all values floating) were used for data analysis.
(figures excerpted from Applicant's NDA)

Equations

Equation 1 **% inhibition = ((U – C1)/(C2 – C1)) * 100**

Where U is the unknown value, C1 is the average of the high signal (0% inhibition) control wells and C2 is the average of the low signal (100% inhibition) control wells.

Equation 2 $Y = A + [(B - A)/(1 + (10^{x}/10^{c})^{D})]$

Where A = minimum response; B = maximum response; c = log(IC_{50}); D = slope factor; x = log(Molar compound concentration); pIC_{50} values equal -c in the above equation.

Equation 3 $$y = v_s \cdot t + \frac{v_i - v_s}{k_{obs}}(1 - e^{-k_{obs} \cdot t}) + background$$

where v_i and v_s are the initial and steady state velocities of the reaction in the presence of the inhibitor, k_{obs} is the apparent first-order rate constant for the transition from v_i to v_s, and t is time. Under the experimental conditions, k_{obs} approximates the dissociation rate constant (k_{off}) of the EI complex. For this analysis, v_s was fixed at the expected v_s, calculated using Equation 4 [Morrison, 1988]:

Equation 4 $$v_s = \frac{VS}{K_m\left(1 + \frac{I}{K_i^*}\right) + S}$$

where v_s is the steady state velocity, V is the maximum velocity (uninhibited reaction), S is the substrate (ATP) concentration, K_m is the Michaelis constant for ATP, I is the inhibitor concentration, and K_i^* is the inhibition constant. The enzyme-inhibitor dissociation half life ($t_{1/2}$) is related to k_{off} by Equation 5

Equation 5 $$t_{\frac{1}{2}} = \frac{0.693}{k_{off}}$$

(equations excerpted from Applicant's NDA)

UH2010-00028: *In Vitro* Biochemical Activity of GSK2118436 against Multiple BRAF Mutant Proteins at Amino Acid V600.

The BRAMA assay (as previously described in Study No. UH2008-00147) was utilized to evaluate the activity of GSK2118436 against several BRAF proteins with mutations at position V600. As summarized in the following table, GSK2118436 inhibited with the kinase activity of BRAFV600E, BRAFV600K and BRAFV600D with IC_{50} values of 0.65, 0.5, and 1.84 nM, respectively.

Inhibition of BRAFV600E, BRAFV600K and BRAFV600D by GSK2118436

Cmpd	Enzyme	pIC50 ave	pIC50 stdev	IC50 ave (nM)
GSK2118436	$BRAF^{V600E}$	9.19	0.0438	0.65
	$BRAF^{V600K}$	9.30	0.0078	0.50
	$BRAF^{V600D}$	8.73	0.0035	1.84

All pIC50 values represent the mean ± standard deviation.
(table excerpted from Applicant's NDA)

The anti-proliferative activity of GSK2118436A was tested using the CellTiter-Glo™ luminescent cell viability assay. As shown in the table below, 3-day continuous exposure to GSK2118436 inhibited the growth of SK-MEL-28 cells (human melanoma cells encoding a BRAFV600E protein) and WM115 cells (human melanoma cells with a BRAFV600D) with growth IC_{50}s (gIC_{50}) of 3 and 5 nM, respectively. GSK2118436 was inactive against HN5 cells (head and neck cell line encoding a wild type BRAF protein) with a gIC_{50} >2000 nM. The anti-proliferative activity of GSK2118436A in a BRAFV600K-containing cell line was not tested.

Activity of GSK2118436 against Human Cancer Cell Lines Encoding BRAFV600E, BRAFV600D and Wild-type (WT) BRAF

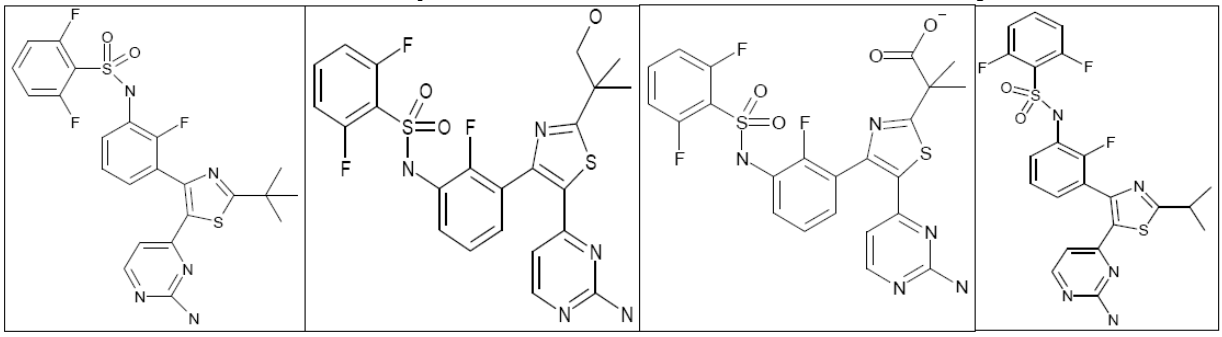
Cmpd	Cell line	BRAF	IC50 ave (nM)
GSK2118436	SK-MEL-28	V600E	3
	WM115	V600D	5
	HN5	WT	>2000

(table excerpted from Applicant's NDA)

UH2010-00045: *In Vitro* Characterizations of GSK2285403, GSK2298683, and GSK2167542: Active Metabolites of B-Raf Inhibitor GSK2118436.

In the isolated perfused rat liver model (IPRL), oral administration of ^{14}C-labeled GSK2118436 revealed the presence of three metabolites at concentrations that may contribute to the activity of the parent compound, GSK2118436. Mass spectrometry identified the t-butyl group as the site of initial hydroxylation (GSK2285403), which can further be hydroxylated (GSK2298683) and decarboxylated (GSK2167542). The structures of these metabolites, also referred as M7, M4, and M8, respectively, are illustrated in the following figure, in comparison to the parent compound GSK2118436.

Structures of Parent Compound GSK2118436 and its Three Major Metabolites



GSK2118436 Parent | GSK2285403 M7 | GSK2298683 M4 | GSK2167542 M8

(figure excerpted from Applicant's NDA)

Using the BRAF/CRAF activated MEK ATPase (BRAMA/CRAMA) coupled assays, as previously described in Study No. UH2008-00147, GSK2118436 and its three major metabolites were shown to be active against wild-type (WT) BRAF and CRAF kinases, as demonstrated in the table below. Specifically, while GSK2167542 (M8) showed similar activity than parent (1.2X and 1.5X less active than parent against CRAF and BRAF, respectively), GSK2285403 (M7) and GSK2298683 (M4) demonstrated ~6X and ~20-50X lower activity than parent against CRAF and BRAF WT enzymes, respectively.

GSK2118436 and Metabolites Inhibitory Activity against Human BRAF and CRAF Enzymes

Assays	parent GSK2118436	M7 GSK2285403	M4 GSK2298683	M8 GSK2167542
CRAF truncated human, pIC50 (stdev)	8.60 (0.09)	7.83 (0.01)	7.29 (0.20)	8.51 (0.13)
BRAF human WT, pIC50 (stdev)	8.65 (0.15)	7.91 (0.01)	6.99 (0.17)	8.50 (0.06)

(table excerpted from Applicant's NDA)

The activity of GSK2118436 and its major metabolites against the wild-type BRAF enzyme was also compared across different species (human, rat, dog, and cynomolgus monkey) using the BRAMA assay. As summarized in the following table, the parent compound (GSK2118436) and M8 (GSK2167542) have similar (within 2X) activity while M7 (GSK2285403) showed 6-7X lower activity than parent against BRAF from all species tested. M4 (GSK2298683) showed the lowest activity of the 4 compounds with 47-55X lower activity than parent against BRAF enzymes from all species tested.

Compounds Inhibitory Activity against BRAF Enzymes from Different Species

Assays	parent GSK2118436	M7 GSK2285403	M4 GSK2298683	M8 GSK2167542
BRAF human WT, pIC50 (stdev)	8.65 (0.15)	7.91 (0.01)	6.99 (0.17)	8.50 (0.06)
BRAF rat WT, pIC50 (stdev)	8.67 (0.24)	7.83 (0.02)	6.93 (0.11)	8.47 (0.20)
BRAF dog WT, pIC50 (stdev)	8.70 (0.24)	7.91 (0.04)	6.96 (0.07)	8.49 (0.23)
BRAF monkey WT, pIC50 (stdev)	8.72 (0.16)	7.91 (0.02)	7.02 (0.17)	8.53 (0.17)

(table excerpted from Applicant's NDA)

To evaluate the selectivity of these metabolites and to compare each metabolite's activity to that of the parent compound GSK2118436 (previously reported in Study No UH2008-00147), the Applicant tested the activity of each metabolite against a panel of protein/lipid kinases. While the selectivity of GSK2285403 (M7) and GSK2167542 (M8) was tested against 24 and 40 kinases, respectively, the selectivity of GSK2298683 (M4) was not assessed due to the instability of the compound in DMSO. GSK2167542 (M8), apart from RAF related enzymes, showed IC_{50} values of <1000 nM against ALK5, SIK, DDR2 and LCK (13.7, 125, 243 and 654.6 nM, respectively). GSK2285403 (M7), apart from RAF related enzymes, showed inhibition of ALK5 (IC_{50} of 31.5 nM) and to a lower extent inhibition of LCK (IC_{50} of 921.5 nM). For GSK2285403, the activity against SIK and DDR2 was not measured. For the remaining kinases for which GSK2285403 and GSK2167542 were tested (data not shown), there was at least a 300-fold decrease in potency compared to the potency of the metabolites against BRAFV600E.

The BRAMA assay was also utilized to measure the activity of GSK2118436 and its major metabolites against BRAF proteins with mutations at position V600. As summarized in the following table, the activity of each compound against a specific BRAF mutant enzyme is similar (1-3X difference) except GSK2298683 (M4), which is lower (16-23X) than that of parent and other metabolites (M7 and M8).

Compounds Inhibitory Activity against Mutant BRAF Enzymes at Position V600

Assays	parent GSK2118436	M7 GSK2285403	M4 GSK2298683	M8 GSK2167542
B-Raf^{V600E} human,	9.19 (0.04)	8.72 (0.025)	7.78 (0.05)	8.95 (0.093)
B-Raf^{V600K} human,	9.30 (0.008)	8.89 (0.007)	8.20 (0.03)	9.25 (0.093)
B-Raf^{V600D} human,	8.73 (0.004)	8.20 (0.006)	7.3 (0.02)	8.56 (0.022)

(table excerpted from Applicant's NDA)

The anti-proliferative activity of GSK2118436 and metabolites was tested using the CellTiter-Glo™ luminescent cell viability assay. As shown in the table below, 3-day continuous exposure to either the parent compound GSK2118436, M7, or M8 resulted in anti-proliferative activity against SK-MEL-28 cells (melanoma cells encoding a BRAFV600E protein) and Colo205 cells (colorectal cells encoding a BRAFV600E protein) below a gIC_{50} of 23 nM. The anti-proliferative activity of GSK2298683 (M4) was lower (gIC_{50} of 223 and 320 nM against SK-MEL-28 and Colo205, respectively) than the

anti-proliferative activity of the parent compound, M7, and M8. The gIC_{50} against HN5 cells (head and neck cell line encoding a wild type BRAF protein) was >20,000 nM for all 4 compounds tested.

Antiproliferative Activity of GSK2118436 and Metabolites is Specific to Activated BRAF Mutant Cell Lines

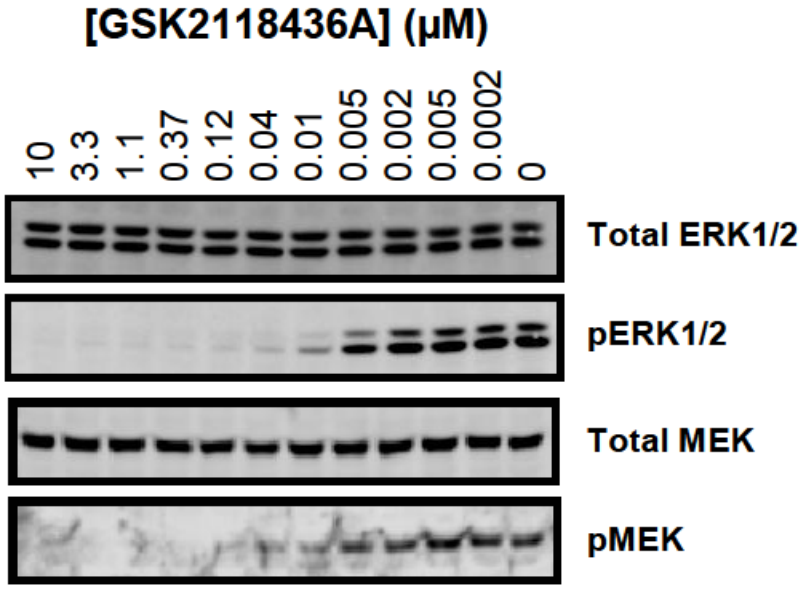
Assays proliferation gIC50, nM	parent GSK2118436	M7 GSK2285403	M4 GSK2298683	M8 GSK2167542
SK-MEL-28	6	17.7	223	9
Colo205	6	23	320	23
HN5	>20000	>20000	>20000	>20000

(table excerpted from Applicant's NDA)

UH2008-00132: Characterization of Cellular Activity of GSK2118436A.

Since MEK and ERK are downstream substrates of RAF kinases, the inhibition of BRAF activity in cells containing mutant BRAFV600E is expected to decrease the phosphorylation of MEK and ERK. As expected, treatment of ES-2 ovarian carcinoma cells, containing the BRAFV600E mutant, with GSK2118435A for 1 hour resulted in a concentration-dependent decrease in pERK and pMEK, with no change in total ERK and MEK protein levels (see figure below).

pMEK and pERK Inhibition in ES-2 cells (BRAFV600E) following 1-hour treatment with Various Concentrations of GSK2118436A



(figure excerpted from Applicant's NDA)

Treating various cell lines with GSK2118436A also resulted in pERK inhibition in other BRAFV600E cell lines, as demonstrated by Meso Scale Discovery (MSD®), in-cell

Western assay, or Western blot assays (see table below). Cell lines containing wild-type B-Raf were less sensitive to GSK2118436A-mediated inhibition of pERK, regardless of Ras status. This suggests that BRAFV600E cells are more sensitive to BRAF inhibition by GSK2118436A and that pERK inhibition could possibly be used as a pharmacodynamic marker.

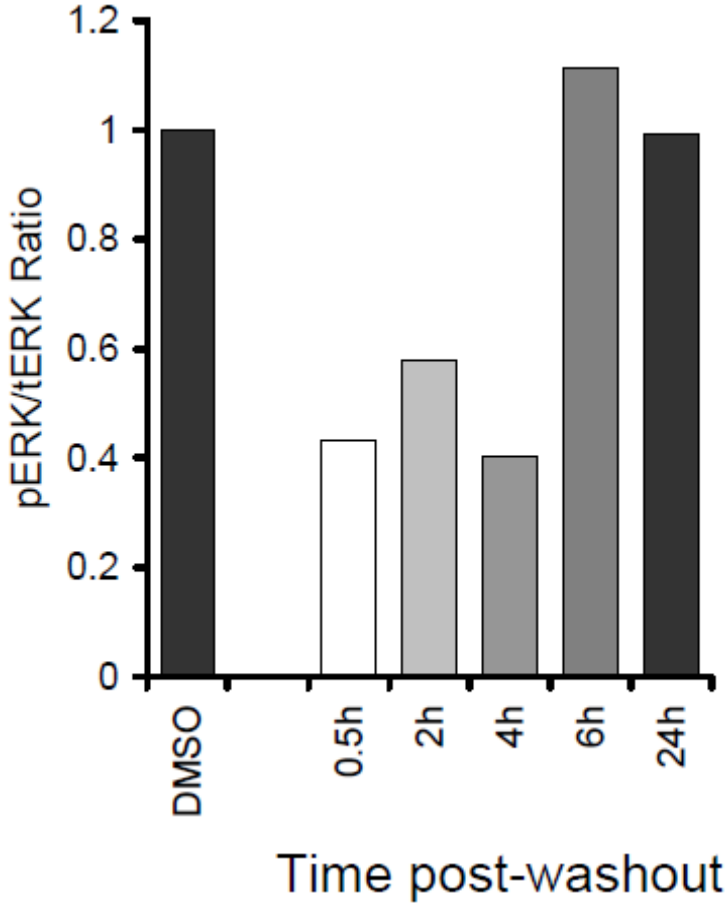
Selectivity of GSK2118436A for pERK inhibition in Human Tumor Cells Containing BRAFV600E by IC_{50} value

CELL LINE	Tissue of Origin	Status	IC_{50} (nM)
A375P F11s	Skin	$BRAF^{V600E}$	3
ES-2	Ovary	$BRAF^{V600E}$	3
SK-MEL-28	Skin	$BRAF^{V600E}$	4
Colo 205	Colon	$BRAF^{V600E}$	14
RKO	Colon	$BRAF^{V600E}$	<120
HCT-116	Colon	$KRAS^{G13D}$	7450
HepG2	Liver	$NRAS^{Q61L}$	904
SK-MEL-2	Skin	$NRAS^{Q61R}$	4000
HN5	Head and Neck	WT	>2500
HFF	Foreskin	WT	3000
HeLa	Cervix uteri	WT	>10000
LNCaP	Prostate	WT	>10000

(table excerpted from Applicant's NDA)

The duration and reversibility of pERK inhibition was assessed by exposing human melanoma cells containing the BRAFV600E mutation (SK-MEL-28 cells) to 300 nM GSK2118436A, for 2 hours. As illustrated in the following figure, pERK inhibition persisted for up to 4 hours under the conditions tested, and was reversible, with complete recovery 6 hours following exposure cessation.

Sustained pERK Inhibition following a 1-hour Exposure to GSK2118436A and Compound Removal by Washout



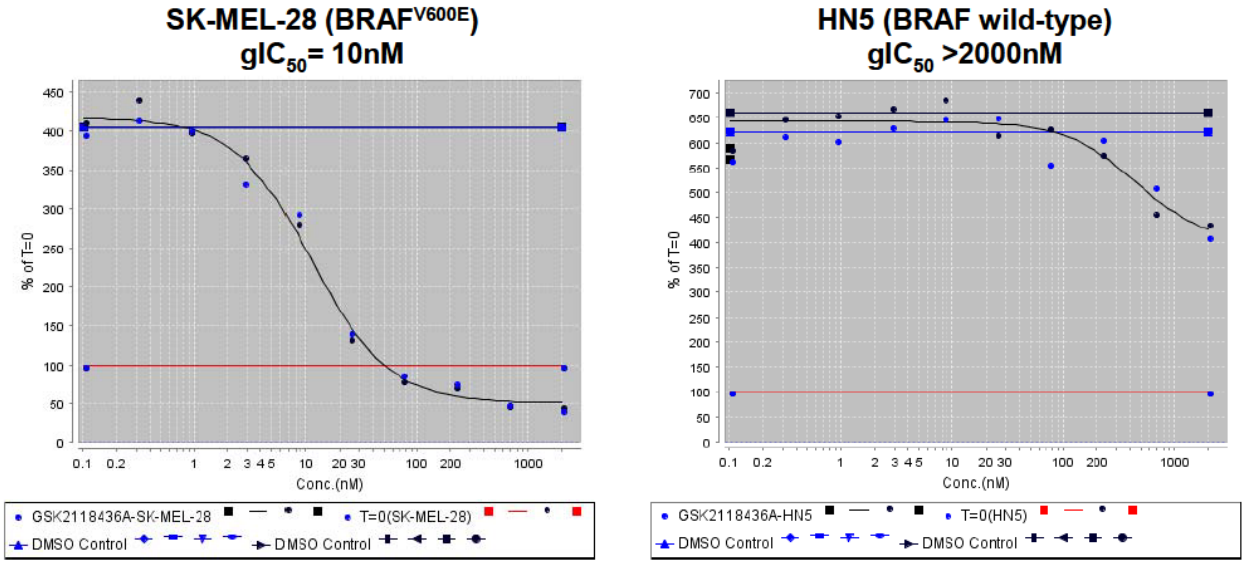
(figure excerpted from Applicant's NDA)

In addition to inhibiting wild-type BRAF, BRAFV600E, and CRAF kinases, *in vitro* kinase profiling suggests GSK2118436A inhibits Alk5/TGFβ1R kinase as well (IC_{50} 17 nM; previously reviewed above; Study UH2008-00147). Therefore, to examine the cellular activity of GSK2118436A against Alk5, the inhibition of SMAD2/3 phosphorylation in TGFβ1-stimulated HepG2 cells was measured. According to the Applicant, under the conditions tested, the IC_{50} of Alk5 cellular inhibition was 3.9 μM. This cellular IC_{50} against Alk5/TGFβ1R is greater than 200-fold higher than cellular inhibition of BRAF (pERK) in HepG2 cells, suggesting a reduced likelihood of Alk5-related activity *in vivo* at concentrations required to modulate BRAF activity.

As previously described above, SK-MEL-28 cells, a human melanoma cell line carrying the BRAFV600E mutation, are sensitive to GSK2118436A-induced pERK inhibition. In contrast, HN5 cells, a human head and neck cancer cell line which contain wild-type BRAF, are relatively insensitive to GSK2118436A. To determine if GSK2118436-induced pERK inhibition correlated with the anti-proliferative activity of GSK2118436 in these two cell lines, a CellTiter-Glo™ 3-day continuous inhibitor exposure growth-death assay was performed. As shown in the figure below, GSK2118436 inhibited the growth

of SK-MEL-28 cells with a growth IC_{50} (gIC_{50}) of 10 nM, while HN5 cells were relatively resistant (gIC_{50} >2 μM). Therefore, the anti-proliferative activity of GSK2118436 appears to correlate to the ability of GSK2118436 to inhibit pERK activity.

Selective Anti-proliferative Effect of GSK2118436A on Human Tumor cells containing BRAFV600E by 3-day Growth-death Assay



(figure excerpted from Applicant's NDA)

GSK2118346A was also profiled against a panel of 110 human tumor cell lines, each with confirmed BRAF mutational status, using 3-day growth-death assay. Cell line sensitivities to GSK2118436A are shown as gIC_{50} values in the following table and grouped as sensitive (gIC_{50} <100 nM), intermediate (gIC_{50} 100 nM – 1 μM), and resistant (gIC_{50} >1 μM). Mutation status of cell lines is also shown, where available, for BRAF, HRAS, KRAS, and NRAS.

GSK2118436A inhibited (gIC_{50} <100 nM) proliferation of 73% of the BRAFV600E containing cell lines and showed little to no activity against all other cancer cell lines tested. Sensitivity to GSK2118436A significantly correlated with BRAFV600E presence (p = 3x10-14), but did not extend to other BRAF mutants or cell lines containing activated Ras. A total of four cell lines containing the BRAFV600E mutation (RKO A673, GCT, and NCI-H292) were insensitive to GSK2118436A. These BRAFV600E containing insensitive cell lines were derived from colon carcinoma, sarcoma, and lung tissue, respectively. Based on these data, the patient population most likely to receive clinical efficacy from GSK2118436A would be those patients with melanoma.

Anti-proliferative Activity (gIC50) of Human Tumor Cell Lines to GSK2118436A Correlates with the Presence of BRAFV600E

SENSITIVE						
CELL LINE	Tissue Origin	*BRAF*	*NRAS*	*HRAS*	*KRAS*	GSK2118436A Mean gIC_{50} (nM)
MALME-3M	Skin	V600E	WT	WT	WT	1
UACC-62	Skin	V600E	WT	WT	WT	1
C32TG	Skin	V600E	WT	WT	WT	1
SK-MEL-1	Skin	V600E	WT	WT	WT	2
UCLA-SO-M14	Skin	V600E	WT	WT	WT	2
SK-MEL-28	Skin	V600E	WT	WT	WT	3
DU4475	Breast	V600E	WT	WT	WT	5
WM115	Skin	V600D, V600E	WT	WT	WT	5
UACC-257	Skin	V600E	WT	WT	WT	6
COLO 205	Colon	V600E	WT	WT	WT	7
SK-MEL-3	Skin	V600E	WT	WT	WT	7
A375P F11s	Skin	V600E	WT	WT	WT	8
SH-4	Skin	V600E	WT	WT	WT	8
A101D	Skin	V600E	WT	WT	WT	9
OV-90	Ovary	Del 486-490	WT	WT	WT	29
HuT78	Peripheral blood	WT	Q61K	WT	WT	52
ES-2	Ovary	V600E	WT	WT	WT	53
HT-29	Colon	T119S, V600E	WT	WT	WT	66

INTERMEDIATE						
CELL LINE	Tissue Origin	*BRAF*	*NRAS*	*HRAS*	*KRAS*	GSK2118436A Mean gIC_{50} (nM)
SW1417	Colon	V600E	WT	WT	WT	158
RPMI-8226	Peripheral blood	WT	WT	WT	G12A	263
BC-3	Lymph node	WT	WT	WT	WT	277
ACHN	Kidney	WT	WT	WT	WT	294
SW872	Connective tissue	V600E	WT	WT	WT	377
CESS	Peripheral blood	WT	WT	WT	WT	497
DB	Lymph node	WT	WT	WT	WT	873
RPMI-6666	Peripheral blood	WT	WT	WT	WT	928

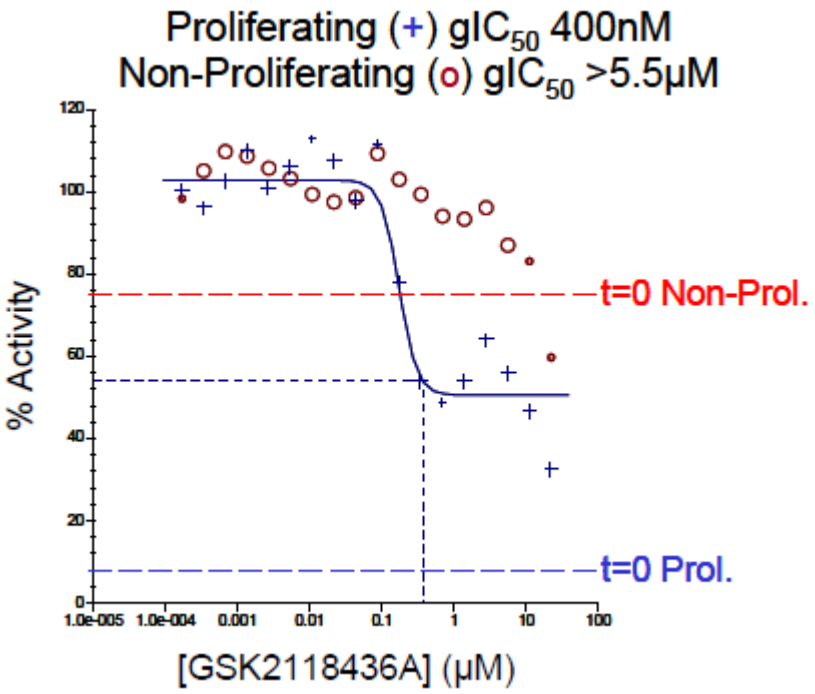
RESISTANT						
CELL LINE	Tissue Origin	*BRAF*	*NRAS*	*HRAS*	*KRAS*	GSK2118436A Mean gIC_{50} (nM)
MC/CAR	Peripheral blood	WT	WT	WT	WT	1002
SNU-1	Stomach	A400V	WT	WT	G12D	1501
CEM/C1	Peripheral blood	WT	WT	WT	G12D	1773
GDM-1	Peripheral blood	*WT*	WT	WT	WT	*1997*
HT-1080	Skin	WT	Q61K	WT	WT	*2004*
HL-60	Peripheral blood	WT	Q61L	WT	WT	2178
RKO	Colon	V600E	WT	WT	WT	2522
MES-SA	Uterus	WT	WT	WT	WT	6989
Daudi	Peripheral blood	E26D	WT	WT	WT	>10000
MDA-MB-468	Breast	E26D	WT	WT	WT	>10000
C33A	Cervix uteri	A115V	WT	WT	WT	>10000
NCI-H508	Colon	G596R	WT		WT	>10000
22Rv1	Prostate	L597R	WT	WT	WT	>10000
KM12	Colon	A712T	WT	WT	WT	>10000
A673	Muscle	V600E	WT	WT	WT	>10000
GCT	Skin	V600E	WT	WT	WT	>10000
NCI-H292	Lung	T119S, V600E	WT	WT	WT	>10000
LS-174T	Colon	D211G	WT	WT	G12D	>10000
MDA-MB-231	Breast	G464V	WT	WT	G13D	>10000
CAL-27	Tongue	WT	R68T, D92N		WT	>10000
SCC-13	Skin	*WT*	WT	G12N	WT	>10000
MiaPaCa	Pancreas	WT	WT	WT	G12C	>10000
NCI-H358	Lung	WT	WT	WT	G12C	>10000
UM-UC-3	Bladder	WT	WT	WT	G12C	>10000
SW1990	Pancreas	*WT*	WT	WT	G12D	>10000
NCI-H157	Lung	WT	WT	WT	G12R	>10000
SW480	Colon	WT	WT	WT	G12V	>10000
DLD-1	Colon	WT	WT	WT	G13D	>10000
HCT-116	Colon	WT	WT	WT	G13D	>10000
NCI-H460	Lung	WT	WT	WT	Q61H	>10000
NCI/ADR-RES	Breast	WT	WT	WT	P121H	>10000
HCC-2998	Colon	WT	WT	WT	A146T	>10000
647-V	Bladder	*WT*	WT	WT		>10000
786-O	Kidney	WT	WT	WT	WT	>10000
A204	Muscle	*WT*	WT	WT	WT	>10000
A431	Skin	WT	WT	WT	WT	>10000
A2780	Ovary	WT	WT	WT	WT	>10000
ARH-77	Peripheral blood	WT	WT	WT	WT	>10000
BT-20	Breast	WT	WT		WT	>10000
BxPC3	Pancreas	*WT*	WT	WT	WT	>10000
C-4 I	Cervix uteri	WT	WT	WT	WT	>10000
CaOv-3	Ovary	WT	WT	WT	WT	>10000
CHL-1	Skin	WT	WT	WT	WT	>10000
COLO-320DM	Colon	WT	WT	WT	WT	>10000
CRO-AP2	Lymph node	*WT*	WT	WT	WT	>10000
DoTc2-4510	Cervix uteri	WT	WT	WT	WT	>10000
EFM-19	Breast	WT	WT	WT	WT	>10000
FaDu	Pharynx	*WT*	WT			>10000
G401	Kidney	WT	WT	WT	WT	>10000

RESISTANT						
CELL LINE	Tissue Origin	*BRAF*	*NRAS*	*HRAS*	*KRAS*	GSK2118436A Mean gIC_{50} (nM)
HCC-1954	Breast	WT	WT	WT	WT	>10000
HeLa	Cervix uteri	*WT*	WT	WT	WT	>10000
Hep3B	Liver	WT	WT	WT	WT	>10000
HN5	Tongue	*WT*	WT	WT	WT	>10000
HS746T	Stomach	WT	WT	WT	WT	>10000
J82	Bladder	WT	WT	WT	WT	>10000
KATO III	Stomach	WT	WT	WT	WT	>10000
LNCaP	Prostate	WT	WT	WT	WT	>10000
MCF-7	Breast	WT	WT	WT	WT	>10000
NCI-H69	Lung	WT	WT	WT	WT	>10000
NCI-H82	Lung	WT	WT	WT	WT	>10000
NCI-N87	Stomach	WT	WT	WT	WT	>10000
NCI-H322	Lung	WT		WT	WT	>10000
NCI-H526	Lung	*WT*	WT	WT	WT	>10000
NCI-H661	Lung	WT	WT	WT		>10000
NCI-H2052	Lung	*WT*	WT	WT	WT	>10000
OVCAR-3	Ovary	WT	WT	WT	WT	>10000
OVCAR-4	Ovary	WT	WT	WT	WT	>10000
P3HR-1	Peripheral blood	WT	WT	WT	WT	>10000
PC-3	Prostate	WT	WT	WT	WT	>10000
Raji	Bone	WT	WT	WT	WT	>10000
RD-ES	Bone	WT	WT	WT	WT	>10000
SaOS2	Bone	WT	WT	WT	WT	>10000
SCLC-3	Lung	*WT*	WT	WT	WT	>10000
SF-268	Brain	WT	WT	WT	WT	>10000
SK-BR-3	Breast	WT	WT	WT	WT	>10000
SK-N-BE(2)C	Brain	WT	WT	WT	WT	>10000
SK-OV-3	Ovary	WT	WT	WT	WT	>10000
SNU-5	Stomach	*WT*	WT	WT	WT	>10000
ST486	Peripheral blood	WT	WT	WT	WT	>10000
SW579	Thyroid	*WT*	WT			>10000
SW1088	Brain	*WT*	WT	WT	WT	>10000
T-47D	Breast	WT	WT	WT	WT	>10000
U-2 OS	Bone	WT	WT	WT	WT	>10000
ZR-75-1	Breast	WT		WT	WT	>10000

Sensitive (gIC_{50} <100 nM), intermediate (gIC_{50} 100 nM – 1 μM), resistant (gIC_{50} >1 μM).
Wild-type proteins are denoted WT and those for BRAF in italics signify incomplete sequence data but wild-type at residue-600 (V).
Empty cell denotes that the sequence information for the gene in the specific cell line is not available.
(table excerpted from Applicant's NDA)

To determine of specificity of GSK2118436A for proliferating cells, non-proliferating normal human umbilical vein endothelial cells (HUVEC) and proliferating HUVEC were exposed to GSK2118436A for 3 days. A representative assay from 4 independent experiments is shown in the following figure. These data demonstrated weak GSK2118436-induced inhibition of HUVEC proliferation (gIC_{50} 400 nM), and even weaker inhibition of non-proliferating HUVEC (gIC_{50} > 5.5 μM).

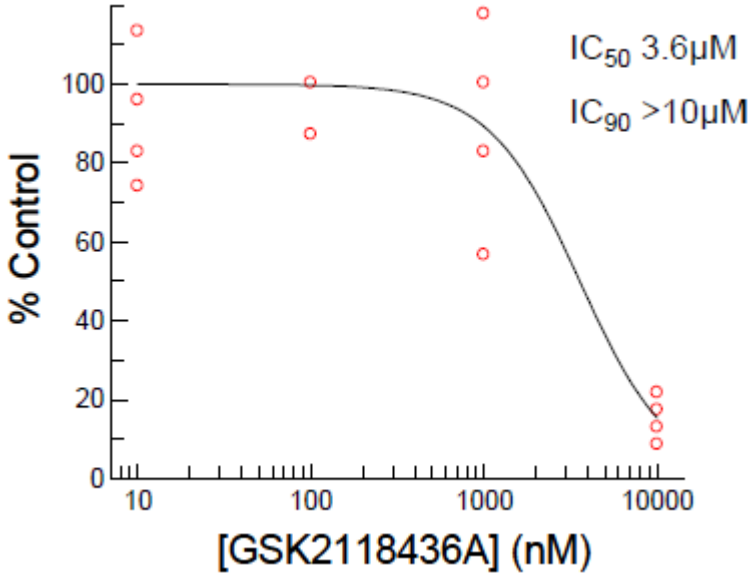
Specificity of GSK2118436A for Proliferating Normal Human Umbilical Vein Endothelial Cells



(figure excerpted from Applicant's NDA)

To determine the potential for GSK2118436A-induced myelosuppression, the effect of GSK2118436A on GM-CSF-stimulated human progenitor cell (neutrophil & monocyte) growth was tested in a standard CFU-GM colony forming assay. GSK2118436A inhibited CFU-GM colony formation at high concentrations with an IC_{50} of 3.6 µM (see figure below), which is approximately 100- to 3000-fold higher than the gIC_{50} values seen for the majority of BRAFV600E cell lines in the 3-day growth-death assay (previously shown in table above). Furthermore, according to the Applicant, the IC_{90}, which may be a better correlate with potential for clinical neutropenia [Foti, 2003], was not measurable over the concentration range tested, suggesting a high likelihood that neutropenia may be avoidable at clinically efficacious concentrations of GSK2118436A.

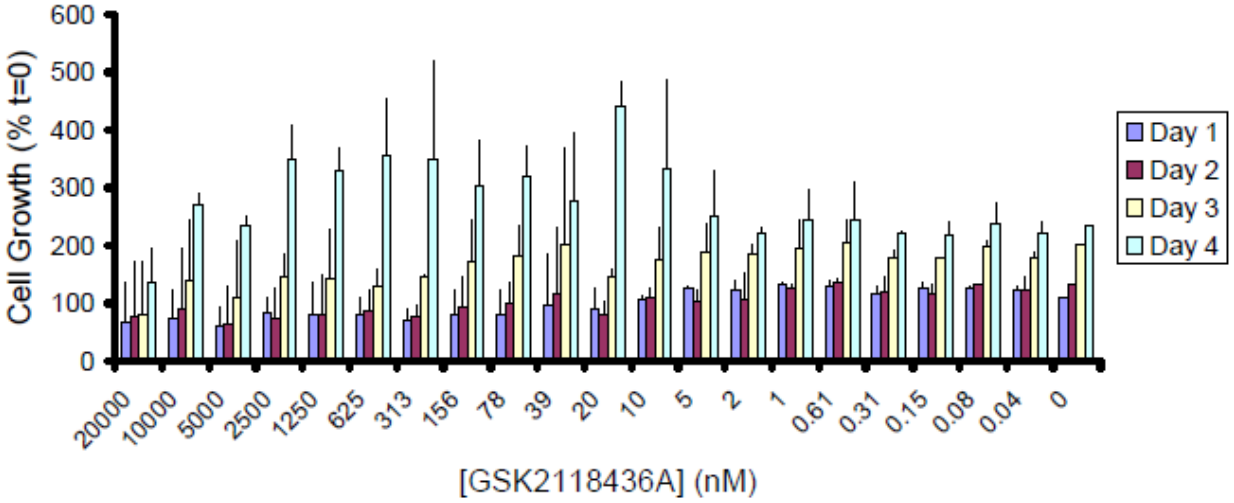
Inhibition of Human GM-CSF-stimulated Bone Marrow Progenitor Colony Growth by GSK2118436A



(figure excerpted from Applicant's NDA)

The reversibility of GSK2118436A-induced cell growth inhibition was examined in a compound washout assay. Exponentially growing tumor cells were treated for an initial 72-hour period with GSK2118436A, followed by removal of compound from cells by washing with medium containing 20% serum, trypsinization, and re-plating into new plates. Cell numbers were determined by CellTiter-Glo™ assay at time zero (i.e. after the initial 72-hour exposure and immediately after washout and re-plating), as well as at 1, 2, 3, and 4 days subsequent to the time of re-plating following washout. As illustrated in the following figure, reversibility of growth inhibition was demonstrated over a 2- to 4-day period following compound washout in the human melanoma BRAFV600E-containing cell line, SK-MEL-28. Reversibility of growth inhibition was particularly noticeable at days 3 and 4 after washout, as the cells entered an exponential growth phase. Re-growth was also observed with A375P F11s cells following compound washout (data not shown). These data demonstrate that GSK2118436-induced inhibition of cell growth in mutant BRAF cells is reversible, following inhibitor removal, and consequently that sustained compound presence may be required for prolonged tumor growth inhibition.

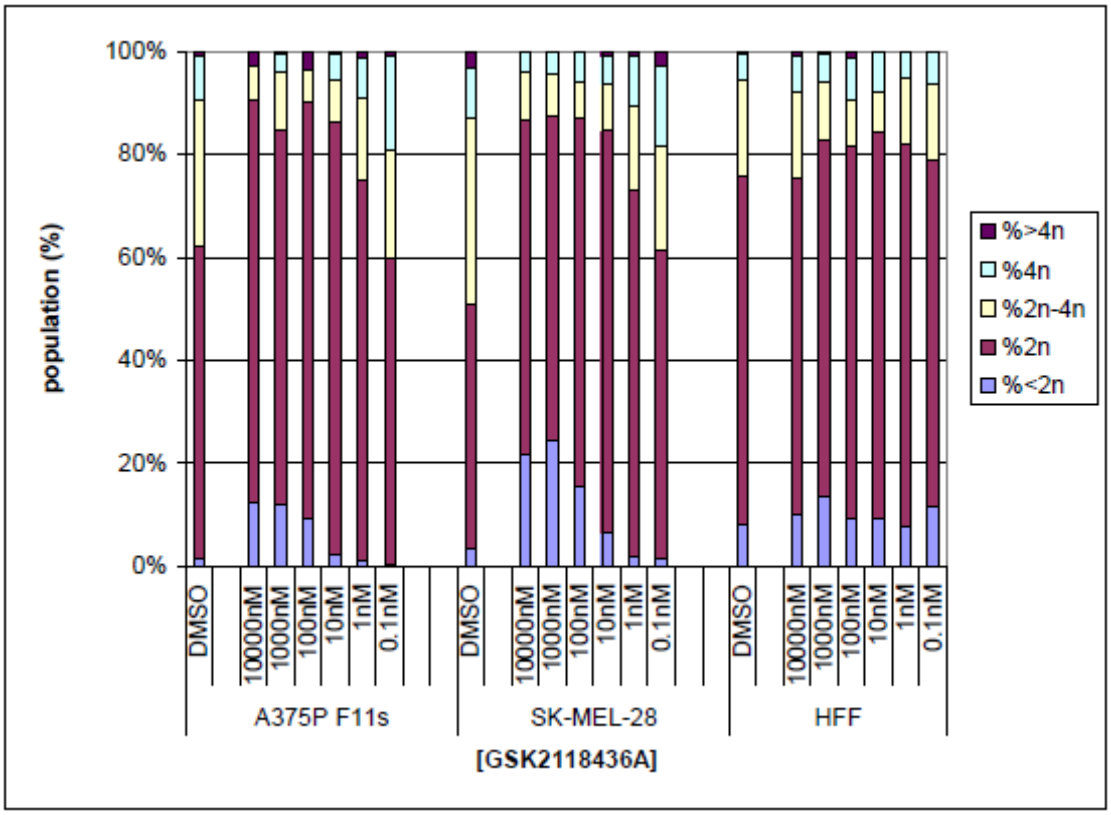
Inhibition of SK-MEL-28 BRAFV600E Cell Proliferation by a 72-hour exposure to GSK2118436A is Reversible following Inhibitor Washout (data at 1, 2, 3, and 4 days after re-plating following compound washout, i.e. t=0)



(figure excerpted from Applicant's NDA)

According to the Applicant, GSK2118436A can induce a G0/G1 (2N DNA) cell cycle arrest in the BRAFV600E-containing cells, A375P F11, Colo205, and SK-MEL-28, following a 24-hour exposure, as demonstrated by flow cytometry (data not shown). This concentration-dependent G0/G1 arrest resulted in the accumulation of cells containing sub-2N DNA by 72 hours in A375PF11s and SK-MEL-28, suggesting induction of cell death (see figure below). The wild-type BRAF-containing cells, HFF (see figure below) and HN5 (data not shown), were not susceptible to either a significant G0/G1 arrest or the induction of sub-2N DNA accumulation. These data reflect the sensitivity of BRAFV600E containing cells to GSK2118436A in the 3-day growth-death assay.

Cell Cycle Effect of GSK2118436A Treatment for 72 hours on BRAFV600E Human Tumor Cell Lines and Normal Human Fibroblasts



(figure excerpted from Applicant's NDA)

The increased sub-2N DNA population seen following cell cycle arrest in these cells correlates to induction of apoptosis, as demonstrated by the Caspase-Glo™ assay, which measures activation of caspases 3 and 7 (caspase 3/7). In this assay the caspase 3/7 induction is corrected for cell number, as concomitantly determined by CTG assay, and expressed as EC200 (nM), which is defined as the concentration of BRAF inhibitor required for a 2-fold signal increase over DMSO control set at 100%. Concentration of GSK2118436 required for caspase-3/7 cleavage in BRAFV600E cells treated for 24 hours was variable, whereas a generally lower concentration of GSK2118436A was required for activation at 48 hours, which was comparable in all three cell lines (see table below). In contrast, little or no measurable caspase-3/7 activation was seen in HFF and HN5 cells following BRAF inhibitor treatment, either at 24 or 48 hours, reflecting the previously observed lack of sensitivity of these cells to GSK2118436A in the growth-death assay. The Caspase-Glo™ assay was performed in duplicate in BRAFV600E cells (data from both assays reported), due to high variability at early (24-hour) time points.

GSK2118436A Selectively Activates Caspase-3/7 after 24 or 48 hours in BRAFV600E Human Tumor Cells but not Cells Containing wild-type BRAF

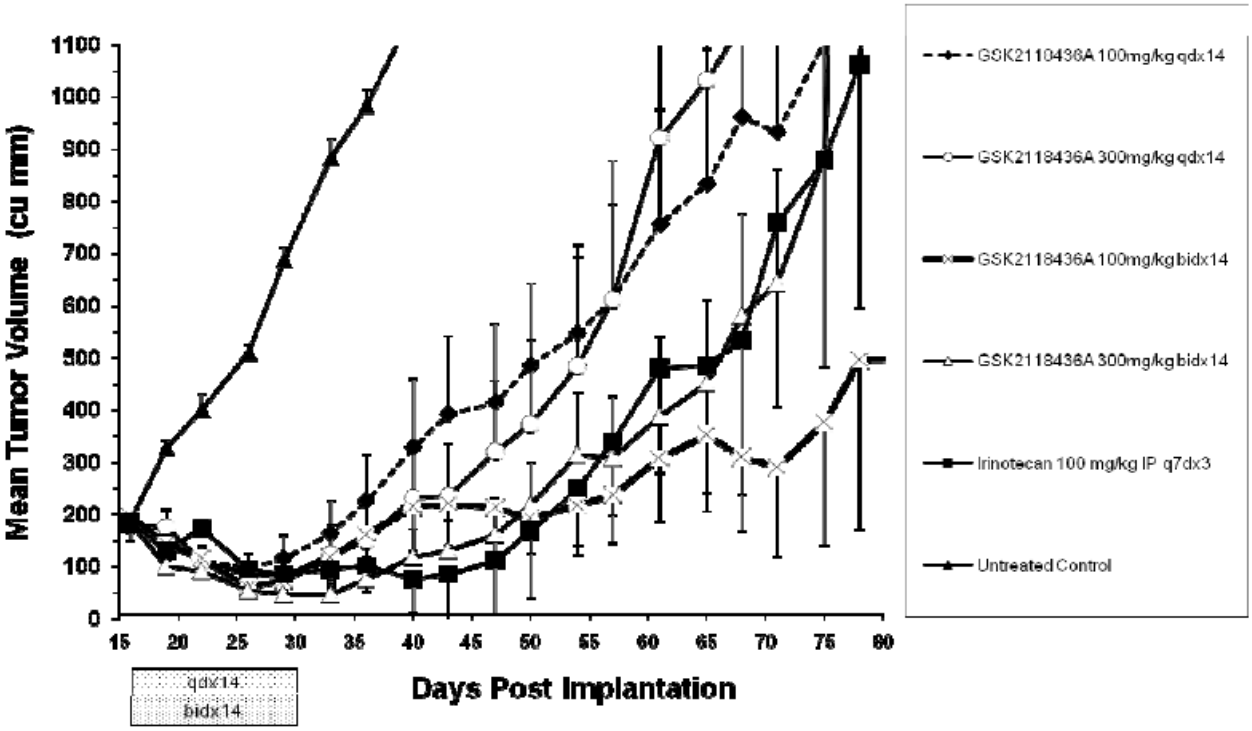
CELL LINE	BRAF Status	Caspase Activation (EC_{200} nM)	
		24hr	48hr
A375P F11s	V600E	87	8
		1813	134
Colo205	V600E	>20000	49
		615	141
SK-MEL-28	V600E	7	8
		65	78
HN5	WT	>13200	>13200
HFF	WT	>20000	>20000
		5476	11901

(table excerpted from Applicant's NDA)

UH2008-00145: Efficacy of the Oral B-Raf Inhibitor GSK2118436 in Various Mouse Xenograft Models of Human Cancer.

Efficacy studies were conducted in CD-1 *nu/nu* mice bearing A375P F11s tumor xenografts since A375P F11s cells contain BRAFV600E and have shown an *in vitro* sensitivity to GSK2118436A. In the first study, GSK2118436A was administered as an oral suspension (in 0.5% HPMC, 0.2% Tween 80, pH 7-8) of 100 or 300 mg/kg, daily for 14 days. Additional animals received GSK2118436A, twice daily for 14 days. As a positive control, irinotecan was administered intraperitoneally at 100 mg/kg, weekly for 3 weeks. Tumor sizes were monitored until groups reached 2000mm^3 or Day 100. As shown in the following figure, both doses and dosing regimes of GSK2118436A resulted in tumor growth inhibition. Tumor growth inhibition ranged from 113% to 127%. Irinotecan also demonstrated expected efficacy with tumor growth inhibition of 120%. According to the Applicant, a durable response was seen at the end of the study (Day 100), as three mice in the 300 mg/kg twice daily group, one mouse in the 300 mg/kg daily group, and one mouse in the 100 mg/kg twice daily group remained tumor-free. Overall, these data demonstrate that under the conditions studied, weekly or twice weekly administration of GSK2118436A results in similar efficacy.

Effect of GSK2118436A on the Growth of A375P F11s Xenografts Treated Orally with 100 and 300 mg/kg Daily or Twice Daily for 14 Days



	% TGI	Mean	SE	CR/PR
GSK2118436A 100mg/kg qdx14	113.1%	116.7	43	1CR/3PR/8
GSK2118436A 300mg/kg qdx14	120.2%	82.0	28	2CR/4PR/8
GSK2118436A 100mg/kg bidx14	124.2%	75.6	19	3CR/2PR/8
GSK2118436A 300mg/kg bidx14	127.4%	47.9	19	3CR/1PR/7
Irinotecan 100 mg/kg IP q7dx3	120.0%	84.9	15	2CR/2PR/7
Untreated Control	0.0%	686.8	27	--

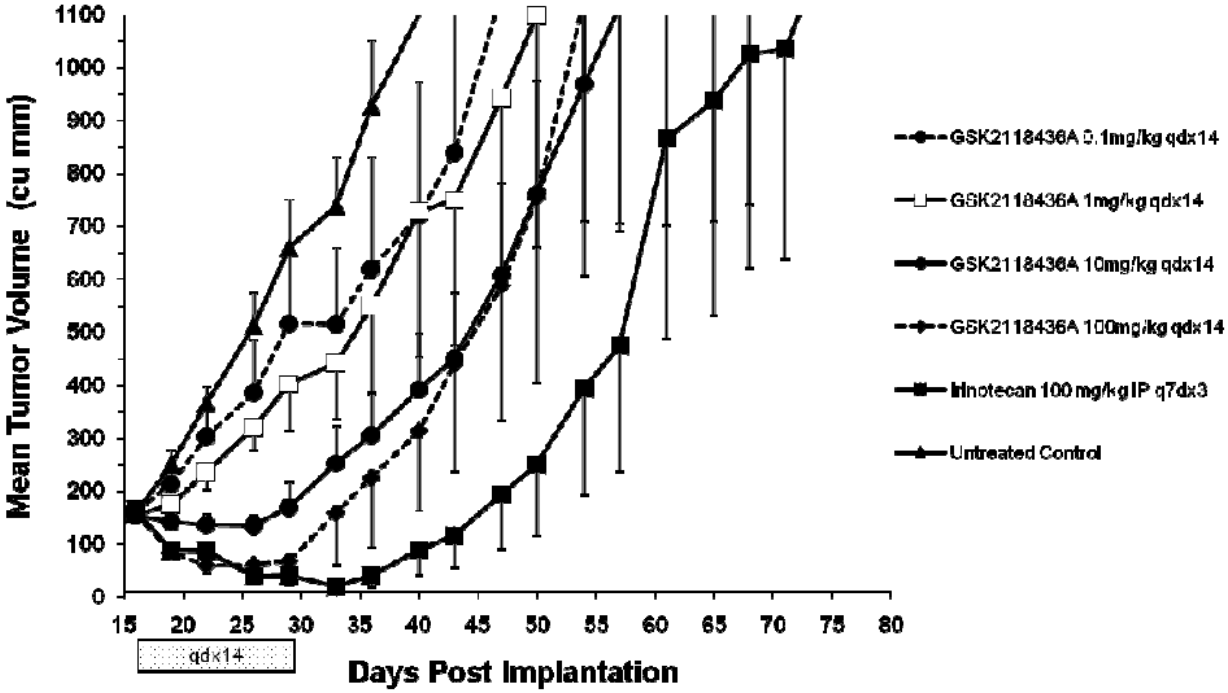
% TGI = % tumor growth inhibition, calculated on Day 29. Mean and SE values are mean and standard error of the mean tumor volumes (mm^3). CR (complete regression) = individual tumor volume < 13 mm^3 for a minimum of three consecutive measurements; PR (partial regression) = individual tumor volume equal to one half the initial starting volume for a minimum of three consecutive measurements.

(figure excerpted from Applicant's NDA)

In the next study, the efficacy of GSK2118436A in CD-1 *nu/nu* mice bearing A375P F11s tumor xenografts was evaluated at lower dose levels of 0.1, 1, 10, and 100 mg/kg. All dose levels were orally administered, daily for 14 days. The results show a dose-responsive increase in tumor growth inhibition. Specifically, as shown in the figure below, 0.1, 1, 10, and 100 mg/kg of GSK2118436A, administered daily for 14 days, resulted in 29, 51, 97, and 118% tumor growth inhibition, respectively. The positive control, irinotecan, administered as previously described above, resulted in a 125% tumor growth inhibition. At the end of the study (Day 100), 2 mice in the 100 mg/kg

group, one mouse in the 10 mg/kg group, and one mouse in the 0.1 mg/kg group remained tumor-free.

Effect of GSK2118436A on the Growth of A375P F11s Xenografts Treated Orally with 100 to 0.1 mg/kg Daily for 14 Days



	% TGI	Mean	SE	CR/PR
GSK2118436A 0.1mg/kg qdx14	29.1%	516.9	133	2CR/8
GSK2118436A 1mg/kg qdx14	50.7%	402.1	89	1CR/8
GSK2118436A 10mg/kg qdx14	97.0%	169.6	47	1CR/2PR/8
GSK2118436A 100mg/kg qdx14	117.7%	69.1	43	4CR/2PR/8
Irinotecan 100 mg/kg IP q7dx3	124.8%	40.9	18	4CR/2PR/8
Untreated Control	0.0%	659.6	90	

% TGI = % tumor growth inhibition, calculated on Day 29. Mean and SE values are mean and standard error of the mean tumor volumes (mm^3). CR (complete regression) = individual tumor volume < 13 mm^3 for a minimum of three consecutive measurements; PR (partial regression) = individual tumor volume equal to one half the initial starting volume for a minimum of three consecutive measurements.

(figure excerpted from Applicant's NDA)

A third study was conducted to compare the efficacy of GSK2118436A in CD-1 *nu/nu* mice bearing A375P F11s tumor xenografts, when treated with 5 mg/kg twice daily for 14 days; 10 mg/kg daily for 14 days, 2 cycles of 14 days, or 90 days; 15 mg/kg twice daily for 14 days; and 30 mg/kg daily for 14 days, 2 cycles of 14 days, or 90 days. For the 2 cycle dosing regimes, at the end of the first cycle, tumors were allowed to re-grow to approximately 500 mm^3 before re-dosing animals. The percentage of tumor growth inhibition at each dose level and dosing schedule is detailed in the following table.

Effect of GSK2118436A on the Growth of A375P F11s Xenografts Dosed at 5, 10, 15, and 30 mg/kg using Different Treatment Schedules

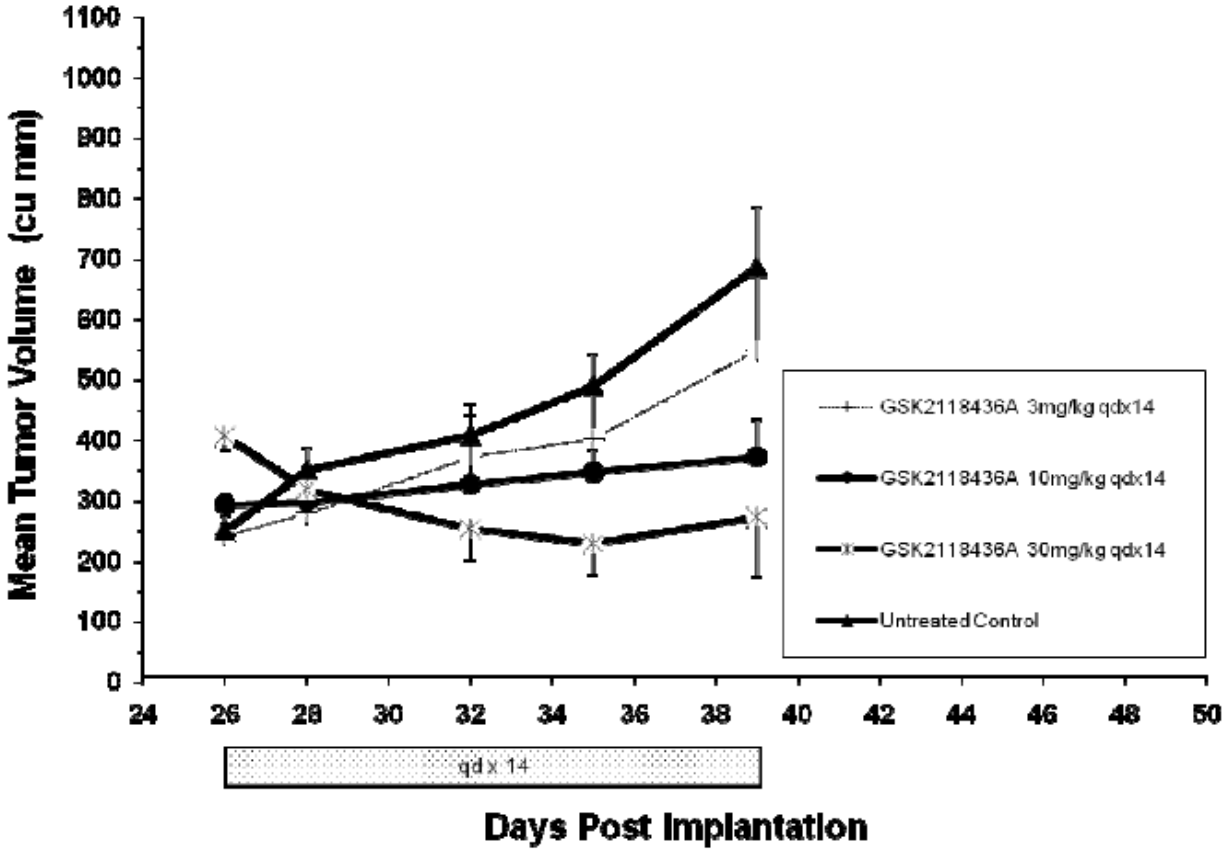
	% TGI	Mean	SE	CR/PR
GSK2118436A 5mg/kg bidx14	93.8%	182.5	63	
GSK2118436A 10mg/kg qdx14 ref	116.2%	122.6	26	2PR/8
GSK2118436A 10mg/kg qdx14 redose	104.7%	158.0	38	
GSK2118436A 10mg/kg qdx90	51.6%	278.4	72	1CR/8
GSK2118436A 15mg/kg bidx14	120.4%	116.4	29	1PR/8 1
GSK2118436A 30mg/kg qdx14 ref	90.2%	189.9	56	1PR/8
GSK2118436A 30mg/kg qdx14 redose	140.8%	75.4	13	5PR/8
GSK2118436A 30mg/kg qdx90	121.5%	113.9	26	1CR/2PR/8
Irinotecan 100 mg/kg IP q7dx3	150.2%	41.4	7	1CR/7PR/8
Untreated Control	0.0%	415.3	62	

% TGI = % tumor growth inhibition, calculated on Day 40. Mean and SE values are mean and standard error of the mean tumor volumes (mm^3). CR (complete regression) = individual tumor volume < 13 mm^3 for a minimum of three consecutive measurements; PR (partial regression) = individual tumor volume equal to one half the initial starting volume for a minimum of three consecutive measurements.

(table excerpted from Applicant's NDA)

The final study to assess the efficacy of GSK2118436A in CD-1 *nu/nu* mice bearing A375P F11s tumor xenografts was performed by administering GSK2118436A at 3, 10, and 30 mg/kg, daily for 14 days. Similar to previous studies, GSK2118436A induced a dose-responsive increase in tumor growth inhibition. As shown in the figure below, the percentage of tumor growth inhibition following 3, 10, and 30 mg/kg of GSK2118436A, administered daily for 14 days, was 30, 82, and 131%, respectively.

Effect of GSK2118436 on the Growth of A375P F11s Xenografts Dosed at 3, 10, and 30 mg/kg, Daily for 14 Days



	% TGI	Mean	SE	CR/PR	Mean AUC(0-24) (ng.h/mL)
GSK2118436A 3mg/kg qdx14	29.9%	549.8	124		108.8
GSK2118436A 10mg/kg qdx14	82.0%	372.9	61		388.8
GSK2118436A 30mg/kg qdx14	130.6%	273.5	99	8PR/12	1604.8
Untreated Control	0.0%	686.8	99		--

% TGI = % tumor growth inhibition, calculated on Day 39. Mean and SE values are mean and standard error of the mean tumor volumes (mm^3). CR (complete regression) = individual tumor volume < 13 mm^3 for a minimum of three consecutive measurements; PR (partial regression) = individual tumor volume equal to one half the initial starting volume for a minimum of three consecutive measurements. AUC(0-24) is the GSK2118436 area under the concentration-time curve from time = zero to 24 hours (average of Day 26, Day 33 and Day 39 values).

(figure excerpted from Applicant's NDA)

4.3 Safety Pharmacology

Study title: GSK2118436A: Acute Neurobehavioral Effects Following Oral Administration in the Conscious Crl:CD(SD) Rat. Reviewed by Kimberly Ringgold, Ph.D. and slightly modified for this NDA review.

Study no:	VD2008-00869
Study report location:	4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 17, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, 081179173, 99.3%

Key study findings:
No GSK2118436A-induced neurobehavioral effects were noted, under the conditions tested.

Methods:

Doses:	0, 5, 20, 200 mg/kg/day
Frequency of dosing:	Single dose with neurobehavioral observations at 1, 2, 4, 8, and 24 hours post-dose
Route of administration:	Oral suspension
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% hydroxypropyl methylcellulose with 0.1% Tween 80 at 0.5, 2, and 20 mg/mL
Species/Strain:	Crl:CD(SD) Rats
Number/Sex/Group:	8 males
Age:	10-12 weeks
Weight:	375 to 440 grams
Satellite groups:	None
Dose justification:	Same doses used in 4-week rat toxicology study (Study No. CD2008-01511)

Observations and Results:

Motor activity: unremarkable

Behavior: unremarkable

Pupil size: unremarkable

Lacrimation: unremarkable

Salivation: unremarkable

Body temperature: unremarkable

Study title: GSK2118436A: Acute Effects on Respiratory Function Following Oral Administration in the Conscious Crl:CD(SD) Rat. Reviewed by Kimberly Ringgold, Ph.D. and slightly modified for this NDA review.

Study no:	CD2008-01279
Study report location:	4.2.1.3
Conducting laboratory and location:	GlaxoSmithKline, King of Prussia, PA
Date of study initiation:	October 30, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, 081179173, 99.3%

Key study findings:
No GSK2118436A-induced effects on respiratory function or body temperature were noted, under the conditions tested.

Methods:

Doses:	0, 5, 20, 200 mg/kg/day
Frequency of dosing:	Single dose with neurobehavioral observations at 1, 2, 4, 8, and 24 hours post-dose
Route of administration:	Oral suspension
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% hydroxypropyl methylcellulose with 0.1% Tween 80 at 0.5, 2, and 20 mg/mL
Species/Strain:	Crl:CD(SD) Rats
Number/Sex/Group:	4 males
Age:	13 weeks
Weight:	363 to 394 grams
Satellite groups:	None
Dose justification:	Same doses used in 4-week rat toxicology study (Study No. CD2008-01511)

Observations and Results:

Tidal volume: unremarkable

Respiratory rate: unremarkable

Minute volume: unremarkable

Pulmonary resistance: unremarkable

Body temperature: unremarkable

Study title: GSK2118436A: Acute Effects on Cardiovascular Function Following Oral Administration of in the Conscious Beagle Dog. Reviewed by Kimberly Ringgold, Ph.D. and slightly modified for this NDA review.

Study no:	FD2008-01280
Study report location:	4.2.1.3
Conducting laboratory and location:	GlaxoSmithKline, Hertfordshire, UK
Date of study initiation:	November 6, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, 081179173, 99.3%

Key study findings:
No toxicologically significant GSK2118436A-induced effects were seen on arterial pressure, heart rate, body temperature, or electrocardiographic intervals, under the conditions tested.

Methods:

Doses:	0, 1, 5, 50 mg/kg/day
Frequency of dosing:	Single dose with monitoring up to 24 hours post-dose
Route of administration:	Oral suspension
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% hydroxypropyl methylcellulose with 0.1% Tween 80 at 0.2, 1, and 10 mg/mL
Species/Strain:	Dog / Beagle
Number/Sex/Group:	4 males
Age:	1-2 years
Weight:	8 to 13 kg
Satellite groups:	None
Dose justification:	Same doses used in 4-week dog toxicology study (Study No. CD2008-01503)

Observations and Results:

Heart rate: unremarkable

Mean arterial pressure: unremarkable

Systolic pressure: unremarkable

Diastolic pressure: unremarkable

Pulse pressure: unremarkable

Electrocardiograph: unremarkable

QT/QTc intervals: unremarkable

Body temperature: unremarkable

Study title: GSK2118436A: Effect on hERG Tail Current Recorded from Stably Transfected HEK-293 Cells. Reviewed by Kimberly Ringgold, Ph.D. and slightly modified for this NDA review.

Study no:	FD2008-00376
Study report location:	4.2.1.3
Conducting laboratory and location:	GlaxoSmithKline, Hertfordshire, UK
Date of study initiation:	November 17, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, 081179173, 99.3%

Key study findings:
Under the conditions tested, GSK2118436 was a weak hERG blocker, with a low potential to induce QT prolongation.

Methods:

Strains/species/cell line:	Human Embryonic Kidney Cells
Controls:	Vehicle: HEPES-buffered physiological saline (HB-PS) solution + 0.3% DMSO Reference: E-4031
Concentrations:	1.5, 5, 15, 30 µM
Test system:	Standard hERG assay

Results:
The IC_{25} for the inhibitory effect of GSK2118436 on hERG potassium current was 11.7 µM.

5 Pharmacokinetics/ADME/Toxicokinetics

Reviewed by Shawna L. Weis, Ph.D.

5.1 PK/ADME

UH2008/00115/02: Preliminary Drug Metabolism and Pharmacokinetics of GSK2118436.

This summary report provides the results of a panel of studies performed to assess the ADME profile of GSK2118436 (dabrafenib) in the mouse, rat, monkey, and dog. An

additional panel of *in vitro* studies was completed to assess plasma protein binding and stability, the potential for metabolic inhibition, and permeability and inhibition of transport by transporter molecules involved in distribution and elimination. The following summarizes the conclusions of these studies as stated in the Applicant's report; data were not presented.

Bioavailability in animals ranged from 46 ± 4% in the monkey to 82 ± 12% in the dog. The mouse, rat, and monkey were considered moderate clearance species (43.5%, 32% and 50% of hepatic blood flow, respectively). The dog was considered a low clearance species (12% of hepatic blood flow). The steady state volume of distribution was low in all species (0.4-1.0 L/kg).

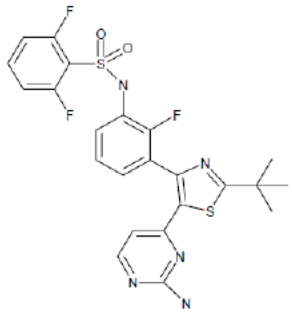
Stability assays were conducted in microsomal preparations from each species, and the drug was found to be stable following a 2-hour incubation at 37°C. Dabrafenib was stable in whole blood, highly protein bound in the rat, mouse, dog, monkey, and human (> 98%), and was not found to partition to blood cells in any species. Dabrafenib is a substrate of human P-gp and murine Bcrp1 *in vitro* and the apparent permeability was high across MDCK monolayers (148 mm/sec in MDR1-MDCK cells and 415 ± 63 mm/sec in Bcrp-MDCK cells.

Dabrafenib is a moderate inhibitor of CYP3A4, CYP2C9 and CYP2C19, but a weak inhibitor of CYP1A2 and CYP2D6.

CD2009-0041: An Evaluation of the Systemic Exposure of GSK2118436 Following Oral Gavage Administration of Micronized GSK211843A (free Base, (b) (4) in Suspension at 5 or 10 mg/kg to Male Crl:CD(SD) Rats.

The purpose of this study was to assess dose-linearity and pharmacokinetic profiles of different pharmaceutical forms (polymorph) of GKS2118436 free base in rats, when administered as a suspension, by oral gavage. The properties of the test article administered are summarized in the figure below.

CD2009-0041 Test article Summary

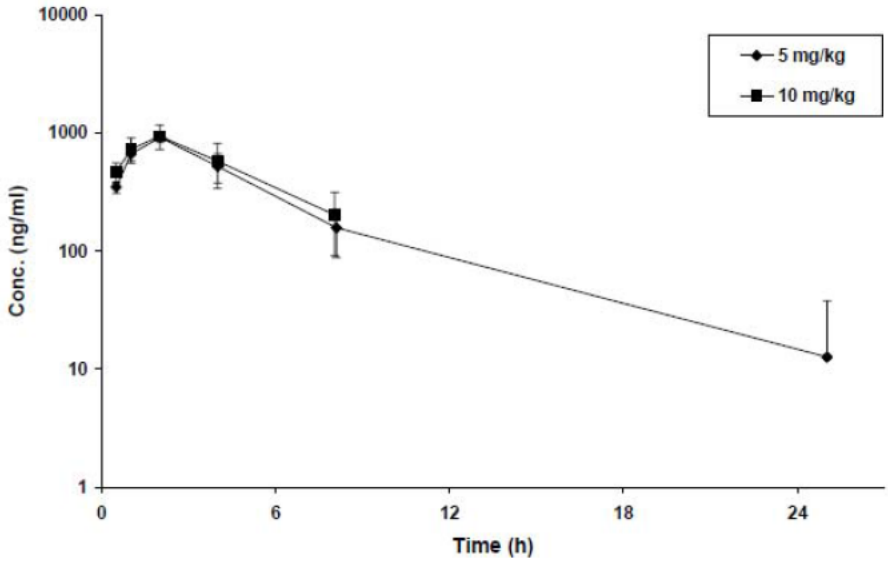
Compound structure	Compound [form]	Batch Number	Particle size (μm)	Assigned Purity (%w/w)	Molecular Weight	Physical Form
 GSK2118436A free base	GSK2118436 [Form 2]	EE293267-1	(b) (4)	99.58	519.571	Solid

a. As measured by laser diffraction (Malvern).

(figure excerpted from Applicant's NDA)

The test article was administered by oral gavage to 4 male rats per group in a suspension of 0.5% HPMC (10 mL/kg). As illustrated in the figure below, exposure was sub-proportional with dose, as exposure at 10 mg/kg was essentially identical to exposure at 5 mg/kg.

Concentration-Time Profile of 2 Doses of GSK2118436



(figure excerpted from Applicant's NDA)

2011N126179: The Assessment of the Systemic Exposure of GSK2118436 and its Metabolites GSK229683 (M4), GSK2285403 (M7) and GSK2167542 (M8) following a Single Oral Administration of GSK2118436B Suspension at 20 mg/kg to Male Crl:CD(SD) Rats.

The purpose of this study was to evaluate the systemic exposure of GSK2118436 and the M4, M7, and M8 metabolites following a single 20 mg/kg oral dose to male rats. Test article was prepared as a suspension in 0.5% HPMC at a concentration of 2 mg/mL and administered by oral gavage (10 mL/kg) to 5 male rats. Plasma concentrations were evaluated by UHPLC-MS/MS. The LLQ of the assay was 5 ng/mL, and the HLQ was 5000 ng/mL.

As shown below, exposure to GSK2118436 (parent) and its metabolites, M4 (GSK229683), M7 (GSK2285403), and M8 (GSK2167542), was achieved. The relative exposure (AUC) to M4 was approximately equal to that of the parent, while the exposure to M7 was greater than 2-fold higher than parent. Exposure to M8 was approximately 0.7% of parent. The dose-linearity of metabolite exposure was not explored in this study. Thus, it is unclear whether higher doses would saturate the metabolic processes and increase or decrease relative metabolite exposures.

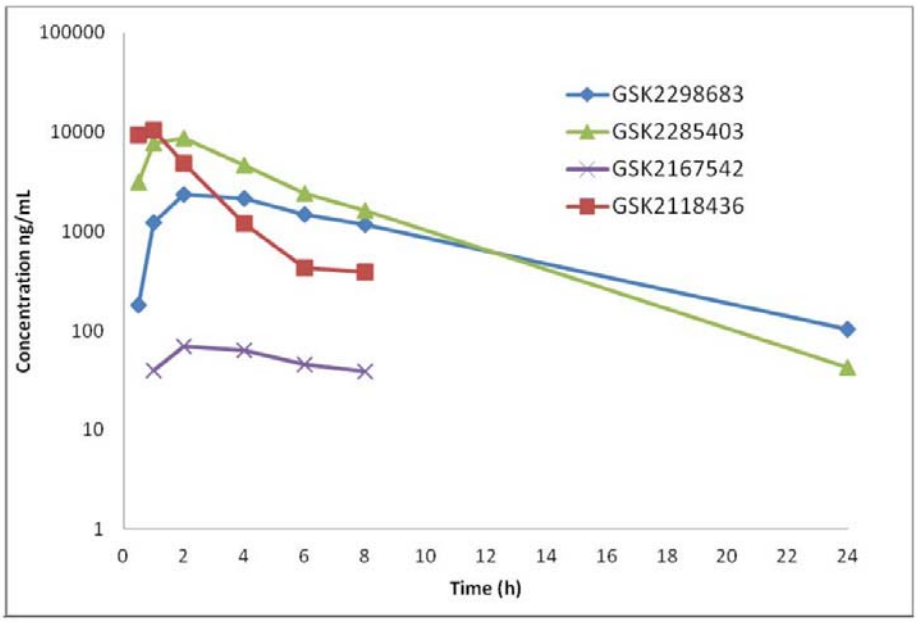
Pharmacokinetic Analysis of GSK2118436 and its M4, M7 and M8 Metabolites in the SD rat

Mean (or Median) Plasma Pharmacokinetic Parameters [Range]			
Analyte	AUC_{0-t} (µg.h/mL)	C_{max} (µg/mL)	T_{max} (h)
GSK2118436	22.1 [19.2-24.3]	10.6 [9.4-11.8]	1 [0.5-1]
GSK2298683	19.9 [7.79-26.4]	2.36 [1.02-2.90]	2 [2.0-4.0]
GSK2285403	42.4 [39.5-46.8]	8.73 [7.03-10.9]	2 [2.0-4.0]
GSK2167542	0.39 [0.15-0.57]	0.07 [0.03-0.09]	4 [2.0-4.0]

T_{max} is the Median value. n = 4 animals.

Row 2: parent; Row 3: M4; Row 4: M7; Row 5: M8
(table excerpted from Applicant's NDA)

Concentration-Time Profiles of GSK2118436 and its M4, M7 and M8 Metabolites



(figure excerpted from Applicant's NDA)

2011N119114: GSK2118436 Oral Bioavailability in the Beagle Dog; Comparison of HPMC and Gelatin Capsule Performance Using PillCam™ Swallowable Camera and Pharmacokinetic Analysis.

The purpose of this study was to evaluate the pharmacokinetic properties of two different capsule formulations to better understand the high pharmacokinetic variability in human subjects. Two capsule formulations were evaluated in Beagle dogs; a HPMC capsule and a gelatin capsule. Dogs also received a PillCam™ swallowable camera immediately prior to dose administration to provide visual data about the dissolution kinetics, which the Applicant hoped to correlate with pharmacokinetic data.

By visual observation, there was no difference between the two formulations in physical behavior during erosion and content-release in the stomach. In both preparations, the contents were released into the gastric fluid as a slurry, with larger particulate or chunk-like material.

As detailed in the table below, modest differences in the pharmacokinetic profile were noted. The peak and overall exposures were generally ~ 2X higher with the HPMC capsule than with the gelatin capsule. The Applicant suggests that the HPMC capsule may have stabilized the physical state of the solubilized material leading to a super-saturated gastric solution, and a more rapid absorption. Whether this two-fold difference could account for the observed clinical exposure variability is unclear.

GSK2118436 Pharmacokinetic Comparison of Gelatin and HPMC Capsule Formulations in Beagles

Dog	Regimen	T(1/2)	Tmax (hr)	Cmax (ng/mL)	Cmax ratio HPMC/GEL	AUC(0-inf) (ng-hr/mL)	AUC ratio HPMC/GEL
1	GELATIN CAPS	5.4	1.5	5618.1		24572.8	
2	GELATIN CAPS	3.7	4.0	7582.4		46035.7	
3	GELATIN CAPS	3.7	4.0	5093.7		28009.0	
4	GELATIN CAPS	3.6	2.0	6143.3		37426.1	
Mean		**4.1**	**2.9**	**6109.4**		**34010.9**	
1	HPMC CAPS	3.5	2.0	9428.6	1.7	54392.6	2.2
2	HPMC CAPS	3.3	1.5	15846.6	2.1	101919.9	2.2
3	HPMC CAPS	3.3	2.0	10055.5	2.0	49759.4	1.8
4	HPMC CAPS	3.2	1.5	8509.4	1.4	47087.0	1.3
Mean		**3.3**	**1.8**	**10960.0**	**1.8**	**63289.7**	**1.9**

(table excerpted from Applicant's NDA)

RD2009-00091: Quantitative Tissue Distribution of Radioactivity Using Whole-Body Autoradiography Following a Single Oral Administration of [^{14}C]GSK2118436 (10 mg/kg) to Partially Pigmented Male Rats

The purpose of this study was to evaluate the distribution of GSK2118436 following administration of a single 10 mg/kg oral dose to 7 male, partially-pigmented (Long-Evans) rats, using quantitative whole body audioradiography (QWBA). Sampling was performed at 2, 4, 8, and 24 hours, and at 3, 7, and 35 days post-dose. The relative doses were all between 12.65 - 13.22 mg/kg (48.7-54.3 µCi/rat).

As summarized in the following table, GSK2118436 exhibited widespread tissue distribution; however, most tissue concentrations were lower than those observed in blood. Tissue concentrations declined rapidly. By 24 hours post-dose, all tissues except for kidney, renal cortex, renal medulla, liver, and adrenal medulla, were below the limit of quantitation (BLQ). All tissues were BLQ by 35 days post-dose.

The Applicant claims that there was no association of [^{14}C]GSK2118436-related radioactivity with melanin in the male rat (as demonstrated by the similarity of distribution between pigmented and non-pigmented skin). The Applicant also claims

that there was no radioactivity detected in the brain at any timepoint (all BLQ by this method); however, meninges and choroid plexus both had demonstrable exposure.

Tissue Distribution of [^{14}C]GSK2118463 in Partially-Pigmented Male Rats

Tissue Type	Tissue	Concentration of Radioactivity (µg equiv/g)						
		Time Point						
		2 h	4 h	8 h	24 h	3 Days	7 Days	35 Days
Vascular/ Lymphatic	Aorta	0.211	0.675	0.089	BLQ	BLQ	BLQ	BLQ
	Blood (cardiac)	0.412	0.856	0.233	0.052	BLQ	BLQ	BLQ
	Bone Marrow	0.287	0.467	0.090	BLQ	BLQ	BLQ	BLQ
	Mandibular Lymph Nodes	0.268	0.399	0.154	BLQ	BLQ	BLQ	BLQ
	Spleen	0.278	0.346	0.074	BLQ	BLQ	BLQ	BLQ
	Thymus	0.184	0.349	0.094	BLQ	BLQ	BLQ	BLQ
Excretory/ Metabolic	Kidney	0.683	1.259	0.413	0.374	0.272	BLQ	BLQ
	Renal Cortex	0.934	1.555	0.710	0.624	0.256	BLQ	BLQ
	Renal Medulla	0.378	0.892	0.255	0.185	0.103	BLQ	BLQ
	Liver	12.207	12.872	5.399	0.696	0.426	0.055	BLQ
Central Nervous System	Brain	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Choroid Plexus	0.324	0.468	0.138	BLQ	BLQ	BLQ	BLQ
	Meninges	0.307	0.660	0.254	BLQ	BLQ	BLQ	BLQ
Endocrine	Adrenal Cortex	0.552	0.732	0.131	0.044	0.043	BLQ	BLQ
	Adrenal Medulla	0.447	0.680	0.216	BLQ	BLQ	BLQ	BLQ
	Pineal Gland	0.370	0.540	0.187	BLQ	BLQ	BLQ	BLQ
	Pituitary Gland	0.295	0.470	0.229	BLQ	BLQ	BLQ	BLQ
	Thyroid Gland	0.387	0.522	0.167	BLQ	BLQ	BLQ	BLQ
Secretory	Exorbital Lachrymal Gland	0.282	0.459	0.105	BLQ	BLQ	BLQ	BLQ
	Intra-orbital Lachrymal Gd.	0.331	0.432	0.171	BLQ	BLQ	BLQ	BLQ
	Harderian Gland	0.699	0.582	0.192	BLQ	BLQ	BLQ	BLQ
	Pancreas	0.321	0.406	0.122	BLQ	BLQ	BLQ	BLQ
	Salivary Gland	0.324	0.464	0.159	BLQ	BLQ	BLQ	BLQ
Fatty	Fat (brown)	0.750	0.889	0.260	BLQ	BLQ	BLQ	BLQ
	Fat (abdominal)	0.152	0.200	0.064	BLQ	BLQ	BLQ	BLQ
Dermal	Skin (non-pigmented)	0.206	0.412	0.087	BLQ	BLQ	BLQ	BLQ
	Skin (pigmented)	0.227	0.333	0.122	BLQ	BLQ	BLQ	BLQ
Reproductive	Epididymis	0.098	0.261	0.120	BLQ	BLQ	BLQ	BLQ
	Prostate	0.196	0.214	0.080	BLQ	BLQ	BLQ	BLQ
	Testis	0.076	0.187	0.063	BLQ	BLQ	BLQ	BLQ
Skeletal/ Muscular	Muscle (skeletal)	0.148	0.188	0.061	BLQ	BLQ	BLQ	BLQ
	Myocardium (heart)	0.398	0.551	0.146	BLQ	BLQ	BLQ	BLQ
Respiratory Tract	Lung	0.395	0.495	0.207	BLQ	BLQ	BLQ	BLQ
	Nasal Turbinates	0.179	0.135	0.088	BLQ	BLQ	BLQ	BLQ
Alimentary Canal	Cecum Mucosa	0.386	29.186	173.563	1.230	0.529	BLQ	BLQ
	Esophagus	0.175	0.391	0.097	0.072	BLQ	BLQ	BLQ
	Large Intestine Mucosa	0.247	0.414	79.192	0.488	0.770	BLQ	BLQ
	Large Intestine Contents	0.062	BLQ	1114.324*	12.871	10.820	BLQ	BLQ
	Rectum Mucosa	0.240	0.421	0.048	0.049	BLQ	BLQ	BLQ
	Small Intestine Mucosa	5.659	11.930	3.302	0.869	0.611	BLQ	BLQ
	Small Intestine Content	1300.869*	1718.614*	12.744	5.339	1.920	BLQ	BLQ
	Stomach Mucosa	0.397	2.273	0.166	0.921	BLQ	BLQ	BLQ
Ocular	Lens	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Uveal Tract	0.246	0.126	BLQ	BLQ	BLQ	BLQ	BLQ

BLQ: Below the limit of quantitation (< 0.040 µg equiv/g) or tissue could not be visually identified because of non-detectable radioactivity
*= above the limit of quantitation (>344.569 µg equiv/g)

(table excerpted from Applicant's NDA)

2010N108408: Quantitative Tissue Distribution of Radioactivity Using Whole-Body Autoradiography Following a Single Oral Administration of [^{14}C]GSK2118436 (10 mg/kg) to Partially Pigmented Female Rats.

The purpose of this study was to evaluate the distribution of GSK2118436 following administration of a single 10 mg/kg oral dose to 7 female, partially-pigmented (Long-Evans) rats, using quantitative whole body audioradiography (QWBA). Sampling was performed at 2, 4, 8, and 24 hours, and at 3, 7, and 14 days post-dose. The calculated specific activity of [^{14}C]GSK2118436 was 16.68 µCi/mg. The relative doses were all between 10.32 - 10.66 mg/kg (35.7-37.3 µCi/rat).

As summarized in the following table, GSK2118436 exhibited widespread tissue distribution; however, most tissue concentrations were lower than those observed in blood. Tissue concentrations declined rapidly. By 24 hours post-dose, all tissues except for kidney, renal cortex, liver, harderian gland, vagina, and intestinal tract were below the limit of quantitation (BLQ). All tissues were BLQ by 35 days post-dose.

The Applicant claims that there was no clear association of [^{14}C]GSK2118436-related radioactivity with melanin in the female rat (the relative exposure difference between pigmented and non-pigmented skin was between 0.76-2X). The Applicant also claims that was no radioactivity detected in the brain at any timepoint (all BLQ by this method), despite high meningeal exposure and demonstrable exposure in the choroid plexus.

Tissue Distribution of [^{14}C]GSK2118436 in Partially-Pigmented Female Rats

Tissue Type	Tissue	Concentration of Radioactivity (µg equiv/g)						
		Time Point						
		2 h	4 h	8 h	24 h	3 Days	7 Days	14 days
Vascular/ Lymphatic	Aorta	0.826	0.634	0.533	BLQ	BLQ	BLQ	BLQ
	Blood (cardiac)	0.938	1.073	0.559	BLQ	BLQ	BLQ	BLQ
	Bone Marrow	0.467	0.529	0.279	BLQ	BLQ	BLQ	BLQ
	Mandibular Lymph Nodes	0.397	0.680	0.207	BLQ	BLQ	BLQ	BLQ
	Spleen	0.443	0.487	0.265	BLQ	BLQ	BLQ	BLQ
	Thymus	0.330	0.369	0.205	BLQ	BLQ	BLQ	BLQ
Excretory/ Metabolic	Renal Cortex	1.166	1.314	0.851	0.153	BLQ	BLQ	BLQ
	Renal Medulla	0.732	0.854	0.565	BLQ	BLQ	BLQ	BLQ
	Liver	12.359	13.729	8.399	0.606	0.085	BLQ	BLQ
Central Nervous System	Brain	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Choroid Plexus	0.515	0.397	0.250	BLQ	BLQ	BLQ	BLQ
	Meninges	0.528	0.860	0.323	BLQ	BLQ	BLQ	BLQ
Endocrine	Adrenal Cortex	1.080	1.105	0.601	BLQ	BLQ	BLQ	BLQ
	Adrenal Medulla	0.922	0.934	0.562	BLQ	BLQ	BLQ	BLQ
	Pineal Gland	0.370	0.883	0.223	BLQ	BLQ	BLQ	BLQ
	Pituitary	0.509	0.613	0.282	BLQ	BLQ	BLQ	BLQ
	Thyroid	0.600	0.800	0.293	BLQ	BLQ	BLQ	BLQ
Secretory	Exorbital Lachrymal Gland	0.586	0.580	0.295	BLQ	BLQ	BLQ	BLQ
	Intra-orbital Lachrymal Gd.	0.499	0.516	0.268	BLQ	BLQ	BLQ	BLQ
	Harderian Gland	1.074	1.098	0.579	0.054	BLQ	BLQ	BLQ
	Pancreas	0.488	0.594	0.340	BLQ	BLQ	BLQ	BLQ
	Salivary Gland	0.543	0.705	0.302	BLQ	BLQ	BLQ	BLQ
Fatty	Fat (brown)	1.142	1.329	0.622	BLQ	BLQ	BLQ	BLQ
	Fat (abdominal)	0.319	0.338	0.066	BLQ	BLQ	BLQ	BLQ
Dermal	Skin (pigmented)	0.235	0.430	0.124	BLQ	BLQ	BLQ	BLQ
	Skin (non-pigmented)	0.474	0.363	0.162	BLQ	BLQ	BLQ	BLQ
	Mammary Gland (or region)	0.227	0.328	0.150	BLQ	BLQ	BLQ	BLQ
Reproductive	Ovary	0.746	0.713	0.440	BLQ	BLQ	BLQ	BLQ
	Uterus	0.485	0.590	0.239	BLQ	BLQ	BLQ	BLQ
	Vagina	0.383	0.729	0.312	0.051	BLQ	BLQ	BLQ
Skeletal/ Muscular	Muscle (skeletal)	0.191	0.242	0.095	BLQ	BLQ	BLQ	BLQ
	Myocardium (heart)	0.789	0.674	0.388	BLQ	BLQ	BLQ	BLQ
Respiratory Tract	Lung	0.885	0.872	0.521	BLQ	BLQ	BLQ	BLQ
	Nasal Turbinates	0.314	0.136	0.060	BLQ	BLQ	BLQ	BLQ
Alimentary Canal	Cecum Mucosa	4.362	0.827	0.578	0.053	BLQ	BLQ	BLQ
	Esophagus	0.277	0.336	0.288	BLQ	BLQ	BLQ	BLQ
	Large Intestine Mucosa	0.527	0.627	0.421	BLQ	BLQ	BLQ	BLQ
	Large Intestine Contents	NI	138.654	*544.939	7.188	0.043	BLQ	BLQ
	Rectum Mucosa	0.443	0.473	0.231	BLQ	BLQ	BLQ	BLQ
	Small Intestine Mucosa	4.397	15.305	2.621	BLQ	BLQ	BLQ	BLQ
	Small Intestine Content	*981.073	140.429	18.470	1.566	BLQ	BLQ	BLQ
	Stomach Mucosa	1.112	0.983	1.754	BLQ	BLQ	BLQ	BLQ
Ocular	Lens	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
	Uveal Tract	0.231	0.292	0.142	BLQ	BLQ	BLQ	BLQ

BLQ: Below the limit of quantitation (< 0.038 µg equiv/g) or tissue could not be visually identified because of non-detectable radioactivity
*= above the limit of quantitation (>396.703 µg equiv/g)

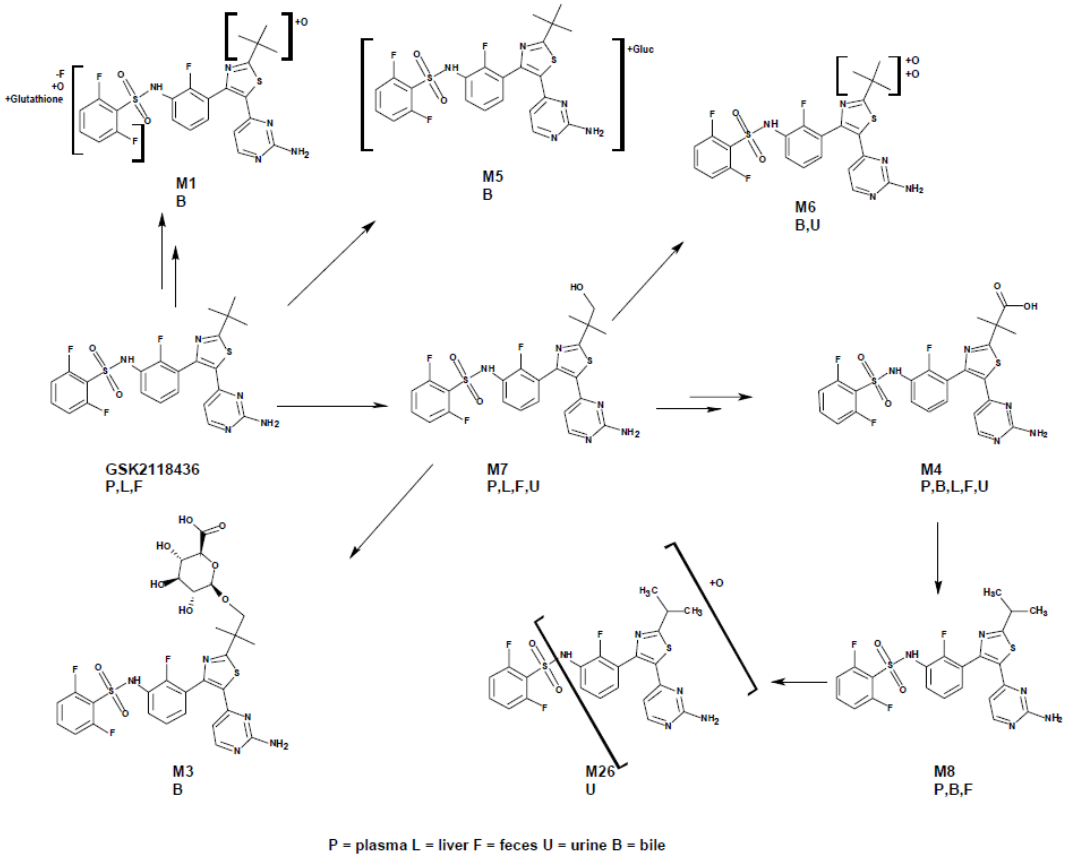
(table excerpted from Applicant's NDA)

2011N111703: Metabolism of GSK2118436 Following a Single Oral Administration (10 mg/kg) of [^{14}C]GSK2118436 to Intact Male and Female and Bile Duct-Cannulated Male Rats.

The purpose of this study was to characterize the metabolism of GSK2118436 in the rat using both intact male and female rats as well as bile duct-cannulated male rats. Based on these data, the following metabolism scheme was elucidated in the rat (see figure

below). Analysis across all matrices yielded eight labeled GSK2118436 derivatives in the rat. These were denoted M1, M3, M4, M5, M6, M7, M8, and M26. Structural elucidation was then performed, and their routes of elimination were characterized.

Proposed Metabolic Scheme for [^{14}C]GSK2118436 in Rats



(figure excerpted from Applicant's NDA)

The elimination profile of GSK2118436 is summarized in the table below. Although the majority (> 90%) of recovered radiolabel was measured in feces, the majority of that was considered to be unabsorbed parent. Based upon data from bile duct cannulated rats, biliary secretion accounted for approximately 32% of the recovered radiolabel, therefore, the fecal route is considered the predominant route of metabolite elimination. Urine accounted for less than 5% of the total radiolabel recovered.

Excretion of [^{14}C]GSK2118436 Following Oral Administration in Intact Male and Female Rats, and Bile Duct Cannulated Male Rats

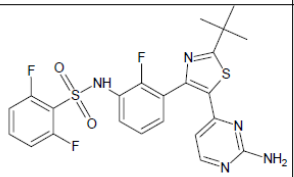
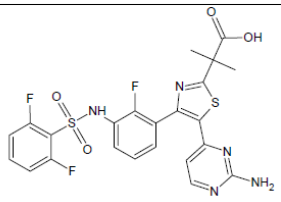
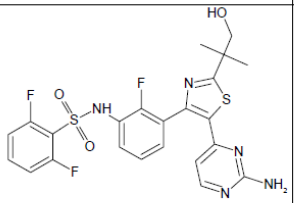
Dose	Route and Status	Excretion Balance (% dose)		
		Bile	Urine	Feces
10 mg/kg	Oral, intact male	NS	0.8	92.9
10 mg/kg	Oral, intact female	NS	3.1	90.2
10 mg/kg	Oral, BDC male	32.2	3.4	64.4

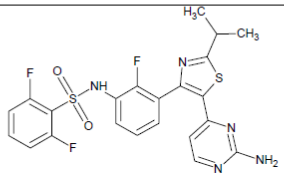
NS – no sample

(figure excerpted from Applicant's NDA)

The majority of the metabolites identified were produced through Phase 1 modifications (oxidation, hydroxylation, dealkylation); these include M4, M6, M7, M8, and M26. M1, M3, and M5 were formed via phase II processes (glucuronide-conjugates. As detailed in the tables below, of the 8 metabolites identified, three observed in plasma (M4, M7, and M8), and of these, two (M4 and M7) were also detected in urine. Two other urinary metabolites were also identified: M6 and M26. The remaining metabolites were identified in feces and/or bile. Metabolites identified in feces included M4, M5, M7 and M8. Of these, only M8 was also isolated from bile. The other biliary species include M1, M5, M4, and M6.

Circulating Metabolites of GSK2118436 in Rats

Metabolite ID	Metabolite Structure	% Radioactivity in Plasma[1] (ng eq. parent/g plasma)[2]					
		Male			Female		
		2 h	4 h	24 h	2 h	4 h	24 h
P	 GSK2118436	23.3 (340)	20.2 (321)	<LLQ (<LLQ)	26.2 (386)	10.9 (180)	3.5 (5.3)
M4[3] GSK2298683	 Oxidation	8.4 (122)	14.8 (235)	11.7 (4.6)	2.7 (39.2)	3.90 (64.9)	8.9 (13.4)
M7[4] GSK2285403	 Oxidation	51.7 (753)	49.1 (781)	51.0 (19.9)	62.5 (919)	77.9 (1285)	69.0 (105)

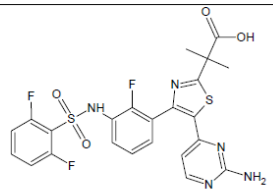
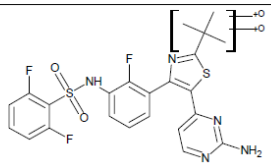
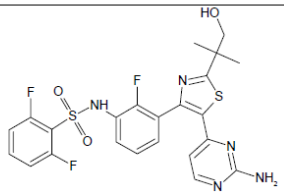
Metabolite ID	Metabolite Structure	% Radioactivity in Plasma[1] (ng eq. parent/g plasma)[2]					
		Male			Female		
		2 h	4 h	24 h	2 h	4 h	24 h
M8[5] GSK2167542	 Oxidation and decarboxylation	3.6 (52.3)	4.1 (65.8)	<LLQ (<LLQ)	<LLQ (<LLQ)	<LLQ (<LLQ)	3.0 (4.5)
Total Quantified[1,2]		87.0 (1268)	88.3 (1403)	62.7 (24.5)	91.4 (1344)	92.7 (1530)	84.3 (128)
Concentration (ng eq parent/g plasma)[6]		1438	1548	24.4	1443	1662	153
% Overall Recovery[7]		100	96.5	100	97.0	100	91.1

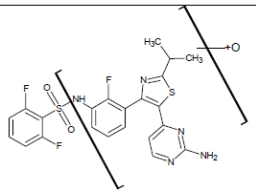
LLQ – Lower Limit of Quantitation

1. Percent radioactivity recovered under each peak, corrected by the overall sample preparation recovery.
2. Expressed as ng equivalents of GSK2118436 per g of plasma.
3. M4 was determined to be identical to the synthetic standard GSK2298683 (batch 993-066 A1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
4. M7 was determined to be identical to the synthetic standard GSK2285403 (batch N8235-64-A3) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
5. M8 was determined to be identical to the synthetic standard GSK2167542 (batch N12759-8-D1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
6. Total radioactivity concentrations in plasma expressed as ng equivalents per g of plasma [RD2008/01676/00].
7. Percent recovery of radioactivity following solvent extraction, evaporation and reconstitution of plasma samples.

(figure excerpted from Applicant's NDA)

Urinary Metabolites of GSK2118436 in Rats

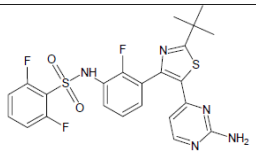
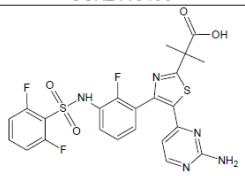
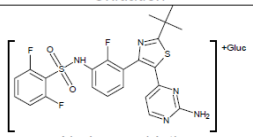
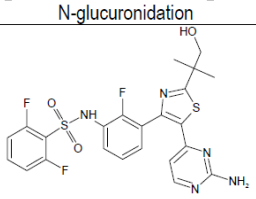
Metabolite ID	Metabolite Structure	Mean % Radioactivity in Urine[1] (Mean % Administered Dose)[2]	
		Intact Female	BDC Male
M4[3] GSK2298683	 Oxidation	4.9 (0.11)	2.6 (0.12)
M6	 Oxidation	24.6 (0.54)	57.8 (2.17)
M7[4] GSK2285403	 Oxidation	40.0 (0.93)	4.4 (0.17)

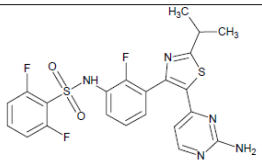
Metabolite ID	Metabolite Structure	Mean % Radioactivity in Urine[1] (Mean % Administered Dose)[2]	
		Intact Female	BDC Male
M26	 (Oxidation-decarboxylation) and oxidation	9.9 (0.22)	0.4 (0.02)
Total Quantified[1,2]		79.4 (1.80)	65.2 (2.47)
% Dose in Sample Analyzed[5]		2.21	3.80
% Dose in Total Sample[6]		3.09	4.22
% Centrifugation Recovery[7]		97.7	97.8

1. Mean percent radioactivity recovered under each peak, corrected for centrifugation recovery for urine.
2. Mean percent of administered dose recovered under each peak, corrected for centrifugation recovery for urine.
3. M4 was determined to be identical to the synthetic standard GSK2298683 (batch 993-066 A1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
4. M7 was determined to be identical to the synthetic standard GSK2285403 (batch N8235-64-A3) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
5. Mean percent of dose in (pooled) samples used for profiling, corrected for centrifugation recovery for urine.
6. Mean percent of dose excreted via urine [RD2008/01676/00].
7. Mean percent recovery of radioactivity following centrifugation for urine.

(figure excerpted from Applicant's NDA)

Quantification of the Major Radioactive Components Recovered in Feces

Metabolite ID	Metabolite Structure	Mean % Radioactivity in Feces[1] (Mean % Administered Dose)[2]		
		Intact Male	Intact Female	BDC Male
P	 GSK2118436	64.0 (59.1)	64.8 (57.7)	86.4 (55.3)
M4[3] GSK2298683	 Oxidation	2.2 (2.0)	1.7 (1.5)	ND (ND)
M5[4]	 N-glucuronidation	ND (ND)	2.3 (2.0)	ND (ND)
M7[5] GSK2285403	 Oxidation	12.5 (11.6)	13.2 (11.8)	9.6 (5.9)

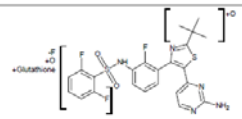
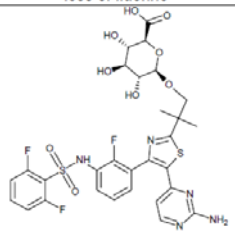
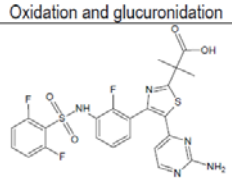
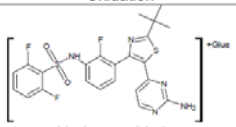
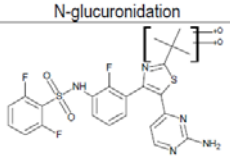
Metabolite ID	Metabolite Structure	Mean % Radioactivity in Feces[1] (Mean % Administered Dose)[2]		
		Intact Male	Intact Female	BDC Male
M8[6] GSK2167542	 Oxidation and decarboxylation	14.5 (13.4)	8.4 (7.6)	1.6 (1.0)
Total Quantified[1,2]		93.1 (86.1)	90.4 (80.6)	97.7 (62.2)
% Dose in Sample Analyzed[7]		91.7	87.6	63.7
% Dose in Total Sample[8]		92.9	90.2	64.4
% Overall Recovery[9]		99.2	98.2	99.9

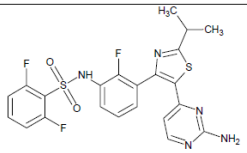
ND – not detected

1. Mean percent radioactivity recovered under each peak, corrected for overall sample preparation recovery for feces.
2. Mean percent of administered dose recovered under each peak, corrected for overall sample preparation recovery for feces.
3. M4 was determined to be identical to the synthetic standard GSK2298683 (batch 993-066 A1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns .
4. M5 in feces was identified based on retention time observed for M5 in bile.
5. M7 was determined to be identical to the synthetic standard GSK2285403 (batch N8235-64-A3) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
6. M8 was determined to be identical to the synthetic standard GSK2167542 (batch N12759-8-D1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
7. Mean percent of dose in (pooled) samples used for profiling, corrected for overall sample preparation recovery for feces.
8. Mean percent of dose excreted via feces [RD2008/01676/00].
9. Mean percent recovery of radioactivity following solvent extraction, evaporation, and reconstitution for feces.

(figure excerpted from Applicant's NDA)

Quantification of the Major Radioactive Components Recovered in Bile from Male Rats

Metabolite ID	Metabolite Structure	Mean % Radioactivity in Bile[1] (Mean % Administered Dose)[2] BDC Male
M1	 Oxidation, glutathione conjugation, loss of fluorine	4.0 (1.3)
M3	 Oxidation and glucuronidation	19.9 (5.7)
M4[3] GSK2298683	 Oxidation	26.0 (8.3)
M5	 N-glucuronidation	5.8 (1.7)
M6	 Oxidation	10.9 (3.6)

Metabolite ID	Metabolite Structure	Mean % Radioactivity in Bile[1] (Mean % Administered Dose)[2] BDC Male
M8[4] GSK2167542	 Oxidation and decarboxylation	6.2 (2.0)
Total Quantified[1,2]		72.8 (22.7)
% Dose in Sample Analyzed[5]		31.2
% Dose in Total Sample[6]		32.2
% Centrifugation Recovery[7]		100

1. Mean percent radioactivity recovered under each peak, corrected for centrifugation recovery for bile.
2. Mean percent of administered dose recovered under each peak, corrected for centrifugation recovery for bile.
3. M4 was determined to be identical to the synthetic standard GSK2298683 (batch 993-066 A1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
4. M8 was determined to be identical to the synthetic standard GSK2167542 (batch N12759-8-D1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
5. Mean percent of dose in (pooled) samples used for profiling, corrected for centrifugation recovery for bile.
6. Mean percent of dose excreted via bile [RD2008/01676/00].
7. Mean percent recovery of radioactivity following centrifugation for bile.

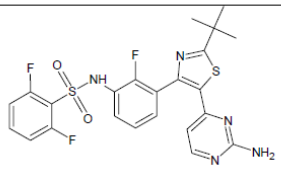
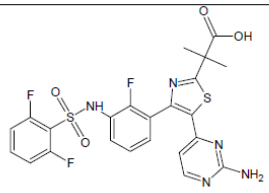
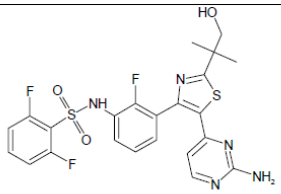
(figure excerpted from Applicant's NDA)

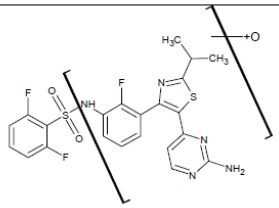
2011N111704: Metabolism of GSK2118436 in Male and Female Intact Dogs Following a Single Oral Administration of [^{14}C]GSK2118436 at a Dose Level of 10 mg/kg.

The purpose of this study was to evaluate the metabolic profile and route of elimination of GSK211436 in intact Beagle dogs following a single oral dose of 10 mg/kg. GSK211436 undergoes relatively little biotransformation in the dog, with the vast majority of radiolabeled material exiting in the feces.

As shown in the following table, the primary circulating species in male and female dogs were (1) unchanged parent and (2) a Phase 1 metabolite (M7) formed by oxidation of the tert-butyl moiety. M26 was also observed in males and females (present in females, but < LLQ) at a low level at 24 hours post-dose.

Metabolite Exposure Time-course in Dog Plasma

Metabolite ID	Metabolite Structure	% Radioactivity in Plasma[1] (ng eq. parent/g plasma)[2]							
		Male				Female			
		2 h	4 h	8 h	24 h[3]	2 h	4 h	8 h	24 h[3]
P	 GSK2118436	72.0 (702)	68.1 (364)	56.2 (48.9)	63.9 (273)	80.4 (470)	75.7 (314)	53.5 (50.8)	39.9 (44.8)
M4[4] GSK2298683	 Oxidation	ND (ND)	<LLQ (<LLQ)	<LLQ (<LLQ)	5.00 (21.6)	ND (ND)	ND (ND)	<LLQ (<LLQ)	<LLQ (<LLQ)
M7[5] GSK2285403	 Oxidation	14.4 (141)	17.8 (94.9)	<LLQ (<LLQ)	20.4 (87.3)	14.3 (83.5)	17.6 (72.7)	24.9 (23.7)	<LLQ (<LLQ)

Metabolite ID	Metabolite Structure	% Radioactivity in Plasma[1] (ng eq. parent/g plasma)[2]							
		Male				Female			
		2 h	4 h	8 h	24 h[3]	2 h	4 h	8 h	24 h[3]
M26	 (Oxidation decarboxylation) and oxidation	ND (ND)	ND (ND)	ND (ND)	7.00 (30.1)	ND (ND)	ND (ND)	ND (ND)	<LLQ (<LLQ)
Total Quantified[1,2]		86.4 (842)	85.9 (459)	56.2 (48.9)	96.3 (412)	94.7 (553)	93.3 (386)	78.4 (74.5)	39.9 (44.8)
Concentration (ng eq parent/g plasma)[6]		975	534	87.0	428	584	414	95.0	112
% Overall Recovery[7]		100	100	100	100	100	100	100	93.6

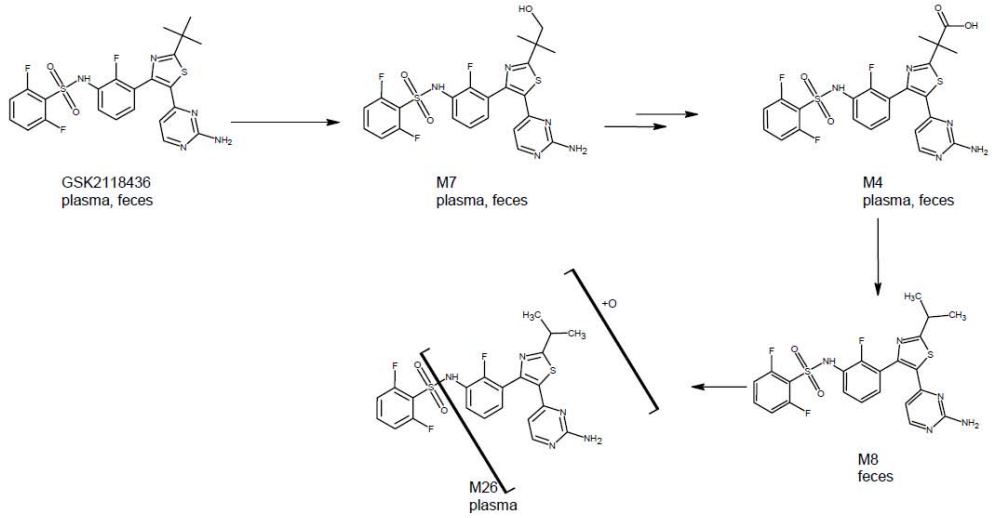
LLQ – Lower Limit of Quantitation, ND – not detected

1. Percent radioactivity recovered under each peak, corrected by the overall sample preparation recovery.
2. Expressed as ng equivalents of GSK2118436 per g of plasma.
3. Individual dog plasma samples (male MH01666, female FH01668).
4. M4 was determined to be identical to the synthetic standard GSK2298683 (batch 993-066 A1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
5. M7 was determined to be identical to the synthetic standard GSK2285403 (batch N8235-64-A3) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
6. Total radioactivity concentrations in plasma expressed as ng equivalents per g of plasma [RD2009/00137/00].
7. Percent recovery of radioactivity following solvent extraction, evaporation and reconstitution of plasma samples

(table excerpted from Applicant's NDA)

Based on these results, the following illustration is the proposed metabolic scheme according to the Applicant.

Proposed Metabolic Scheme for [^{14}C]GSK2118436 in Dogs



(figure excerpted from Applicant's NDA)

GSK2118436 was primarily eliminated in the feces in dogs, as summarized in the table below.

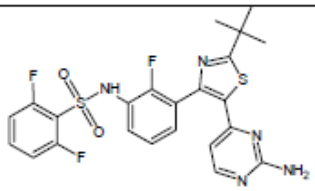
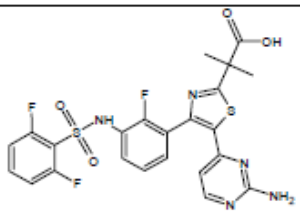
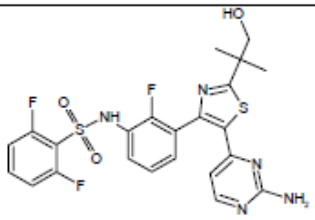
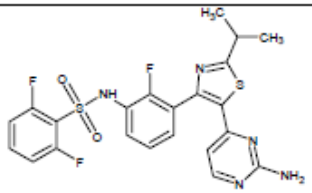
Elimination of GSK2118436 in the Dog

Dose	Route and Gender	Balance Excretion (% dose)	
		Urine	Feces
10 mg/kg	Oral, male	0.6	101
10 mg/kg	Oral, female	0.5	103

(table excerpted from Applicant's NDA)

An evaluation of metabolites in feces showed the predominant species recovered was parent (~ 92-95%). Phase 1 metabolites present at lower levels in feces included: M4, M7 and M8 (see table below). Evaluation of urinary metabolites was not conducted due to the low level of drug-related material present in urine (< 1% of administered dose).

Metabolite Elimination in Dog Feces

Metabolite ID	Metabolite Structure	Mean % Radioactivity in Feces[1] (Mean % Administered Dose)[2]	
		Male	Female
P	 GSK2118436	91.9 (92.3)	93.2 (95.1)
M4[3] GSK2298683	 Oxidation	0.63 (0.64)	0.81 (0.84)
M7[4] GSK2285403	 Oxidation	1.36 (1.37)	1.37 (1.41)
M8[5] GSK2167542	 Oxidation and decarboxylation	1.54 (1.54)	1.43 (1.48)
Total Quantified[1,2]		95.4 (95.9)	96.8 (98.8)
% Dose in Sample Analyzed[6]		98.6	101
% Dose in Total Sample[7]		101	103
% Overall Recovery[8]		98.2	98.4

1. Mean percent radioactivity recovered under each peak, corrected for overall sample preparation recovery for feces.
2. Mean percent of administered dose recovered under each peak, corrected for overall sample preparation recovery for feces.
3. M4 was determined to be identical to the synthetic standard GSK2298683 (batch 993-066 A1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
4. M7 was determined to be identical to the synthetic standard GSK2285403 (batch N8235-64-A3) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
5. M8 was determined to be identical to the synthetic standard GSK2167542 (batch N12759-8-D1) based on chromatographic retention time, accurate mass and MS/MS fragmentation patterns.
6. Mean percent of dose in (pooled) samples used for profiling, corrected for overall sample preparation recovery for feces.
7. Mean percent of dose excreted via feces [RD2009/00137/00].
8. Mean percent recovery of radioactivity following solvent extraction, evaporation, and reconstitution for feces.

(table excerpted from Applicant's NDA)

CD2009-00079: An *in vitro* Investigation into the Human Oxidative Enzymology of GSK2115436.

Human liver microsomes and recombinant cytochrome (CYP) P450 enzymes were used to evaluate the enzymes responsible for oxidative metabolism of GSK2118436. Based on data from recombinant enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP3A4), a model of intrinsic clearance was proposed and subsequently compared with the intrinsic clearance obtained from human liver microsomes, in the presence and absence of the CYP3A4 inhibitor, azamullin.

As summarized in the following table, the oxidative metabolism of GSK2118436 is primarily attributable to the actions of CYP2C8, CYP3A4, and CYP2C9.

Intrinsic Clearance of GSK2118436 in Recombinant CYP Enzyme Incubations

CYP Enzyme	CLint (mL/min/pmole)	CLi[1]RAF (pmole/mg protein)	Scaled CLint (mL/min/mg protein)	Percent to total scaled CLint of CYP450
CYP1A2	0.0008	6.1	0.005	1.9%
CYP2B6	0.0006	30	0.018	6.9%
CYP2C8	0.0025	59	0.147	56%
CYP2C9	0.0017	16	0.027	10%
CYP2C19	0.0011	3.7	0.004	1.5%
CYP2D6	0.0004	1.3	0.001	0.4%
CYP3A4	0.0022	27	0.060	23%

1. CLi: rate of CYP metabolism for GSK2118436A

(table excerpted from Applicant's NDA)

RD2008-01676: Elimination of Radioactivity Following a Single Oral Dose Administration of [^{14}C]GSK2118436 to Male and Female Intact and Male Bile Duct-Cannulated Rats at a Target Dose Level of 10 mg/kg.

Following administration of a single 10 mg/kg oral dose of [^{14}C]GSK2118436 to male and female SD rats, plasma, urine and feces were collected for up to 168 hours post-dose. The specific activity of the test article was 18.5 µCi/mg. Mass balance was achieved in this study; the total amount of radiation recovered ranged from 93.4-100% across treatment groups.

As detailed in the table below, the relative contribution of the three routes was: fecal >> biliary > urine. Urinary elimination accounted for approximately 1-3% of the total eliminated material, whereas feces accounted for approximately 90% of eliminated material. While some fecal exposure could be attributed to unabsorbed parent, a significant proportion is likely to be the result of biliary excretion, as 32% of administered dose was observed in bile over the 96 hour collection interval in the bile-duct-cannulated animals group.

Summary of the Elimination of Total Radioactivity Following Oral Administration of [^{14}C]GSK2118436 (10 mg/kg) to Intact Male and Female Rats and Bile Duct-Cannulated Male Rats

	Percent of Administered Dose Recovered					
Sample	Group 1 Intact Males		Group 2 Intact Females		Group 3 BDC Males	
	Mean	SD	Mean	SD	Mean	SD
Urine	0.76	0.14	3.09	0.09	3.39	1.93
Feces	92.9	1.25	90.2	1.32	64.4	13.3
Bile	NA		NA		32.2	13.3
Cage wash	0.02	0.01	0.16	0.11	0.16	0.17
Total	93.6	1.10	93.4	1.40	100	13.7

SD = Standard Deviation, n=3

BDC = bile duct-cannulated

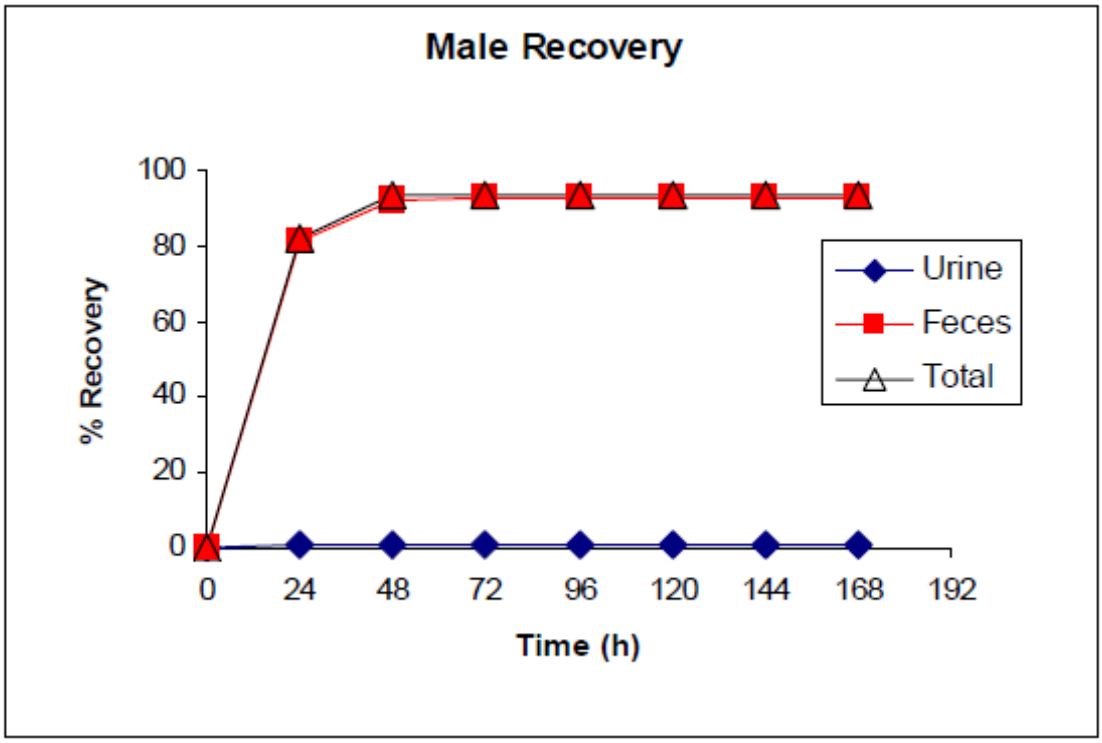
NA = not applicable

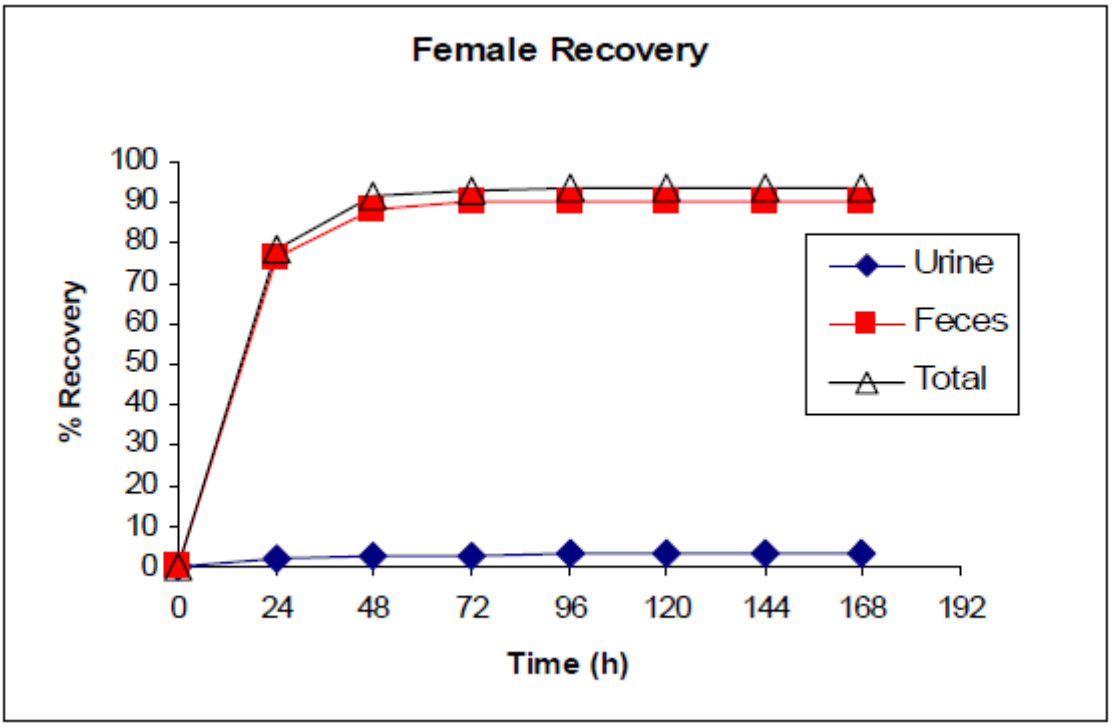
Individual elimination data are shown in Appendix 2 and Appendix 3 for intact males and females, respectively, and in Appendix 4 for BDC males

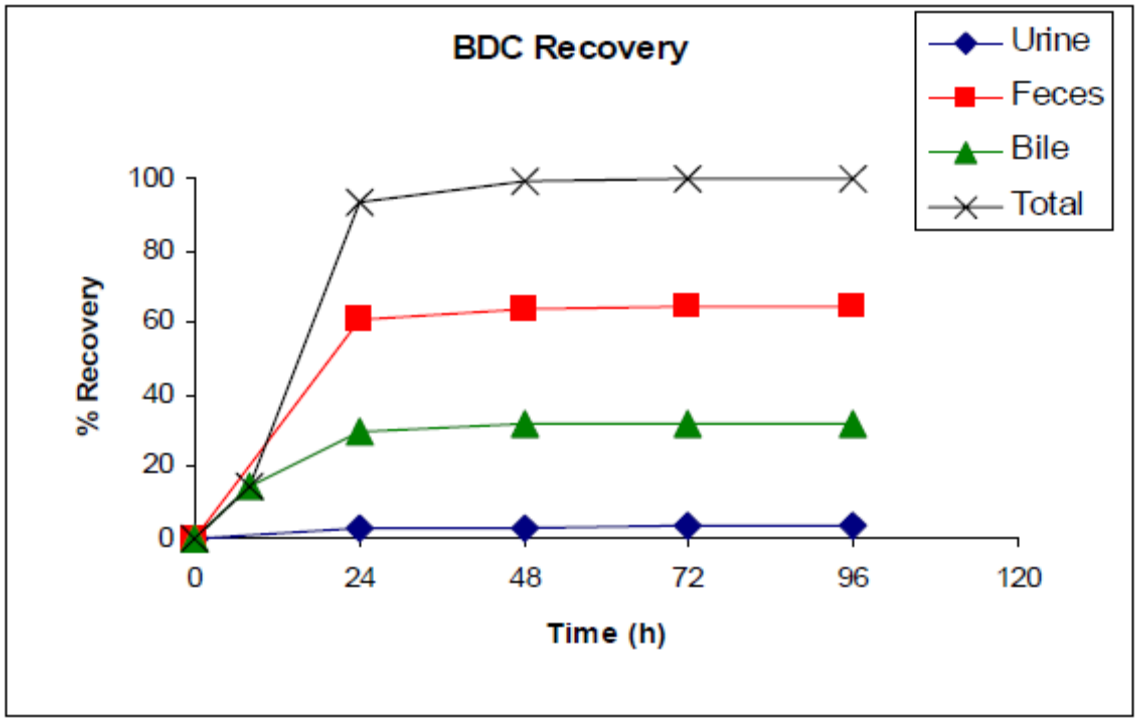
(table excerpted from Applicant's NDA)

As shown in the following figure, elimination was nearly complete within 48-hours of dose administration in intact male and female rats, and bile duct-cannulated (BDC) male rats.

Recovery of Radioactivity from Intact Male and Female Rats, and Bile Duct-cannulated (BDC) Male Rats







(figure excerpted from Applicant's NDA)

RD2008-00137: Elimination of Radioactivity Following a Single Oral (10 mg/kg) Administration of [^{14}C]GSK2118436 to Male and Female Dogs.

The purpose of this mass balance study was to evaluate the mechanism, rate, and extent of [^{14}C]GSK2118436 elimination in intact male and female Beagle dogs following a 10 mg/kg single oral dose (5 mL/kg). The specific activity was 3.95 µCi. Blood samples were taken at 2, 4, 6, 24, and 168 hours post dose. Feces and urine were collected at 24 hour various intervals up to 168 hours post-dose. After each fecal collection, cage debris was collected and pooled for each animal and the cage pan was rinsed with water. All samples, including cage wash, cage screen, and cage wipes, were analyzed for radioactivity.

Mass balance was achieved in this study; total recoveries were 102% and 104% from males and females, respectively. As detailed in the following table, the majority of the radiolabel was recovered in the feces (101% and 103% for males and females, respectively. Urinary excretion accounted for < 1% of the total dose administered in both genders.

Total Mean Recovery of Radioactivity Following Oral Administration of [^{14}C]GSK2118436 (10 mg/kg) to Male And Female Dogs

Matrix	Percent of Administered Dose	
	Males	Females
Feces	101 ± 0.00	103 ± 4.42
Urine	0.57 ± 0.37	0.46 ± 0.23
Cage Pan/Screen Rinse	0.28 ± 0.13	0.19 ± 0.10
Cage Debris	0.02 ± 0.03	0.08 ± 0.09
Cage Wash	0.05 ± 0.02	0.04 ± 0.03
Cage Wipe	0.39 ± 0.05	0.31 ± 0.20
Vomitus/Emesis	BLQ[a]	NS
Total	102 ± 0.58	104 ± 4.73

Note: Values are the mean ± standard deviation (n=3).
BLQ Below the limit of quantitation.
NS No samples collected since no emesis was observed for female animals.
a: Vomitus was collected from one male animal only.

(table excerpted from Applicant's NDA)

Elimination was essentially complete by 24 hours, with over 96% of total radiolabel recovered by the end of the first collection interval in both sexes (see table below). All samples were below the limit of quantitation by 168 hours post-dose. There was no evidence for partitioning of the radiolabel to red blood cells.

Mean Recovery of Radioactivity in Urine and Feces During Each Collection Interval Following Oral Administration of [^{14}C]GSK2118436 (10 mg/kg) to Male and Female Dogs

Collection Interval (h)	Percent of Administered Dose			
	Male		Female	
	Urine	Feces	Urine	Feces
0-12	0.08 ± 0.07	-	0.12 ± 0.05	-
12-24	0.11 ± 0.07	-	0.10 ± 0.09	-
0-24	-	96.5 ± 5.06	-	98.3 ± 4.16
24-48	0.18 ± 0.18	4.14 ± 4.81	0.14 ± 0.13	4.28 ± 4.97
48-72	0.08 ± 0.07	0.16 ± 0.04	0.04 ± 0.04	0.25 ± 0.16
72-96	0.04 ± 0.03	0.14 ± 0.09	0.02 ± 0.02	0.12 ± 0.07
96-120	0.03 ± 0.02	0.08 ± 0.05	0.01 ± 0.00	0.06 ± 0.01
120-144	0.02 ± 0.01	0.06 ± 0.03	0.01 ± 0.01	0.05 ± 0.02
144-168	0.02 ± 0.02	0.04 ± 0.02	0.01 ± 0.01	0.05 ± 0.03
Total	0.57 ± 0.37	101 ± 0.00	0.46 ± 0.23	103 ± 4.42

Note: Values are the mean ± standard deviation (n=3).
- Not determined.

(table excerpted from Applicant's NDA)

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: GSK2118436A: 4-Week Oral Toxicity in Rats Followed by a 2-Week Recovery Period. Reviewed by Kimberly Ringgold, Ph.D. and slightly modified for this NDA review.

Study no.:	CD2008-01511
Study report location:	4.2.3.2
Conducting laboratory and location:	GlaxoSmithKline King of Prussia, PA
Date of study initiation:	October 22, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, 081179173, 99.3%

Key study findings:

- One 200 mg/kg/day high-dose male was sacrificed in moribund condition.

- Target organs of toxicity included the stomach, eye, and liver.

Methods:

Doses:	0, 5, 20, or 200 mg/kg/day
Frequency of dosing:	Daily for 29 days, with 2-week recovery period
Route of administration:	Oral suspension
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% hydroxypropyl methylcellulose with 0.1% Tween 80 at 0.5, 2, and 20 mg/mL
Species/Strain:	Rat / Crl:CD(SD)
Number/Sex/Group:	10 main group 4 recovery group for control and high-dose
Age:	~11 weeks
Weight:	Males: 327-433 g Females: 216-279 g
Satellite groups:	3/sex/group
Dose justification:	The dose levels were selected by the Applicant based on previous dose range finding toxicity data (study CD2008/00951/01).

Observations and Results:

Mortality [observations made twice daily]

One 200 mg/kg/day high-dose male was sacrificed in moribund condition on day 9. Clinical signs included a red swollen left hind paw, dry scab on left cheek, and crust on face above left eye.

Clinical Signs [pre-dose; day 28 and 42]

Unremarkable

Body Weights [pre-dose; 3 x weekly]

Unremarkable

Food Consumption [weekly]

Unremarkable

Ophthalmoscopy [pre-dose; at sacrifice]

Unremarkable

Hematology [at sacrifice]

Unremarkable

Coagulation [at sacrifice]

Unremarkable

Clinical Chemistry [at sacrifice]

Unremarkable

Urinalysis [at sacrifice]

Unremarkable

Immunotoxicology [at sacrifice]

Changes in T-cell populations (CD4+/CD8-; CD4+/CD8+; CD4-/CD8-) were examined. The results were unremarkable.

Gross Pathology [at sacrifice]

No treatment-related macroscopic changes were noted in the recovery animals.

Treatment-Related Macroscopic Findings: Terminal Necropsy

Tissue and Findings		No. of animals affected							
		Males				Females			
	Dose (mg/kg/day)	0	5	20	200	0	5	20	200
	Number of animals examined	10	10	10	10	10	10	10	10
Stomach	Red discoloration, junctional ridge	-	-	-	1	-	-	-	1
	Irregular surface, junctional ridge	-	-	-	1	-	-	-	-

Organ Weights [at sacrifice]

Unremarkable

Histopathology [at sacrifice]

Adequate Battery: Yes
Peer Review: Yes

With the exception of findings in the liver, the following treatment-related microscopic findings were reversible by the end of the recovery period.

Treatment-Related Microscopic Findings Early Death Animal	
Doses (mg/kg/day)	
Number of animals examined	
Epididymides	Mild sperm granuloma
Hind limb	Vesicle, epidermis, moderate
	Inflammation, chronic-active, marked
	Hemorrhage, mild
Liver	Degeneration, focal, portal, vascular, mild
Skin	Ulcer, focal, mild

Treatment-Related Microscopic Findings Terminal Necropsy		**No. of animals affected**							
		Males				**Females**			
Doses (mg/kg/day)		0	5	20	200	0	5	20	200
Number of animals examined		10	10	10	10	10	10	10	10
Eyes	Retinal rosette, min	-	-	-	2	-	-	-	1
Liver	Necrosis, subcapular, focal, min	-	-	-	-	-	-	-	1
Lung	Inflammation, mixed cell, moderate	-	-	-	-	-	-	-	1
	Inflammatory, mixed cell, infiltrate, min	-	-	-	-	-	-	-	1
Spleen	Macrophage infiltration, red pulp, min	-	-	-	1	-	-	-	-
Stomach	Degeneration, junctional ridge, keratinocyte, focal, mild	-	-	2	2	-	-	2	3
Testes	Degeneration, seminiferous tubules, bilateral, very marked	-	-	-	1	-	-	-	-

Treatment-Related Microscopic Findings Recovery		**No. of animals affected**							
		Males				**Females**			
Doses (mg/kg/day)		0	5	20	200	0	5	20	200
Number of animals examined		10	10	10	10	10	10	10	10
Liver	Fibrosis, subcapsular, focal	-	-	-	-	-	-	-	1
	Mineralization; focal	-	-	-	-	-	-	-	1
	Necrosis, focal	-	-	-	-	-	-	-	1

Toxicokinetics [day 1 and 28 at 0, 0.5, 1, 2, 4, 8, and 24 hrs post-dose]

- Exposure levels (Cmax and AUC) were less than dose-proportional between all doses in males and females.
- No drug accumulation was noted.

- No sex differences were seen.
- Details are shown in Applicant's tables below.

Mean Toxicokinetic Parameters on Day 1 and 28 Following Daily Oral Administration of GSK2118436 to Rats

Parameter [a]	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	200
AUC_{0-t} (µg.h/mL)	Day 1	3.64 [2.83-4.98]	9.43 [6.61-13.6]	23.0 [13.3-28.4]
	Day 28	1.91 [1.39-2.60]	4.39 [3.27-5.90]	12.6 [5.68-18.7]
C_{max} (µg/mL)	Day 1	0.983 [0.790-1.23]	1.58 [1.48-1.69]	2.50 [1.73-2.95]
	Day 28	0.755 [0.713-0.784]	1.27 [0.876-1.64]	1.62 [1.23-2.07]
Median T_{max} (h)	Day 1	1.00 [1.00-2.00]	2.00 [1.00-2.00]	2.00 [2.00-4.00]
	Day 28	1.00 [1.00-1.00]	1.00 [1.00-2.00]	1.00 [1.00-2.00]
Parameter [a]	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	200
AUC_{0-t} (µg.h/mL)	Day 1	2.96 [2.29-3.79]	7.74 [6.88-8.98]	27.1 [23.0-33.1]
	Day 28	3.42 [2.87-3.80]	6.01 [4.00-8.61]	28.3 [22.6-38.5]
C_{max} (µg/mL)	Day 1	1.01 [0.803-1.24]	1.80 [1.45-2.29]	3.73 [2.86-4.32]
	Day 28	1.07 [0.858-1.33]	1.53 [1.35-1.70]	3.80 [2.95-4.96]
Median T_{max} (h)	Day 1	1.00 [1.00-1.00]	2.00 [2.00-2.00]	2.00 [2.00-2.00]
	Day 28	1.00 [1.00-1.00]	1.00 [1.00-2.00]	2.00 [1.00-2.00]

a. Results are reported as mean unless stated otherwise and [range].

(table excerpted from Applicant's NDA)

Study title: GSK2118436A: 4-Week Oral Toxicity in Dogs Followed by a 2-Week Recovery Period. Reviewed by Kimberly Ringgold, Ph.D. and slightly modified for this NDA review.

Study no.:	CD2008-01503
Study report location:	4.2.3.2
Conducting laboratory and location:	GlaxoSmithKline King of Prussia, PA
Date of study initiation:	October 22, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, 081179173, 99.3%

Key study findings:

- All doses of GSK2118436A were well tolerated.
- Target organs of toxicity included the heart and skin.

Methods:

Doses:	0, 1, 5, or 50 mg/kg/day
Frequency of dosing:	Daily for 29 days, with 2-week recovery period
Route of administration:	Oral suspension
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% hydroxypropyl methylcellulose with 0.1% Tween 80 at 0.2, 1, and 2 mg/mL
Species/Strain:	Dog / Beagle
Number/Sex/Group:	3 main group 2 recovery group for control and high-dose
Age:	10-14 months
Weight:	Males – 7.6 to 10 kg; Females – 5.7 to 7.9 kg
Satellite groups:	3/sex/group
Dose justification:	The dose levels were selected by the Applicant based on previous dose range finding toxicity data (study CD2008/01229/00)

Observations and Results:

Mortality [observations made daily]

None

Clinical Signs [pre-dose; day 28 and 42]

Unremarkable

Body Weights [pre-dose; twice weekly]

Unremarkable

Food Consumption [weekly]

Unremarkable

Ophthalmoscopy [pre-dose; day 24]

Unremarkable

Electrocardiography [pre-dose; day 23]

Unremarkable

Hematology [at sacrifice]

Unremarkable

Coagulation [at sacrifice]

Unremarkable

Clinical Chemistry [at sacrifice]

Unremarkable

Urinalysis [at sacrifice]

Unremarkable

Gross Pathology [at sacrifice]

The following treatment-related macroscopic changes were reversible by the end of the recovery period.

Treatment-Related Macroscopic Findings - Primary Necropsy		**No. of animals affected**							
		Males				**Females**			
Dose (mg/kg/day)		0	1	5	50	0	1	5	50
Number of animals examined		3	3	3	3	3	3	3	3
Skin	Raised areas, muzzle	-	-	-	1	-	-	-	1
	Small pedunculated areas (chin)	-	-	-	1	1	-	-	1
	Thickening, external ear	-	-	-	-	-	-	-	1
	Nodular appearance, external ear	-	-	-	-	-	-	-	1

Organ Weights [at sacrifice]

The following treatment-related changes in organ weights were reversible by the end of the recovery period.

MALES	Percent Change From Control (Relative to Bodyweight)							
	0 mg/kg/day		1 mg/kg/day		5 mg/kg/day		50 mg/kg/day	
	W4	W6	W4	W6	W4	W6	W4	W6
No. of Animals	5	2	3	NA	3	NA	5	2
Final Body Weight	8.808	8.385	-	NA	-	NA	-	-
Prostate	0.097	0.085	↓21	NA	↓25	NA	↓35	-

FEMALES	Percent Change From Control (Relative to Bodyweight)							
	0 mg/kg/day		1 mg/kg/day		5 mg/kg/day		50 mg/kg/day	
	W4	W6	W4	W6	W4	W6	W4	W6
No. of Animals	5	2	3	NA	3	NA	5	2
Final Body Weight	6.378	6.585	-	NA	-	NA	-	-
Ovaries	0.0197	0.0140	↓39	NA	↓39	NA	↓48	↓39

Histopathology [at sacrifice]

Adequate Battery: Yes
Peer Review: Yes

The following treatment-related microscopic findings were reversible by the end of the recovery period.

Treatment-Related Microscopic Findings - Primary Necropsy		**No. of animals affected**							
		Males				**Females**			
	Dose (mg/kg/day)	0	1	5	50	0	1	5	50
	Number of animals examined	3	3	3	3	3	3	3	3
Heart	Hemorrhage; tricuspid valve; focal, mild	-	-	-	1	-	-	-	-
	Hypertrophy, tricuspid valve, marked	-	-	-	1	-	-	-	-
Skin	Acanthosis; external ear; focal, mild	-	-	-	1	-	-	-	-
	Acanthosis; muzzle; mild	-	-	-	-	-	-	-	1
	Hyperkeratosis, external ear, minimal	-	-	-	-	-	-	-	1
	Inflammatory cell infiltrate, mononuclear cell, chin, sebaceous gland, minimal	-	-	-	1	-	-	-	-
	Ulcer, external ear	-	-	-	-	-	-	-	1
	Ulcer, chin	-	-	-	-	-	-	-	1
	Papilloma, squamous cell, external ear, benign	-	-	-	-	-	-	-	1
	Papilloma, squamous cell, chin, benign	-	-	-	-	1	-	-	1

Toxicokinetics [day 1 and 28 at 0, 0.5, 1, 2, 4, 8, and 24 hrs post-dose]

- Exposure levels (Cmax and AUC) were less than dose-proportional between all doses in males and females.
- No drug accumulation was noted.
- No sex differences were seen.
- Details are shown in Applicant's tables below.

Mean Toxicokinetic Parameters on Day 1 and 28 Following Daily Oral Administration of GSK2118436 to Dogs

Parameter [a]	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		1 [b]	5 [b]	50 [c,d]
AUC_{24} (µg.h/mL)	Day 1	4.47 [3.62-5.07]	19.2 [15.2-23.6]	48.9 [23.5-72.4]
	Day 28	4.10 [3.34-4.68]	10.5 [8.32-14.4]	40.2 [26.4-46.8]
C_{max} (µg/mL)	Day 1	1.13 [0.754-1.55]	3.69 [2.00-5.36]	8.87 [3.53-15.0]
	Day 28	0.893 [0.599-1.04]	2.73 [1.49-3.87]	8.41 [4.88-11.4]
Median T_{max} (h)	Day 1	1.00 [1.00-1.00]	2.00 [1.00-2.00]	1.00 [0.50-4.00]
	Day 28	1.00 [1.00-1.00]	1.00 [1.00-1.00]	1.00 [1.00-4.00]
Parameter [a]	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		1 [b]	5 [b]	50 [c]
AUC_{24} (µg.h/mL)	Day 1	5.83 [1.60-11.2]	17.3 [8.68-31.7]	35.7 [17.2-51.3]
	Day 28	4.48 [2.86-7.44]	14.0 [7.35-21.4]	45.2 [21.0-74.5]
C_{max} (µg/mL)	Day 1	1.21 [0.593-2.09]	3.23 [1.57-5.53]	6.58 [4.45-9.11]
	Day 28	0.841 [0.727-0.904]	2.62 [1.51-3.21]	6.85 [4.28-9.06]
Median T_{max} (h)	Day 1	1.00 [1.00-2.00]	1.00 [1.00-1.00]	1.00 [1.00-2.00]
	Day 28	1.00 [1.00-1.00]	1.00 [1.00-2.00]	1.00 [1.00-1.00]

a. Results are reported as mean unless stated otherwise and [range].
b. n=3.
c. n=5.
d. One of the 5 male animals at 50 mg/kg/day had an episode of emesis on Day 2 (prior to the 24-hour timepoint).

(table excerpted from Applicant's NDA)

Study title: GSK2118436A: A 13 Week Oral Gavage Toxicity Study in the Rat Followed by a 4-week Recovery.

Study no.:	CD2010-00052
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	November 30, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, Batch 081391, 99.3% (used Day 1-30) Batch 092099, 99.5% (used Day 31-91)

Key study findings:

- One 400 mg/kg/day high-dose female was found dead on day 22.
- A dose-responsive decrease in body weight and food consumption was noted.
- Target organs of toxicity were skin, stomach, and male reproductive organs.
- A dose-responsive increase in the incidence of skin lesions (inflamed, dry, red, scabbing, and flaking), was seen on the paws. Skin papules were also observed on the forepaws of one 400 mg/kg/day high-dose male. Skin toxicity corresponded with histopathological hyperkeratosis.
- Histopathological hyperplasia, epithelial down-growth, and infiltration were noted in the stomach of male and female rats, at all doses tested.
- 100% of male rats showed degeneration/depletion in the testis and aspermia in the epididymis, irrespective of dose.

Methods:

Doses:	0, 20, 200, or 400 mg/kg/day
Frequency of dosing:	Daily for 13-weeks with a 4-week recovery period
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% hydroxypropyl methylcellulose with 0.1% Tween 80 at 0.5, 2, and 20 mg/mL
Species/Strain:	Rat / Crl:CD(SD)
Number/Sex/Group:	12 - main group 6 - recovery group for control, mid-dose, and high-dose
Age:	≥ 10 weeks
Weight:	Males – 351 to 434 g; Females – 222 to 269 g
Satellite groups:	3/sex/group
Dose justification:	The dose levels were selected by the Applicant based on previous dose range finding toxicity data (study CD2008/00951/01).

Observations and Results:

Mortality [observations made twice daily]

One 400 mg/kg/day high-dose female was found dead on Day 22. According to the Applicant, the likely cause of death was procedural since it occurred following the jugular blood collection and the animal showed locally extensive hemorrhage in the cervical/thoracic region. However, test-article-related histopathological changes (hyperplasia of the non-glandular mucosa with vacuolar degeneration and inflammation) were seen in the forestomach of this animal.

Clinical Signs [daily]

A dose-responsive increase in the incidence of skin lesions (dry, red, scabbing, flaking, and inflamed skin) was noted, primarily on the hindpaws. For 5 males including one control, the condition of the hindpaws required supportive care with veterinary treatments for up to 5 days. Skin papules were also observed on the forepaws of one 400 mg/kg/day high-dose male. Dry, red, and inflamed skin was still observed in a few animals given ≥ 200 mg/kg/day following the 4-week recovery period.

Body Weights [pre-dose; weekly]

Animals began showing a decrease in body weight gain by Day 3. By the end of the 13-week dosing period, 20, 200, and 400 mg/kg/day males and females showed a 12, 17, and 18% and 10, 28, and 22% decrease in body weight gain, respectively. This reduction in body weight gain was reversible by the end of the recovery period.

Food Consumption [weekly]

Dose-response decreases in food consumption were noted primarily during the first three weeks of doing in males (up to a 20% decrease) and in females (up to a 37% decrease). This reduction in food consumption was reversible by the end of the recovery period.

Ophthalmoscopy [pre-dose; week 12]

Unremarkable

Hematology [pre-dose; week 4 and 13]

The following changes in hematological parameters were reversible by the end of the recovery period.

Week 13 - % change in hematological parameters vs. control

	Males			Females		
	20 mg/kg	200 mg/kg	400 mg/kg	20 mg/kg	200 mg/kg	400 mg/kg
Lymphocytes	-14	+6	+24	+47	+40	+42
Eosinophils	+7	+43	+57	X	+17	+25

x denotes no change

Coagulation [pre-dose; week 4 and 13]

Unremarkable

Clinical Chemistry [pre-dose; week 4 and 13]

Unremarkable

Urinalysis [pre-dose; week 4 and 13]

Unremarkable

Gross Pathology [at sacrifice]

Except skin scaling in males (increased in incidence during the recovery period), the following treatment-related macroscopic changes were reversible or on a trend of reversibility by the end of the recovery period.

Treatment-related Macroscopic Findings: Terminal Necropsy

Tissue and Finding	No. of animals affected							
	Males				Females			
Dose (mg/kg/day)	0	20	200	400	0	20	200	400
# of animals	12	12	12	12	12	12	12	12
Lymph Node *- Enlargement*	0	1	4	3	0	3	1	3
Skin *- Scaling*	0	7	9	11	0	3	8	7
Stomach *- Thickening*	0	6	3	7	0	3	2	4
Epididymis *- Small*	0	0	1	3	-	-	-	-
Testis *- Small*	0	1	6	8	-	-	-	-

Organ Weights [at sacrifice]

The following changes in testis weight in males and spleen weight in females were irreversible by the end of the recovery period.

% change in organ weights vs. control

	Males			Females		
	5 mg/kg	200 mg/kg	400 mg/kg	5 mg/kg	20 mg/kg	200 mg/kg
Spleen	+19	+38	+31	+15	+20	+25
Testis	X	-23	-31	-	-	-

x denotes no change

Histopathology [at sacrifice]

Adequate Battery: Yes
Peer Review: Yes

Degeneration/depletion in the testes was irreversible by the end of the recovery period in 200 and 400 mg/kg/day animals. Aspermia was also present at the end of the recovery period in approximately 50% of the 200 and 400 mg/kg/day animals. All other treatment-related microscopic findings were reversible by the end of the recovery period.

Treatment-related Microscopic Findings: Terminal Necropsy

Tissue and Finding (grade)	No. of animals affected							
	Males				Females			
Dose (mg/kg/day)	0	20	200	400	0	20	200	400
# of animals	12	12	12	12	12	12	12	12
Lymph Node								
- hyperplasia (1-3)	0	1	4	3	0	3	1	3
Skin								
- hyperkeratosis: forepaw (1-4)	0	4	9	10	0	0	4	4
- hyperkeratosis: hindpaw (1-4)	1	9	10	10	0	5	9	9
Stomach								
- hyperplasia: non-gland. mucosa (1-4)	0	9	8	8	0	7	7	10
- epithelial down-growth (1-2)	0	1	2	4	0	0	1	4
- infiltration: mixed cell (1-2)	0	6	6	8	0	3	2	5
Thymus								
- hemorrhage (1-3)	0	0	0	3	1	0	2	0
Testis								
- degeneration/depletion: seminiferous epithlelium (1-5)*	0	12	12	12	-	-	-	-
Epididymis								
- aspermia (1-4)*	0	12	12	12	-	-	-	-

Grade 1, 2, 3, 4, and 5 refers to minimal, mild, moderate, marked, and severe, respectively
**Severity of findings in testis and epididymis were dose-dependent.*

Toxicokinetics [pre-dose, and 0.5, 1, 2, 4, 8, and 24 hrs post-dose on day 1, week 4, and week 13]

- Exposure levels (Cmax and AUC) to GSK2118436 were generally less than dose-proportional between all doses in males and females.
- No drug accumulation was noted.
- No sex differences were seen.
- Ratios of gender averaged metabolite/parent AUC_{0-t} values ranged from 0.650 to 3.00 for GSK2298683 (M4), 2.34 to 4.08 for GSK2285403 (M7), and 0.016 to 0.311 for GSK2167542 (M8).
- Details are shown in Applicant's tables below.

Mean Toxicokinetic Parameters on Day 1, Week 4, and Week 13 for GSK2118436 (Parent Compound) Following Daily Oral Gavage Administration of GSK2118436 to Rats

Parameter	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		20	200	400
AUC_{0-t} (µg.h/mL)	Day 1	11.7 [9.01 – 15.1]	14.5 [13.8 – 14.9]	31.5 [19.8 – 42.0]
	Week 4	4.54 [2.81 – 6.11]	6.41 [5.24 – 7.31]	8.64 [6.97 – 10.9]
	Week 13	6.32 [3.19 – 9.41]	18.4 [15.2 – 22.8]	16.8 [15.0 – 17.9]
C_{max} (µg/mL)	Day 1	1.42 [1.38 – 1.49]	1.46 [1.33 – 1.68]	1.76 [1.12 – 2.36]
	Week 4	0.840 [0.648 – 0.958]	0.924 [0.744 – 1.08]	0.824 [0.591 – 1.07]
	Week 13	1.16 [0.794 – 1.39]	1.94 [1.71 – 2.20]	1.69 [1.63 – 1.74]
Parameter	**Period**	**Female**		
		Dose of GSK2118436 (mg/kg/day)		
		20	**200**	**400**
AUC_{0-t} (µg.h/mL)	Day 1	11.2 [6.32 – 16.1]	17.3 [14.9 – 18.7]	24.6 [8.35 – 40.9]
	Week 4	6.32 [4.71 – 8.62]	7.39 [3.87 – 9.87]	12.6 [7.32 – 21.1]
	Week 13	10.1 [8.73 – 12.3]	21.2 [11.2 – 30.9]	26.8 [21.9 – 36.6]
C_{max} (µg/mL)	Day 1	1.78 [1.49 – 2.17]	1.95 [1.42 – 2.47]	1.76 [1.08 – 2.17]
	Week 4	1.48 [1.28 – 1.71]	1.36 [1.11 -1.53]	1.75 [1.16 – 2.15]
	Week 13	1.93 [1.45 – 2.24]	5.83 [3.09 – 8.28]	3.19 [2.73 – 3.67]

(table excerpted from Applicant's NDA)

Mean Toxicokinetic Parameters on Day 1, Week 4, and Week 13 for GSK2298683 (Metabolite 4 (M4)) Following Daily Oral Gavage Administration of GSK2118436 to Rats

Parameter	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		20	200	400
AUC_{0-t} (µg.h/mL)	Day 1	8.11 [7.65 – 8.41]	14.1 [11.2 – 18.2]	25.4 [22.9 – 30.0]
	Week 4	5.75 [3.76- 7.70]	17.7 [9.69 – 24.5]	12.8 [6.90 – 16.9]
	Week 13	12.9 [8.06 – 20.0]	50.5 [30.7 – 62.3]	31.1 [20.3 – 39.6]
C_{max} (µg/mL)	Day 1	0.617 [0.540 – 0.689]	0.971 [0.774 – 1.26]	1.90 [1.33 – 2.83]
	Week 4	0.466 [0.322 – 0.620]	1.25 [0.873 – 1.65]	0.916 [0.572 – 1.24]
	Week 13	0.902 [0.587 – 1.39]	2.72 [2.30 – 3.17]	1.67 [1.22 – 2.05]
Parameter	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		20	200	400
AUC_{0-t} (µg.h/mL)	Day 1	6.77 [3.41 – 13.3]	16.1 [9.48 – 23.8]	17.1 [7.81 – 22.7]
	Week 4	5.86 [2.38 – 8.26]	23.6 [7.10 – 51.6]	15.1 [11.4 – 18.2]
	Week 13	11.4 [7.36 – 13.9]	55.4 [21.9 – 98.3]	39.4 [25.7 – 48.6]
C_{max} (µg/mL)	Day 1	0.366 [0.213 – 0.663]	1.26 [0.641 – 2.23]	1.36 [0.434 – 2.36]
	Week 4	0.469 [0.165- 0.654]	1.62 [0.618 – 3.27]	0.999 [0.831 – 1.28]
	Week 13	0.870 [0.580 – 1.20]	4.67 [2.27 – 7.81]	2.41 [1.86 – 3.03]

(table excerpted from Applicant's NDA)

Mean Toxicokinetic Parameters on Day 1, Week 4, and Week 13 for GSK2285403 (Metabolite 7 (M7)) Following Daily Oral Gavage Administration of GSK2118436 to Rats

Parameter	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		20	200	400
AUC_{0-t} (µg.h/mL)	Day 1	30.2 [28.1 – 32.9]	41.0 [33.1 – 52.9]	89.2 [54.4 – 113]
	Week 4	14.7 [10.9 – 18.2]	16.8 [10.1 – 23.1]	19.1 [16.2 – 24.5]
	Week 13	12.4 [7.66 – 15.3]	34.5 [25.8 – 43.0]	34.9 [32.3 – 39.8]
C_{max} (µg/mL)	Day 1	2.65 [2.43 – 2.79]	3.59 [3.13 – 4.41]	5.16 [2.54 – 6.56]
	Week 4	1.45 [0.926 – 1.73]	1.51 [1.18 – 1.94]	1.58 [1.28 – 2.05]
	Week 13	1.30 [0.910 – 1.57]	2.72 [2.56 – 2.95]	2.58 [2.20 – 3.01]
Parameter	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		20	200	400
AUC_{0-t} (µg.h/mL)	Day 1	61.4 [43.0 – 85.8]	88.5 [82.3 – 95.5]	105 [53.3 – 142]
	Week 4	27.2 [22.3 – 30.2]	33.9 [18.1 – 46.0]	37.6 [33.3 – 45.8]
	Week 13	34.6 [27.7 – 45.3]	57.9 [32.7 – 74.7]	74.0 [49.1 – 112]
C_{max} (µg/mL)	Day 1	5.01 [3.67 – 7.10]	6.67 [5.04 – 7.89]]	5.98 [4.75 – 7.35]
	Week 4	2.56 [1.82 – 3.06]	3.15 [2.11 – 3.74]	2.76 [2.06 – 3.40]
	Week 13	2.97 [2.15 – 3.52]	8.41 [4.62 – 11.3]	5.51 [3.92 – 6.70]

(table excerpted from Applicant's NDA)

Mean Toxicokinetic Parameters on Day 1, Week 4, and Week 13 for GSK2167542 (Metabolite 8 (M8)) Following Daily Oral Gavage Administration of GSK2118436 to Rats

Parameter	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		20	200	400
AUC_{0-t} (µg.h/mL)	Day 1	1.53 [0.995 – 1.88]	2.18 [1.74 – 2.47]	7.81 [3.67 – 10.3]
	Week 4	0.281 [0.150 – 0.358]	1.86 [0.949 – 2.35]	2.01 [0.375 – 3.31]
	Week 13	NC [NC]	1.50 [0.713 – 1.99]	0.621 [0.334 – 0.775]
C_{max} (µg/mL)	Day 1	0.121 [0.105 – 0.141]	0.202 [0.186 – 0.230]	0.670 [0.223 – 0.987]
	Week 4	0.059 [0.047 – 0.070]	0.279 [0.164 – 0.410]	0.283 [0.065 – 0.553]
	Week 13	0.016 [0.015 – 0.019]	0.106 [0.052 – 0.168]	0.034 [0.017 – 0.044]
Parameter	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		20	200	400
AUC_{0-t} (µg.h/mL)	Day 1	0.684 [0.508 – 0.895]	1.69 [1.61 – 1.76]	2.32 [2.04 – 2.73]
	Week 4	0.330 [0.256 – 0.404]	2.44 [1.06 – 4.67]	3.21 [2.09 – 5.01]
	Week 13	0.130 [NC]	1.28 [0.598 – 2.23]	1.13 [0.829 – 1.36]
C_{max} (µg/mL)	Day 1	0.078 [0.049 – 0.127]	0.144 [0.108 – 0.214]	0.207 [0.198 – 0.225]
	Week 4	0.113 [0.098 – 0.129]	0.488 [0.187 – 1.03]	0.346 [0.308 – 0.407]
	Week 13	0.020 [0.017 – 0.022]	0.121 [0.053 – 0.220]	0.065 [0.054- 0.082]

NC: Not Calculated: Insufficient data

(table excerpted from Applicant's NDA)

Study title: GSK2118436B: A 13 Week Twice Daily Oral Capsule Toxicity Study in the Beagle Dog Followed by a 4-Week Recovery.

Study no.:	CD2010-00051
Study report location:	4.2.3.2
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	September 30, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436B (mesylate salt of GSK2118436A) Batch 091231057, 99.5%

Key study findings:

- The 60 (males) and 100 (females) mg/kg/day high-dose was intolerable due to body weight loss and severity of clinical signs.
- Target organs of toxicity were skin, heart, and male reproductive organs.
- Skin lesions and papules were observed at all doses on various areas including the muzzle, pinna, lower jaw, inguinal, scrotum, and ventral thoracic. Histopathological correlates included acanthosis, infiltration, and erosions/crust.
- Hemorrhage of the atrioventricular value was noted in 20 mg/kg/day mid-dose females and 60 (males) and 100 (females) mg/kg/day high-dose animals.
- Additional toxicities included dose-responsive lymphoid depletion in the thymus and inflammation in the lungs.
- 100% of male dogs showed degeneration/depletion in the testis and aspermia in the epididymis, irrespective of dose. Partial granular development was also noted in the prostate following the 20 mg/kg/day mid-dose.

Methods:

Doses:	0, 5, 20, or 60 (males) / 100 (females) mg/kg/day
Frequency of dosing:	Twice daily for 13-weeks with a 4-week recovery period
Route of administration:	Oral
Formulation/Vehicle:	Capsule containing mesylate salt form of GSK2118436
Species/Strain:	Dog / Beagle
Number/Sex/Group:	4 - main group 3 - recovery group for control and high-dose
Age:	13-14 months
Weight:	Males – 8.6 to 11.6 kg; Females – 5.4 to 8.3 kg
Dose justification:	The dose levels were selected by the Applicant based on previous 4-week toxicity data in the dog (study CD2008-01503, reviewed above)

Observations and Results:

Mortality [observations made twice daily]

The 60 (males) and 100 (females) mg/kg/day dose of GSK2118436B was not tolerated. Due to the severity of clinical signs and body weight loss (up to a 17% decrease in body weight by Day 14, compared to pre-treatment), dosing of animals given 60/100 mg/kg/day was discontinued after 14/15 days. Main study animals given 60/100 mg/kg/day were subsequently euthanized on Day 22/23 after one week off-dose, while recovery animals were euthanized on Day 46/47 after 4 weeks off-dose. Clinical signs included thin body condition, inappetence, body weight loss, prominent backbone, dehydration (slight to severe), increased incidence of liquid feces, red gums/gingivitis (and for one animal, bilateral gingival erosion and ulceration including exposed bone), occasional/transient emesis and eye discharge.

Clinical Signs [twice daily]

Following 5 and 20 mg/kg/day, test article-related clinical signs were observed in all animals generally starting during Week 3. Skin papules were observed on various areas including the muzzle, pinna, lower jaw, inguinal, scrotum, and ventral thoracic. Skin lesions/red skin/scab were noted mostly ventral cervical. Swollen hindpaws and/or forepaws, and ear discharge, were also seen. These adverse clinical signs prompted supportive care with veterinary treatments over several days for some animals.

In addition, two 20 mg/kg/day males showed shallow and/or labored breathing and tremors on two occasions between Days 71 and 77.

Body Weights [pre-dose; weekly]

Due to body weight loss, food supplementation was administered to affected animals, as early as Week 3 of dosing. At the end of the dosing period, males and females

showed a 4 and 13% reduction in body weight following 20 mg/kg/day, respectively. Reversibility was not assessed.

Food Consumption [pre-dose; weekly]

Due to body weight loss, food supplementation was administered to affected animals, as early as Week 3 of dosing. At the end of the dosing period, males and females showed a 15 and 23% reduction in food consumption following 20 mg/kg/day, respectively. Reversibility was not assessed.

Ophthalmoscopy [pre-dose; week 13]

Unremarkable

Electrocardiography [pre-dose; week 4 and 13]

Unremarkable

Echocardiogram [pre-dose; week 4 and 13]

Unremarkable

Hematology [pre-dose; week 4 and 13]

Reversibility was not assessed.

Week 13 - % change in hematological parameters vs. control

	Males			Females		
	5 mg/kg	20 mg/kg	60/100 mg/kg	5 mg/kg	20 mg/kg	60/100 mg/kg
Neutrophils	+57	+157	NA	-32	+40	NA
Monocytes	X	+75	NA	-22	+38	NA

x denotes no change
NA refers to not available since dose level was intolerable

Coagulation [pre-dose; week 4 and 13]

Unremarkable

Clinical Chemistry [pre-dose; week 4 and 13]

Reversibility was not assessed.

Week 13 - % change in clinical chemistry vs. control

	Males			Females		
	5 mg/kg	20 mg/kg	60/100 mg/kg	5 mg/kg	20 mg/kg	60/100 mg/kg

Alkaline phosphatase	+8	+62	NA	+59	+397	NA
Creatinine	-25	-34	NA	+9	-17	NA
Triglycerides	+17	+63	NA	+21	+21	NA
Phosphorus	X	-20	NA	X	-26	NA

x denotes no change
NA refers to not available since dose level was intolerable

Urinalysis [pre-dose; week 4 and 13]

Unremarkable

Serum Cardiac Troponin I [pre-dose; week 4 and 13]

Unremarkable

Gross Pathology [at sacrifice]

Reversibility was not assessed.

Treatment-related Macroscopic Findings: Terminal Necropsy

Tissue and Finding	No. of animals affected							
	Males				Females			
Dose (mg/kg/day)	0	5	20	60/100	0	5	20	60/100
# of animals	4	4	4	0	4	4	4	0
Lymph Node								
- enlargement	0	0	3	NA	0	0	0	NA
Skin								
- raised	0	4	4	NA	0	4	4	NA
- thickening	0	4	3	NA	0	0	3	NA
- ulceration	0	1	2	NA	0	0	0	NA
Lung								
- adhesion	0	0	2	NA	0	0	1	NA
- thickening	0	0	2	NA	0	0	0	NA
Thymus								
- small	0	1	3	NA	1	1	4	NA

NA refers to not available since dose level was intolerable

Organ Weights [at sacrifice]

Reversibility was not assessed.

% change in organ weights vs. control

	Males			Females		
	5 mg/kg	20 mg/kg	60/100 mg/kg	5 mg/kg	20 mg/kg	60/100 mg/kg
Thymus	-14	-43	NA	-22	-67	NA

x denotes no change
NA refers to not available since dose level was intolerable

Histopathology [at sacrifice]

Adequate Battery: Yes
Peer Review: Yes

Reversibility was not assessed.

Treatment-related Microscopic Findings: Terminal Necropsy

Tissue and Finding (grade)	No. of animals affected							
	Males				Females			
Dose (mg/kg/day)	0	5	20	60/100*	0	5	20	60/100*
# of animals	4	4	4	4	4	4	4	4
Bone Marrow								
- hypercellularity: myeloid (1-2)	0	0	1	4	0	0	1	3
Cecum or Colon								
- hemorrhage (1)	0	1	1	1	0	1	1	0
Heart								
- hemorrhage: atrioventricular valve (1)	0	0	0	1	0	0	1	2
Skin: miscellaneous#								
- acanthosis (1-3)	0	0	4	0	0	0	4	0
- infiltration: mixed cell (1-2)	0	3	3	0	0	0	2	0
- erosion/crust (1-2)	1	1	3	0	0	0	2	0
Lung								
- infiltration: mixed cell (1-2)	0	0	2	0	0	0	2	2
- inflammation: bronchoalveolar (2-4)	0	0	3	1	0	0	1	0
Thymus								
- lymphoid depletion (1-4)	0	1	4	4	1	0	4	4
Testis								
- degeneration/depletion: seminiferous epithlelium (1-3)	0	4	4	3	-	-	-	-
Prostate								
- partial glandular development (1-4)	0	0	2	2	-	-	-	-
Epididymis								
- aspermia (1-5‡)	1	4	4	1	-	-	-	-
- cellular debris: intratubular (1-3)	0	4	4	3	-	-	-	-

Grade 1, 2, 3, 4, and 5 refers to minimal, mild, moderate, marked, and severe, respectively.
**60/100 mg/kg/day dosing was stopped on Day 14/15 and animals were euthanized on Day 22/23.*
#additional areas of the skin affected with similar incidence include the muzzle, paws, pinna, prepuce, and scrotum.
‡Severity of findings in epididymis were dose-dependent.

Toxicokinetics [pre-dose, and 0.5, 1, 2, 4, 8, and 24 hrs post-dose on day 1, week 4, and week 13]

- On Day 1, exposure levels (AUC) were approximately dose proportional from 5 to 20 mg/kg/day but less than proportional from 5 to 60 (males)/100 (females) mg/kg/day.
- On Week 13, from 5 to 20 mg/kg/day, exposure levels (AUC) were approximately dose proportional in males and greater than dose proportional in females

- No drug accumulation was noted.
- In regard to sex differences, following 5 mg/kg/day, exposure levels (AUC) were 2.5- and 2.1-fold higher in males than females on Day 1 and Week 13, respectively.
- Ratios of metabolite/parent AUC_{0-t} values ranged from 0.035 to 0.200 for M4, 0.219 to 0.662 for M7 and 0.004 to 0.042 for M8. Concentrations of GSK2118436 and metabolites M4, M7 and M8 were below the lower limit of quantification in plasma samples collected from each dog in the group given 60/100 mg/kg/day after approximately one week off dose.
- Details are shown in Applicant's tables below.

Mean Toxicokinetic Parameters on Day 1, Week 4, and Week 13 for GSK2118436 (Parent Compound) Following Daily Oral Administration of GSK2118436 to Dogs

Parameter	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	60
AUC_{0-t} (µg.h/mL)	Day 1	21.9 [9.93 - 31.1]	76.3 [36.0 - 122]	120 [33.1 - 207]
	Week 4	19.9 [11.5 - 26.1]	92.8 [74.2 - 107]	NA
	Week 13	28.7 [16.9 - 38.3]	79.4 [56.8 - 123]	NA
C_{max} (µg/mL)	Day 1	2.08 [1.16 - 2.51]	8.27 [4.85 - 14.4]	10.7 [4.04 -15.5]
	Week 4	1.88 [1.15 - 2.27]	8.72 [7.75 - 9.88]	NA
	Week 13	2.65 [1.38 - 3.27]	8.07 [5.24 - 9.95]	NA
Parameter	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	100
AUC_{0-t} (µg.h/mL)	Day 1	8.88 [6.86 - 10.2]	49.3 [36.7 - 71.9]	116 [64.8 - 166]
	Week 4	11.1 [9.44 - 12.4]	70.8 [63.4 - 88.0]	NA
	Week 13	13.4 [10.3 - 15.9]	118 [46.9 - 156]	NA
C_{max} (µg/mL)	Day 1	1.13 [0.734 - 1.51]	5.09 [3.46 - 6.88]	12.0 [7.08 - 20.1]
	Week 4	1.89 [1.42 - 2.53]	7.66 [5.52 - 9.11]	NA
	Week 13	1.96 [1.56 - 2.29]	11.6 [5.85 - 16.9]	NA
NA: Not Applicable. Dosing was stopped on study Day 14/15				

(table excerpted from Applicant's NDA)

Mean Toxicokinetic Parameters on Day 1, Week 4, and Week 13 for GSK2298683 (Metabolite 4 (M4)) Following Daily Oral Administration of GSK2118436 to Dogs

Parameter	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	60
AUC_{0-t} (µg.h/mL)	Day 1	0.973 [0.510 - 1.30]	3.42 [2.84 - 4.72]	4.18 [3.10 - 5.37]
	Week 4	1.03 [0.724 - 1.35]	5.04 [3.48 - 6.14]	NA
	Week 13	1.37 [0.832 - 1.88]	4.52 [2.52 - 5.49]	NA
C_{max} (µg/mL)	Day 1	0.0713 [0.0354 - 0.0953]	0.211 [0.155 - 0.263]	0.261 [0.185 - 0.349]
	Week 4	0.0647 [0.0448 - 0.0973]	0.265 [0.191 - 0.313]	NA
	Week 13	0.0919 [0.0576 - 0.134]	0.251 [0.152 - 0.297]	NA
Parameter	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	100
AUC_{0-t} (µg.h/mL)	Day 1	1.78 [1.43 - 2.04]	2.25 [1.82 - 2.52]	5.71 [3.94 - 7.63]
	Week 4	1.88 [1.49 - 2.17]	3.25 [2.62 - 4.21]	NA
	Week 13	2.37 [1.63 - 2.87]	5.00 [2.93 - 7.26]	NA
C_{max} (µg/mL)	Day 1	0.132 [0.118 - 0.160]]	0.138 [0.104 - 0.161]	0.369 [0.237 - 0.440]
	Week 4	0.143 [0.109 - 0.194]	0.236 [0.156 - 0.408]	NA
	Week 13	0.194 [0.123 - 0.260]	0.287 [0.168 - 0.420]	NA
NA: Not Applicable. Dosing terminated at study Day 14/15				

(table excerpted from Applicant's NDA)

Mean Toxicokinetic Parameters on Day 1, Week 4, and Week 13 for GSK2285403 (Metabolite 7 (M7)) Following Daily Oral Administration of GSK2118436 to Dogs

Parameter	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	60
AUC_{0-t} (µg.h/mL)	Day 1	5.05 [2.86 - 6.64]	27.8 [11.0 - 34.8]	52.3 [22.8 - 74.9]
	Week 4	4.82 [3.17 - 6.04]	30.3 [19.4 - 38.4]	NA
	Week 13	6.29 [4.14 - 7.79]	22.5 [20.0 - 25.0]	NA
C_{max} (µg/mL)	Day 1	0.424 [0.192 - 0.663]	2.04 [1.56 - 2.30]	3.92 [2.15 - 4.89]
	Week 4	0.376 [0.227 - 0.512]	2.17 [1.70 - 2.68]	NA
	Week 13	0.505 [0.324 - 0.687]	1.60 [1.25 - 2.17]	NA
Parameter	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	100
AUC_{0-t} (µg.h/mL)	Day 1	4.69 [3.29 - 6.69]	20.9 [16.9 - 26.3]	77.0 [50.4 - 107]
	Week 4	5.25 [4.06 - 6.61]	23.8 [19.0 - 27.4]	NA
	Week 13	6.17 [4.61 - 8.39]	36.5 [16.9 - 55.5]	NA
C_{max} (µg/mL)	Day 1	0.491 [0.301 - 0.681]	1.66 [1.19 - 2.08]	5.85 [3.72 - 9.36]
	Week 4	0.689 [0.530 - 0.849]	2.13 [1.69 - 2.50]	NA
	Week 13	0.766 [0.576 - 1.04]	2.66 [1.98 - 3.36]	NA
NA: Not Applicable. Dosing terminated at study Day 14/15				

(table excerpted from Applicant's NDA)

Mean Toxicokinetic Parameters on Day 1, Week 4, and Week 13 for GSK2167542 (Metabolite 8 (M8)) Following Daily Oral Administration of GSK2118436 to Dogs

Parameter	Period	Male		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	60
AUC_{0-t} (µg.h/mL)	Day 1	NC	0.362 [0.180, 0.544]	0.448 [0.189 - 0.755]
	Week 4	0.130 [0.0267 - 0.354]	1.21 [0.466 - 2.07]	NA
	Week 13	0.462 [0.117 - 0.707	1.70 [0.768 - 2.78]	NA
C_{max} (µg/mL)	Day 1	0.0155 [0.0107 - 0.0230]	0.0288 [0.0131 - 0.0433]	0.0353 [0.0163 - 0.0593]
	Week 4	0.0203 [0.0148 - 0.0228]	0.107 [0.0452- 0.187]	NA
	Week 13	0.0401 [0.0173 - 0.0553]	0.162 [0.0394 - 0.415]	NA
Parameter	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	100
AUC_{0-t} (µg.h/mL)	Day 1	NC	1.21 [0.471, 1.95]	1.079 [0.766 - 1.56]
	Week 4	0.0556 [NC]	2.16 [0.996 - 3.75]	NA
	Week 13	0.563 [0.235 - 1.03]	2.30 [1.17 - 2.75]	NA
C_{max} (µg/mL)	Day 1	0.0145 [0.0107, 0.0182]	0.0952 [0.0181, 0.291]	0.0914 [0.0508 - 0.183]
	Week 4	0.0171 [0.0125 - 0.0263]	0.206 [0.073 - 0.433]	NA
	Week 13	0.0418 [0.0121 - 0.108]	0.227 [0.153 - 0.286]	NA

NA: Not Applicable. Dosing terminated at study Day 14/15
NC: Not Calculated; Insufficient data

(table excerpted from Applicant's NDA)

7 Genetic Toxicology

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

Study Title: GSK2118436A: Bacterial Mutation Test (AMES Test) with *Salmonella typhimurium* and *Escherichia coli.* Reviewed by Kimberly Ringgold, Ph.D. and slightly modified for this NDA review.

Study no.:	WD2008-01655
Study report location:	4.2.3.3
Conducting laboratory and location:	GlaxoSmithKline Hertfordshire SG12 0DP UK
Date of study initiation:	October 15, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, 081179173, 99.3%

Key Study Findings:

- GSK2118436A (dabrafenib) was not mutagenic in tester strains of *Salmonella* or *E. coli* in the presence and absence of S-9 mix, under the conditions tested.

Methods:

Strains:	Salmonella typhimurium tester strains TA1535, TA1537, TA98, TA100, and E. coli WP2 *uvrA*
Concentrations in definitive study:	5, 15, 50, 150, 500, 1500, 2500, and 5000 µg/plate
Basis of concentration selection:	Levels up to 5000 µg/plate is the standard limit dose recommended by regulatory guidelines
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	With S9: 2-aminoanthracene, benzo[a]pyrene Without S9: sodium azide, ICR-191, 2-nitrofluorene, 4-nitroquinoline N-oxide
Formulation/Vehicle:	Dimethyl sulfoxide (DMSO)
Incubation & sampling time:	Plate incorporation: 37ºC for 2 to 3 days

Analysis:

After incubation, colonies were counted electronically for bacterial colony formation using a Sorcerer image analysis system. Scoring of some plates was confirmed by manual count due to precipitates. Cytotoxicity was assessed by examining bacterial lawn density and number of spontaneous revertants per plate.

Criteria for positive results:

Results were considered positive if the data for any treatment level showed a response ≥ 2 times the concurrent vehicle control for TA98, TA100, WP2 uvrA or ≥ 3 times the concurrent vehicle control for TA1535 and TA1537.

Study Validity:

- Selection of the tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICHS2A, April 1996).
- The highest concentration tested was 5000 µg/plate, which allowed maximum exposure.
- The vehicle control values were within the laboratory historical ranges.
- The appropriate positive control compounds (± S9 mix) produced clear, unequivocal increases in the number of revertant colonies.

Results:

Precipitation of the test article was observed at 1500 µg/plate; thus plates ≥ 2500 µg/plate were not scored. Plates with GSK2118436A concentrations ≤ 1500 µg/plate showed no signs of bacterial mutagenicity. As shown in the table below, GSK2118436A was not mutagenic in tester strains of *Salmonella* or *E. coli* in the presence or absence of S-9 mix, under the conditions tested.

Ames Test Results: Mean Number of Revertant Colonies per Plate

Metabolic Activation	Test article	Dose Level[1] (µg/plate)	Main Plate Incorporation Test Mean Number of Revertant Colonies per Plate				
			TA100	TA1535	TA1537	TA98	WP2*uvrA* (pKM101)
Without Activation	DMSO	100 µl/plate	110.7	22.5	12.8	23.7	113.3
	GSK2118436	15	130.7	18.7	14.7	25.3	148.0
	GSK2118436	50	137.0	17.0	17.7	24.7	140.7
	GSK2118436	150	136.7	17.7	19.3	21.3	122.3
	GSK2118436	500	131.0	15.0	19.0	23.0	110.0
	GSK2118436	1500	140.7[3]	17.0[3]	16.3[3]	17.3[3]	112.3[3]
	2-Nitrofluorene	1	-	-	-	217.5[2]	-
	Sodium azide	2	733.5[2]	1211.5[2]	-	-	-
	ICR-191	1	-	-	135.0[2]	-	-
	4NQO	2	-	-	-	-	3601.5[2]
With Activation	DMSO	100 µl/plate	134.0	16.5	19.8	39.5	179.2
	GSK2118436	15	130.7	17.0	17.0	36.7	182.7
	GSK2118436	50	139.3	17.7	18.0	32.3	199.3
	GSK2118436	150	148.3	19.3	19.3	28.7	191.3
	GSK2118436	500	140.3	24.0	13.3	30.3	186.0
	GSK2118436	1500	151.0[3]	14.3[3]	13.0[3]	36.0[3]	170.0[3]
	Benzo[a]pyrene	10	-	-	-	963.0[2]	-
	2-Aminoanthracene	5	3422.5[2]	544.5[2]	266.5[2]	-	-
	2-Aminoanthracene	10	-	-	-	-	1603.0[2]

1. Expressed in terms of parent compound
2. ≥ two fold increase in revertants for TA98, TA100 and WP2*uvrA*(pKM101) and ≥ three fold increase for TA1535 and TA1537
3. Precipitation observed by eye on the test plates at the end of incubation

(table excerpted from Applicant's NDA)

7.2 *In Vitro* Assays in Mammalian Cells

Study title: GSK2118436A: *In Vitro* Mutation Assay with L5178Y/TK +/- Mouse Lymphoma Cells at the TK Locus. Reviewed by Kimberly Ringgold, Ph.D. and slightly modified for this NDA review.

Study no.:	WD2008-01806
Study report location:	4.2.3.3
Conducting laboratory and location:	GlaxoSmithKline Hertfordshire SG12 0DP UK
Date of study initiation:	October 13, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, 081179173, 99.3%

Key Study Findings:

- GSK2118436A (dabrafenib) was not mutagenic in the mouse lymphoma L5178Y *tk* locus assay, under the conditions tested.

Methods:

Cell line:	L5178Y/TK +/- Mouse Lymphoma
Concentrations in definitive study:	1, 4, 10, 25, 30, 32, 40, 48, 54 µg/mL
Basis of concentration selection:	A cytotoxicity test was performed to select doses for the definitive assay. The concentrations tested ranging from 10 to 120 µg/mL (3-hr treatment) and 0.25 to 30 µg/mL (24-hr treatment). Cytotoxicity was observed at GSK2118436A at concentrations of 50 and 45 µg/mL with or without S9 mix, respectively; and at concentrations ≥ 4 µg/mL in the absence of S9 (24-hr treatment). Therefore, the maximum concentrations tested were 100 µg/mL in the presence or absence S9 (3-hr treatment) and 50µg/mL in the absence of S9 (24-hr treatment).
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	With S9: Dimethylbenzanthracene Without S9: Methylmethane sulphonate
Formulation/Vehicle:	Dimethyl sulfoxide (DMSO)
Incubation & sampling time:	Exposure period: 37ºC for 3-hr (± S9) & 37ºC for 24-hr (- S9) Expression period: 37ºC for 2 days Cloning: 37ºC for 6 – 8 days (viability plates) or 10 – 12 days (mutant plates)

Analysis:

Cell concentrations were determined using a Coulter counter. Mutant frequency was determined using a Titertek™ mirror box. Colony sizing was performed on the vehicle, positive control, and treatment plates.

Criteria for positive results:

Results were considered positive if the mutant frequency of any test article exceeded the sum of the mean control mutant frequency plus global evaluation factor.

Study Validity:

- The vehicle and positive controls met the assay acceptance criteria for the 24-hr treatment.
- The mutation frequency of the test-article concentrations were less than the sum of the mean control mutation frequency plus the GEF for the 3-hr treatment.

Results:

The maximum test concentrations examined for the 3-hour treatment period were limited by toxicity to 54 and 48 µg/mL in the presence and absence of S9-mix, respectively. At these concentrations relative total growth was reduced to 17.29% and 15.05%. The maximum test concentration examined for the 24-hour treatment period was limited by toxicity to 25 µg/mL. At this concentration relative total growth was reduced to 17.35%.

As shown in the table below, the mutant frequency was less than the sum of the mean control mutant frequency plus the global evaluation factor at all concentrations plated, following a 3-hour (with and without S9) or 24-hour (absence of S9) exposure. Therefore, GSK2118436A (dabrafenib) was not mutagenic in the mouse lymphoma L5178Y *tk* locus assay, under the conditions tested.

Mouse Lymphoma Assay Results: Mean Relative Total Growth (%) and Mean Mutant Frequency

Test Article	Dose Level[1] mcg/mL	3 hr Treatment -S9-mix Mean Relative Total Growth (%)	3 hr Treatment -S9-mix Mean Mutant Frequency ($x10^{-6}$)	3 hr Treatment +S9-mix Mean Relative Total Growth (%)	3 hr Treatment +S9-mix Mean Mutant Frequency ($x10^{-6}$)	24 hr Treatment -S9-mix Mean Relative Total Growth (%)	24 hr Treatment -S9-mix Mean Mutant Frequency ($x10^{-6}$)
Dimethyl sulphoxide	0	100.00[2]	166.64[2]	99.99	128.35	99.76	101.73
GSK2118436	1	NT	NT	NT	NT	90.47	114.08
GSK2118436	4	NT	NT	NT	NT	49.33	129.34
GSK2118436	10	58.89	125.53	77.14	133.93	27.63	125.63
GSK2118436	25	NT	NT	NT	NT	17.35	121.04
GSK2118436	30	68.71	179.77	NT	NT	NT	NT
GSK2118436	32	NT	NT	NT	NT	NT	NT
GSK2118436	40	39.51	212.78	49.83	132.92	NT	NT
GSK2118436	48	15.05	234.61	26.67	185.00	NT	NT
GSK2118436	54	NR	NR	17.29	177.18	NT	NT
Methyl methane sulphonate	20	18.96	1302.06	NT	NT	NT	NT
Methyl methane sulphonate	5	NT	NT	NT	NT	50.86	846.88
Dimethylbenzanthracene	1.1	NT	NT	11.52	1780.39	NT	NT

1. All concentrations are expressed in terms of parent compound
2. Mean Relative Total Growth and Mutation Frequency based on 3 replicate vehicle controls only

NT= Not treated

NR= Not reported

(table excerpted from Applicant's NDA)

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

Study title: GSK2118436A: Oral Bone Marrow Micronucleus Assay in Rats.
Reviewed by Kimberly Ringgold, Ph.D. and slightly modified for this NDA review.

Study no:	WD2009-00233
Study report location:	4.2.3.3
Conducting laboratory and location:	GlaxoSmithKline Hertfordshire SG12 0DP UK
Date of study initiation:	November 11, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436A, 081179173, 99.3%

Key Study Findings:

- GSK2118436A (dabrafenib) was not genotoxic in the rat bone marrow micronucleus assay, under the conditions tested.

Methods:

Doses in definitive study:	0, 100, 1000 mg/kg/day
Frequency of dosing:	Daily for 2 days
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5 (w/v) hydroxypropyl methylcellulose with 0.1% Tween 80
Species/Strain:	Rat / Crl:CD (SD)
Number/Sex/Group:	6 males per group except the positive control group had 3 males (only males were utilized for the main study since, according to the Applicant, there are no differences in systemic toxicity or exposure between males and females)
Satellite groups:	3 males and females per group in dose (as part of range finding study at 2000 mg/kg)
Basis of dose selection:	Dose selection for the main micronucleus assay was based on the range finding study and previous exposure data indicating a plateau in exposure above 1000 mg/kg/day [CD2008/00951/01], in accordance with current guidelines.
Negative control:	0.5 (w/v) hydroxypropyl methylcellulose with 0.1% Tween 80
Positive control:	Cyclophosphamide monohydrate

Analysis:

- For the range finding study, male and female mice were given a single administration of 2000 mg/kg GSK2118436A. Blood samples were taken approximately 1-hr post dosing.
- All rats were sacrificed 24 hours after receiving their final dose and femoral bone marrow smears prepared. Smears were stained with acridine orange prior to analysis with fluorescence microscopy to determine the proportion of polychromatic erythrocytes in the total erythrocyte count (% PCE) and the number of micronucleated polychromatic erythrocytes (MPCE) per 2000 PCE analysed.

Criteria for positive results:

Results were considered positive if any treatment group showed a response (mean frequency of MPCE) which was ≥ 4 times the concurrent vehicle control value.

Study Validity:

The pharmacokinetic assessments demonstrated systemic exposure, dosing appeared to be adequate based upon dose ranging study results, the positive controls exhibited a clear unequivocal positive response, and the vehicle control data for this study were within laboratory historical data ranges.

Results:

In the dose range finding test clinical signs were observed at 2000 mg/kg/day in male rats which included slight coat piloerection, slight subdued behavior and slight red staining around the eyes. In female rats clinical signs included being slightly tense and vocalizing when handled/during dosing and slight red staining around the nose/snout. There was a slight reduction in group mean bodyweight gain in males given 2000 mg/kg/day (group mean reduction of 1% from Day 1 to Day 3) and a group mean body weight loss in females given 2000 mg/kg/day (group mean loss 7.8% from Day 1 to Day 3). Analysis of plasma samples from satellite animals dosed once with 2000 mg/kg/day and sampled 1 hour after dosing, confirmed systemic exposure to GSK2118436A (mean: 5192 ng/mL (male); 3228 ng/mL (female)).

In the micronucleus assay, no clinical signs of toxicity were observed at doses up to 1000 mg/kg/day, with the exception of one male rat (100 mg/kg/day) which showed slight post-dose intermittent head shaking on Day 1. There was a reduction in group mean body weight gain in animals given 1000 mg/kg/day (5.7% group mean reduction when compared with the concurrent group mean vehicle control). Data for the concurrent vehicle control (group mean % PCE and MPCE/2000 PCE) were within the ranges determined from laboratory historical data. The positive controls induced clear unequivocal increases in micronuclei. Group mean values for % PCE for all rats dosed with GSK2118436A, were similar to the concurrent vehicle control. There was no effect on erythroblast proliferation at any of the doses tested and thus no indication of bone marrow toxicity. As shown in the following table, group mean values for MPCE/2000 PCE for male rats dosed at 100 and 1000 mg/kg/day, were similar to the concurrent vehicle control and fell within the range determined from the laboratory historical control data. Therefore, following two (given 24 hours apart) oral doses of 100 or 1000 mg/kg/day in the rat bone marrow micronucleus assay, GSK2118436A was not genotoxic.

Results from Bone Marrow Micronucleus Assay in Rats

Test Article	Dose[1] (mg/kg/day)	No. of Animals Analysed [2]	Group Mean %PCE	Group Mean MPCE [3]
Vehicle control	0	6 M	50	1.49
GSK2118436	100	6 M	49	1.33
GSK2118436	1000	6 M	49	1.66
Cyclophosphamide[4]	20	3 M	44	57.35

1. Expressed in terms of the parent compound
2. M = Male
3. Group mean micronucleated PCE (MPCE) per 2000 PCE analysed
4. Positive control induced an unequivocal positive response

(table excerpted from Applicant's NDA)

7.4 Other Genetic Toxicity Studies

Study Title: GI-147517 (N-Bromosuccinimide): Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli* (screening study).

Study no.:	2010N105217
Study report location:	4.2.3.7.6
Conducting laboratory and location:	GlaxoSmithKline, UK
Date of study initiation:	April 23, 2010
GLP compliance:	No
QA statement:	No
Drug:	GI-147517 (N-Bromosuccinimide)

Key Study Findings:

- GI-147517 (N-Bromosuccinimide) was mutagenic in the TA100 tester strain of Salmonella in the presence of S9-mix, when tested using either water or dimethyl carbonate as the vehicle. GI-147517 was also mutagenic in TA100 and TA1537 tester strains of Salmonella in the absence of S9-mix, when tested using water as the vehicle.

Methods:

Strains:	Salmonella typhimurium tester strains TA1535, TA1537, TA98, TA100, and E. coli WP2 *uvrA*
Concentrations in definitive study:	50, 150, 500, 1500, 2500, and 5000 µg/plate
Basis of concentration selection:	Levels up to 5000 µg/plate is the standard limit dose recommended by regulatory guidelines
Negative control:	Dimethyl carbonate or water
Positive control:	See tables in results section
Formulation/Vehicle:	Dimethyl carbonate or water
Incubation:	Plate incorporation

Background:

(b) (4)

Previous data has shown that N-Bromosuccinimide, when tested using dimethyl sulphoxide (DMSO) as a vehicle, is mutagenic in *Salmonella typhimurium* TA100 in the presence of S9-mix in a Bacterial Mutation Assay (Ames Test) [Seifried, 2006].

The purpose of this study was to assess the potential of GI-147517 (N-Bromosuccinimide) to induce gene mutations in bacterial strains of *Salmonella typhimurium* and *Escherichia coli* using alternative vehicles to DMSO. (b) (4) In this study all formulations of the test article were prepared and all concentrations

expressed in terms of parent compound, which for the purposes of this report is referred to as GI-147517.

Study Design:

Two plate incorporation tests were conducted for each tester strain both in the presence and absence of rat liver S9-mix. In the first test, water was used as the vehicle. Due to the potential for GI-147517 to decompose in water, to fully evaluate the mutagenic potential of GI-147517, a second test was performed using dimethyl carbonate (DMC) as the vehicle. Vehicle, untreated (where appropriate) and positive controls were included in each test.

Study Validity:

- Selection of the tester strains was adequate based upon Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICHS2A, April 1996).
- The highest concentration tested was 5000 µg/plate, which allowed maximum exposure.
- The vehicle control values were within the laboratory historical ranges.
- The appropriate positive control compounds (± S9 mix) produced clear, unequivocal increases in the number of revertant colonies.

Results:

In all tests, the maximum concentration analyzed was limited to 150 and 500 ug/plate in the absence and presence of S9-mix, respectively, due to toxicity.

In the first test (water as the vehicle), increases in revertant colonies were observed at 150 ug/plate with strain TA100 (2.7 maximum fold increase), in the presence of S9-mix, and with strain TA100 and TA1537 (3.6 and 7.5 maximum fold increases, respectively), in the absence of S9-mix.

In the second test (water as the vehicle), increases in revertant colonies were observed at 250 ug/plate with strain TA100 in the presence of S9-mix (2.1 maximum fold increase).

Ames Test Results: Test 1 (water as vehicle) without Metabolic Activation

Strain	Compound	Dose level per plate (µg)	Mean revertants per plate	Standard Deviation	Ratio treated / Vehicle	Individual revertant colony counts
TA100	GI-147517	50	118.5	0.7	1.4	118, 119
		150	314.0	25.5	3.6	332 T3, 296 T3
		500				T1, T1
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		87.5	3.5		90, 91, 84, 85
TA1535	GI-147517	50	16.5	4.9	1.0	13, 20
		150	23.5	7.8	1.4	18 T3, 29 T3
		500				T1, T1
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		16.5	3.1		18, 15, 20, 13
TA1537	GI-147517	50	16.0	4.2	1.9	19, 13
		150	62.0	4.2	7.5	65 T4, 59 T4
		500				T1, T1
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		8.3	3.4		13, 5, 8, 7
TA98	GI-147517	50	38.0	2.8	1.3	40, 36
		150	40.0	5.7	1.4	44 T3, 36 T3
		500				T1, T1
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		28.5	7.3		18, 35, 31, 30
WP2 uvrA (pKM101)	GI-147517	50	69.5	7.8	0.8	75, 64
		150	82.5	12.0	0.9	74, 91
		500				T1, T1
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		91.8	6.7		82, 95, 93, 97
TA100	NaAz	2	1146.0	24.0	13.1	1129, 1163
TA1535	NaAz	2	1372.0	9.9	83.2	1365, 1379
TA1537	ICR-191	1	461.5	43.1	55.9	492, 431
TA98	2NF	1	322.5	9.2	11.3	316, 329
WP2 uvrA (pKM101)	4NQO	2	4333.5	333.0	47.2	4569, 4098

Key to Positive Controls		Key to Plate Postfix Codes	
NaAz	Sodium Azide	T4	Slight Toxicity (slight diminution of background bacterial lawn)
ICR-191	ICR-191	T3	Toxicity Seen (marked diminution of background bacterial lawn)
2NF	2-Nitrofluorene	T1	Toxic (complete killing of all test bacteria)
4NQO	4-Nitroquinoline-1-oxide		

(table excerpted from Applicant's NDA)

Ames Test Results: Test 1 (water as vehicle) with Metabolic Activation

Strain	Compound	Dose level per plate (µg)	Mean revertants per plate	Standard Deviation	Ratio treated / Vehicle	Individual revertant colony counts
TA100	GI-147517	50	75.5	0.7	1.1	75, 76
		150	183.0	1.4	2.7	182, 184
		500	37.5	0.7	0.6	37 T3, 38 T3
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		68.0	11.2		71, 81, 66, 54
TA1535	GI-147517	50	11.0	1.4	0.9	12, 10
		150	16.0	5.7	1.3	12, 20
		500	11.0	2.8	0.9	13 T3, 9 T3
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		12.0	4.8		11, 10, 8, 19
TA1537	GI-147517	50	22.5	6.4	1.4	27, 18
		150	17.0	2.8	1.0	15 T4, 19 T4
		500	7.0	4.2	0.4	4 T2, 10 T2
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		16.5	4.0		16, 19, 11, 20
TA98	GI-147517	50	45.0	9.9	1.1	38, 52
		150	44.5	0.7	1.0	45, 44
		500	14.5	0.7	0.3	14 T3, 15 T3
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		42.5	6.6		44, 33, 45, 48
WP2 uvrA (pKM101)	GI-147517	50	77.5	30.4	0.7	99, 56
		150	66.0	4.2	0.6	69, 63
		500	20.0	12.7	0.2	11 T3, 29 T3
		1500				T1, T1
		2500				T1, T1
		5000				T1, T1
	Water		103.8	9.9		98, 114, 110, 93
TA100	2-AAN	5	3505.5	157.7	51.6	3394, 3617
TA1535	2-AAN	5	324.0	69.3	27.0	275, 373
TA1537	2-AAN	5	138.0	28.3	8.4	158, 118
TA98	B[a]P	10	472.5	27.6	11.1	492, 453
WP2 uvrA (pKM101)	2-AAN	10	1782.5	78.5	17.2	1727, 1838

Key to Positive Controls		Key to Plate Postfix Codes	
2-AAN	2-Aminoanthracene	T4	Slight Toxicity (slight diminution of background bacterial lawn)
B[a]P	Benzo[a]pyrene	T3	Toxicity Seen (marked diminution of background bacterial lawn)
		T2	Severe Toxicity (severe diminution of background bacterial lawn)
		T1	Toxic (complete killing of all test bacteria)

(table excerpted from Applicant's NDA)

Ames Test Results: Test 2 (Dimethyl carbonate as vehicle) without Metabolic Activation

Strain	Compound	Dose level per plate (µg)	Mean revertants per plate	Standard Deviation	Ratio treated / Vehicle	Individual revertant colony counts
TA100	GI-147517	5	80.0	6.2	1.0	73, 85, 82
		15	73.3	0.6	0.9	73, 74, 73
		50	52.3	41.2	0.6	72, 80, 5
		150	86.3	15.7	1.0	81 T2, 74 T2, 104 T2
		250	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		83.3	5.6		86, 86, 84, 73, 82, 89
	Untreated Control		69.0	9.5		86, 65, 68, 60, 73, 62
TA1535	GI-147517	5	16.0	1.7	0.9	15, 15, 18
		15	15.3	2.1	0.8	17, 13, 16
		50	21.7	1.2	1.2	21, 21, 23
		150	7.0	1.0	0.4	6 T3, 8 T3, 7 T3
		250	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		18.2	4.8		16, 27, 16, 13, 19, 18
	Untreated Control		12.2	5.9		19, 16, 13, 9, 4, 1 E
TA1537	GI-147517	5	11.7	4.9	1.4	6, 14, 15
		15	18.0	6.1	2.2	11, 21, 22
		50	18.7	2.5	2.2	19, 16, 21
		150	10.3	4.0	1.2	15 T3, 8 T3, 8 T3
		250	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		8.3	3.7		13, 5, 11, 5, 11, 5
	Untreated Control		6.8	2.6		10, 7, 6, 3, 8, 1 E
TA98	GI-147517	5	31.0	7.8	1.0	26, 27, 40
		15	39.0	5.3	1.2	33, 43, 41
		50	30.7	8.7	1.0	21, 38, 33
		150	15.7	4.2	0.5	17 T2, 11 T2, 19 T2
		250	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		31.7	4.8		32, 35, 32, 38, 24, 29
	Untreated Control		32.4	7.4		41, 39, 27, 31, 24, 1 E
WP2 uvrA (pKM101)	GI-147517	5	95.7	7.1	1.0	88, 97, 102
		15	82.3	8.1	0.8	88, 86, 73
		50	73.0	18.5	0.8	87, 52, 80
		150	47.7	21.2	0.5	65, 54, 24
		250	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		97.0	18.4		76, 109, 82, 102, 88, 125
	Untreated Control		88.0	9.5		89, 79, 104, 81, 93, 82
TA100	NaAz	2	821.5	33.2	9.9	798, 845
TA1535	NaAz	2	923.0	43.8	50.8	954, 892
TA1537	ICR-191	1	346.5	23.3	41.6	330, 363
TA98	2NF	1	455.0	52.3	14.4	418, 492
WP2 uvrA (pKM101)	4NQO	2	1852.5	417.9	19.1	1557, 2148

Key to Positive Controls		Key to Plate Postfix Codes	
NaAz	Sodium Azide	T3	Toxicity Seen (marked diminution of background bacterial lawn)
ICR-191	ICR-191	T2	Severe Toxicity (severe diminution of background bacterial lawn)
2NF	2-Nitrofluorene	T1	Toxic (complete killing of all test bacteria)
4NQO	4-Nitroquinoline-1-oxide	E	Outlier, excluded from analysis

(table excerpted from Applicant's NDA)

Ames Test Results: Test 2 (Dimethyl carbonate as vehicle) with Metabolic Activation

Strain	Compound	Dose level per plate (µg)	Mean revertants per plate	Standard Deviation	Ratio treated / Vehicle	Individual revertant colony counts
TA100	GI-147517	5	67.0	3.6	0.9	71, 66, 64
		15	68.3	5.5	0.9	63, 68, 74
		50	75.0	8.7	1.0	65, 80, 80
		150	100.7	9.5	1.3	108, 104, 90
		250	159.3	33.2	2.1	121, 177, 180
		500	102.0	67.6	1.3	40 T2, 92 T2, 174 T2
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		77.7	8.3		84, 65, 74, 78, 76, 89
	Untreated Control		71.0	3.9		73, 71, 71, 71, 76, 64
TA1535	GI-147517	5	17.0	3.5	0.9	15, 21, 15
		15	18.3	1.2	1.0	17, 19, 19
		50	14.7	3.5	0.8	11, 15, 18
		150	12.3	6.4	0.7	17, 15, 5
		250	13.7	4.5	0.8	9, 18, 14
		500	17.3	8.1	1.0	10 T4, 26 T4, 16 T4
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		18.2	3.2		18, 16, 15, 17, 19, 24
	Untreated Control		14.8	4.2		16, 21, 16, 14, 14, 8
TA1537	GI-147517	5	22.3	1.2	1.1	23, 23, 21
		15	22.3	4.2	1.1	21, 19, 27
		50	22.0	1.7	1.1	21 T4, 24 T4, 21 T4
		150	16.0	2.6	0.8	14 T3, 15 T3, 19 T3
		250	9.0	1.7	0.4	10 T2, 10 T2, 7 T2
		500	6.0	2.6	0.3	4 T2, 9 T2, 5 T2
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		20.8	3.4		21, 22, 18, 19, 18, 27
	Untreated Control		25.3	3.1		27, 23, 25, 21, 26, 30
TA98	GI-147517	5	58.7	5.9	1.3	63, 61, 52
		15	40.3	11.1	0.9	30, 39, 52
		50	53.3	3.5	1.2	57, 50, 53
		150	40.0	1.7	0.9	41, 41, 38
		250	44.7	8.1	1.0	54 T3, 39 T3, 41 T3
		500	22.3	10.1	0.5	17 T2, 34 T2, 16 T2
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		45.0	9.2		46, 56, 48, 33, 35, 52
	Untreated Control		51.7	10.0		53, 49, 65, 61, 42, 40
WP2 uvrA (pKM101)	GI-147517	5	127.7	10.3	0.9	139, 125, 119
		15	110.3	4.6	0.8	113, 105, 113
		50	97.0	4.6	0.7	93, 96, 102
		150	88.0	26.9	0.6	58, 110, 96
		250	79.3	5.5	0.6	79, 85, 74
		500	34.7	20.1	0.2	16, 32, 56
		1500	0.0	0.0	0.0	0 T1, 0 T1, 0 T1
	DMC		139.2	10.1		140, 147, 134, 124, 153, 137
	Untreated Control		156.7	7.1		161, 167, 153, 160, 149, 150
TA100	2-AAN	5	2824.0	120.2	36.4	2739, 2909
TA1535	2-AAN	5	318.0	45.3	17.5	286, 350
TA1537	2-AAN	5	168.0	24.0	8.1	151, 185
TA98	B[a]P	10	403.5	9.2	9.0	410, 397
WP2 uvrA (pKM101)	2-AAN	10	1113.5	6.4	8.0	1109, 1118

Key to Positive Controls		Key to Plate Postfix Codes	
2-AAN	2-Aminoanthracene	T4	Slight Toxicity (slight diminution of background bacterial lawn)
B[a]P	Benzo[a]pyrene	T3	Toxicity Seen (marked diminution of background bacterial lawn)
		T2	Severe Toxicity (severe diminution of background bacterial lawn)
		T1	Toxic (complete killing of all test bacteria)

(table excerpted from Applicant's NDA)

9 Reproductive and Developmental Toxicology

Reviewed by Shawna Weis, Ph.D.

9.1 Fertility and Early Embryonic Development

Study title: GSK2118436A: Oral Female Fertility, Early Embryonic and Embryo-Fetal Development Dose Range Study in Rats.

Study no.:	2010N107959
Study report location:	4.2.3.5
Conducting laboratory and location:	Department of Safety assessment GlaxoSmithKline King of Prussia, PA
Date of study initiation:	01 November 2010 (Day 1 dose)
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	GSK2118436A, 091236623(092099); 95% purity (as free base)

Key Study Findings:

- The study failed to identify a maximum tolerated dose in pregnant rats, which is necessary to enable dose-selection for the pivotal embryo-fetal study.
- Dabrafenib was well tolerated in pregnant rats when administered orally at doses of up to 200 mg/kg/day. There were no treatment-related mortalities, gross or clinical observations, and no apparent decreases in food consumption or body weights (maternal or fetal) in this study.
- There was an apparent increase in pre-implantation loss in mid- and high-dose dams; however, all other indices of fertility were comparable to controls.
- There were no treatment-related malformations in this pilot study.

Methods:

Doses:	0, 5, 20, 200 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Hydroxypropyl methylcellulose (HPMC) K15M, 0.5% and Tween 80, 0.1%
Species/Strain:	Rat / Crl:CD(SD)
Number/Sex/Group:	7 females / group
Satellite groups:	None
Study design:	F0 females were dosed daily with vehicle or test article by oral gavage 2-weeks prior to mating until Day 17 postcoitum (PC). Treated females underwent up to 7 days of cohabitation with untreated males. Caesarean sections were performed on PC Day 21.

Observations and Results:

Mortality

One low-dose (5 mg/kg/day) female was found dead shortly after dosing on PC Day 12. Red serous fluid was found in the cage bedding. The death was not attributed to treatment.

Clinical Signs

- Alopecia
- Reflux of dose in 1 animal on multiple occasions (days -8 and PC day 2)

Body Weight

Unremarkable

Food Consumption

Unremarkable

Toxicokinetics

Not conducted

Dosing Solution Analysis

Not conducted

Necropsy

Unremarkable

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Pre-implantation Loss, etc.)

There were no treatment-related effects on estrus cycling, time to mating, or the number of pregnancies per group. There was a treatment-related increase in pre-implantation loss, at dose levels of 20 and 200 mg/kg/day; however, there was no effect on fetal body weights.

Dose Group (mg/kg	CL	Implant	Pre-Implant Loss (%)	Early Res	Late Res	Dead	PI Loss (#)	Post Impl. Loss (%)	Alive (#)	% Male	Gravid Uterus	Weight (M)	Weight (F)
0	15.4	14.7	4.74	1.3	0.1	0	1.4	9.46	13.3	46.53	102	5.705	5.395
5	16.7	15.7	4.83	1.3	0	0	1.3	8.16	14.3	47.33	101.16	5.328	5.055
20	15.4	13	15.24	1.1	0.1	0	1.3	9.96	11.7	58.27	91.429	5.562	5.379
200	16.7	14.7	11.14	1	0.2	0	1.2	7.5	13.5	49.52	98.33	5.412	5.037

Offspring (Malformations, Variations, etc.)

There were two malformations observed: One 20 mg/kg/day fetus with a diaphragmatic hernia and one 200 mg/kg/day fetus with agenesis of the innominate artery. The Applicant did not provide historical controls; however, given the low-incidence and lack of dose-response, the observed malformations are presumed to be sporadic and unrelated to treatment.

9.2 Embryonic Fetal Development

Study title: Oral Female Fertility, Early Embryonic and Embryo-Fetal Development Study in Rats.

Study no.: 2011N113146
Study report location: 4.2.3.5
Conducting laboratory and location: Department of Safety assessment, GlaxoSmithKline King of Prussia, PA
Date of study initiation: 07 March 2011 (Day 1 dose)
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: GSK2118436, 091236623 (092099) micronized, 99.5% pure

Key Study Findings:

- High-dose 300 mg/kg/day animals showed a reduction in body weight and food consumption.
- A decrease in the number of corpora lutea, implantations, and live fetuses, and an increase in pre- and post-implantation loss were noted at 300 mg/kg/day.
- A decrease in male and female fetal body weight was noted in the 300 mg/kg/day dose group, and in female fetal body weight in the 20 mg/kg/day dose group.
- There was a decrease in female fertility index following 20 and 300 mg/kg/day.
- Additional adverse findings following high-dose dabrafenib included a decrease in the mean number of corpora lutea, implantations, and live fetuses, an increase in pre- and post-implantation loss, and a reduction in fetal body weights.

- Malformations: There was a significant increase in the incidence of ventricular septal defects (% fetuses) in the high dose group.
- Variations: There was an overall increase in skeletal variations at 20 and/or 300 mg/kg/day, which included: Incomplete ossification of numerous structures including the forepaw, skull (mid-dose), sternebrae (high-dose), thoracic vertebrae (mid- and high-dose). Other variations included wavy and knobby ribs (high-dose), and supernumerary ribs (low-dose).

Methods:

Doses:	0, 5, 20, 300 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral Gavage
Formulation/Vehicle:	0.5% HPMC (K15M), 0.1% Tween™ 80
Species/Strain:	Rat / Crl:CD(SD)
Number/Sex/Group:	25 pregnant dams/Group
Satellite groups:	4 pregnant dams/Group
Study design:	Dams were dosed with vehicle or test article by daily oral gavage 2-weeks prior to mating until Day 17 postcoitum (PC). Treated dams underwent up to 14 days of cohabitation with untreated males. Caesarean sections were performed on PC Day 21.

Observations and Results:

Mortality

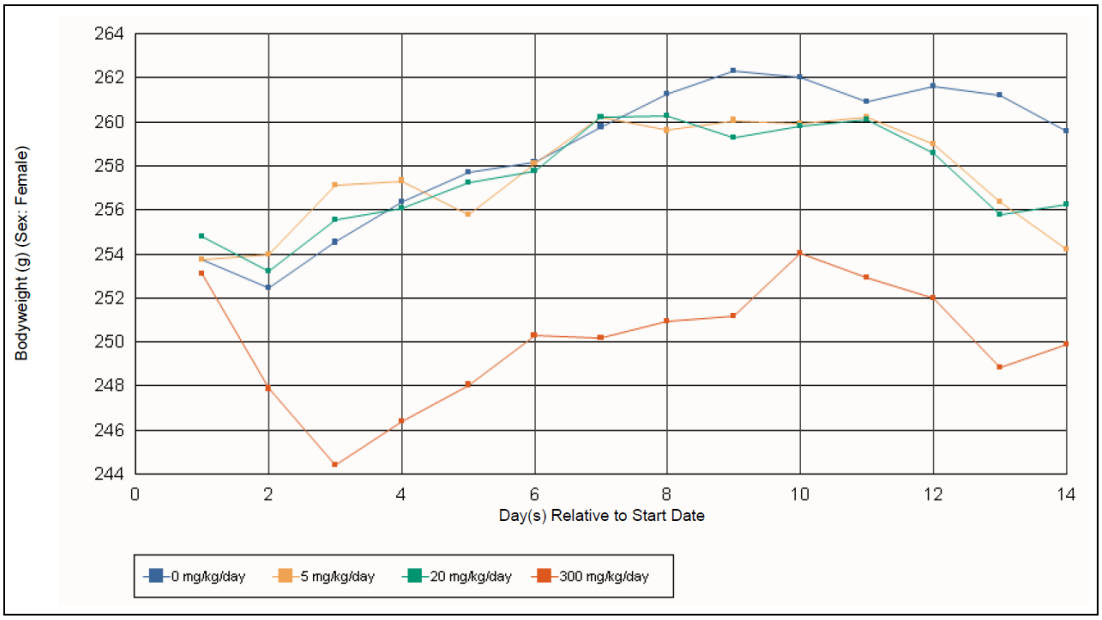
On PC Day 6, one 300 mg/kg/day female was euthanized in extremis (splayed hind limbs, weight loss, dehydration and hyperactivity). On PC Day 15, one control female was euthanized for human reasons (enlargement beneath jaw prevented placement of gavage tube).

Clinical Signs

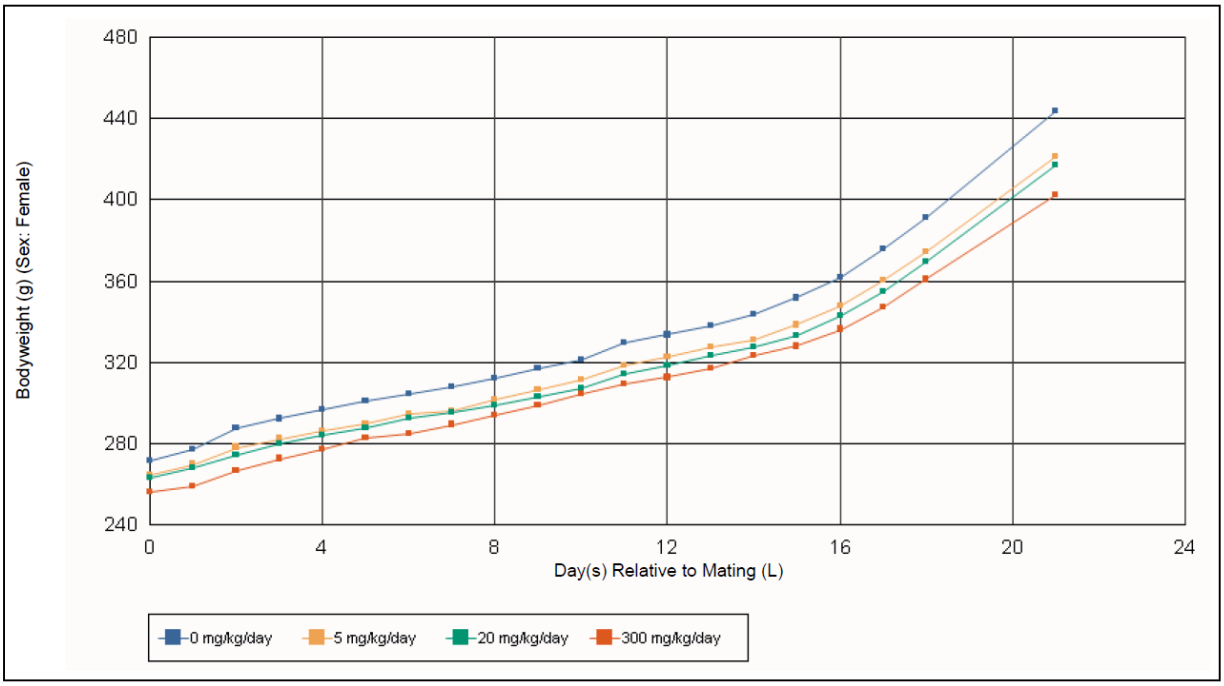
Unremarkable

Body Weight

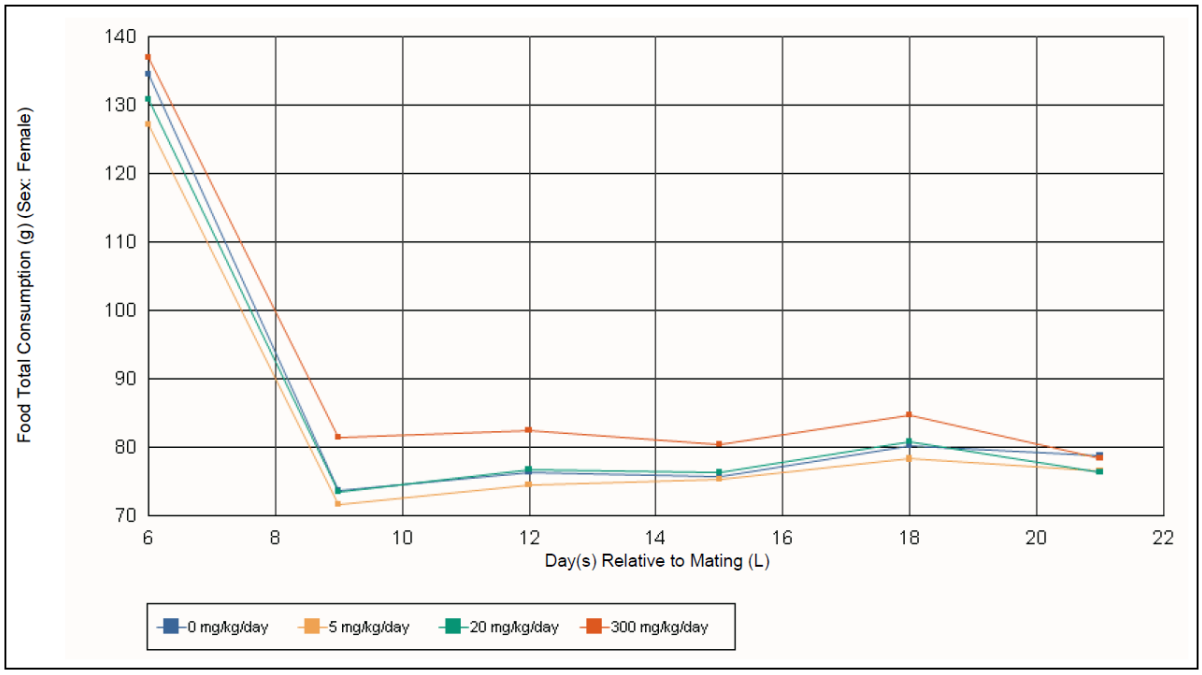
Pre-mating Body Weight Summary



Post-mating Body Weight Summary



Food Consumption



Toxicokinetics

- Fetal concentrations were not evaluated in this study. Therefore, it is unclear whether litter observations were secondary to maternal toxicity.
- Maternal peak and overall (C_{max} and $AUC_{0\text{-}t}$, respectively) exposures in pregnant dams were less than dose-proportional at all timepoints during the study.
- Maternal peak and overall exposures declined on Days 12 and 25 relative to day 1 for all dose groups.
- Maternal exposures (AUC) achieved in this study were up to 3 times those observed clinically at steady-state with the 150 mg BID regimen.

Parameter [a]	Period	Female		
		Dose of GSK2118436 (mg/kg/day)		
		5	20	300
AUC_{0-t} (µg.h/mL)	Day 1	3.93 [3.23 – 4.75]	7.24 [5.62 – 9.84]	38.3 [20.2 – 74.7]
	Day 12	2.04 [1.19 – 2.53]	5.33 [4.35 – 8.03]	16.9 [11.9 – 29.3]
	Day 25[b]	2.62 [1.83 – 3.98]	4.10 [3.44 – 4.95]	22.6 [15.1 – 34.8]
C_{max} (µg/mL)	Day 1	1.18 [0.931 – 1.57]	1.37 [1.08 – 1.77]	3.47 [2.81 – 4.43]
	Day 12	0.677 [0.395 – 0.853]	1.15 [0.859 – 1.33]	2.38 [1.43 – 2.79]
	Day 25[b]	0.765 [0.684 – 0.908]	1.17 [0.672 – 1.47]	2.17 [1.91 – 2.64]
Median T_{max} (h)	Day 1	1.00 [1.00 – 2.00]	1.50 [1.00 – 2.00]	1.50 [0.50 – 2.00]
	Day 12	1.50 [1.00 – 2.00]	2.00 [2.00]	1.00 [1.00 – 2.00]
	Day 25[b]	2.00 [1.00 – 2.00]	2.00 [1.00 – 2.00]	1.00 [0.50 – 2.00]

a. Results are reported as mean unless stated otherwise and [range].
b. Day 10 pc

(table excerpted from Applicant's NDA)

Dosing Solution Analysis

Unremarkable

Reproductive Performance

- Estrus cycle: There were no differences between the groups in the number of estrus cycles observed, nor were there differences between groups in pre- and post-treatment cyclicity.

Study Phase:		-14 to -1 day Pre-treatment Phase	1 to 14 day Treatment Phase
Endpoint		# of time in Estrus	# of time in Estrus
Test Used:		KW	KW
Dose (mg/kg/dag) GSK2118436A			
1	Mean	3.6	3.4
	SEM	0.1	0.1
	N	25	25
2	Mean	3.4	3.6
	SEM	0.1	0.1
	N	25	25
3	Mean	3.5	3.5
	SEM	0.1	0.1
	N	25	25
4	Mean	3.4	3.2
	SEM	0.1	0.1
	N	25	24

- Mating Index: There was no effect of GSK2118436 exposure on mating index at any dose level.

- Fertility Index: There was a decrease in female fertility index at the 20 and 300 mg/kg/day dose level.

Daily Dose (mg/kg/day)	0 (Control)	**5**	**20**	**300**
Mating Index (%)c	100%	100%	100%	100%
Fertility Index (%)d	100%	100%	92.0%	91.7%
No. of Females Mated	25	25	25	24
No. of Pregnant Females	25	25	23	22

Necropsy

Hepatic gross observations in the dams: misshapen liver (described as “tags” on the right posterior and/or median lobes). Whether this finding is sporadic or treatment-related is unclear, as is its toxicological significance.

Liver Tags

	0	**5**	**20**	**300**
Frequency	1/25 (4%)	3/25 (12%)	2/23 (9%)	1/22 (5%)

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

There was a decrease in the number of corpora lutea, the number of implants, and the number of live fetuses at necropsy. There was also a dose-related effect on gravid uterine weights, and in male and female fetal body weights.

Summary of F_0 Reproductive Performance Parameters

Daily Dose (mg/kg/day)	0 (Control)	5	20	300
Mean No. Corpora Lutea	15.9	14.9	14.6	**14****
Mean No. Implantations	15.5	14.4	14.3	**13.2*****
Mean % Pre-implantation Loss	2.24	3.39	2.07	5.08
Mean Total Post-implantation Loss - No. (%)	4.21	7.05	4.89	12.37
Mean No. Live Fetuses	14.9	13.3	13.6	**11.6*****
% Live Males	50.83	53.02	54.3	50.08
Mean Fetal Body Weight (g) Males	5.59	5.60	5.43	**5.34***
Mean Fetal Body Weight (g) Females	5.31	5.24	**5.00***	**4.87***
Gravid Uterus Weight	109.1	**99.6***	**98.5***	**85.4*****

*p < 0.05; **p < 0.01; ***p < 0.001

Offspring

- Visceral Malformations: A statistically significant increase in the number of fetuses with cardiac interventricular septal defects was observed at the highest dose of 300 mg/kg/day, a dose that was maternally toxic.

- Visceral Variations: There were no statistically significant increases in visceral variations among treated fetuses, relative to concurrent control.

- Skeletal Malformations: No skeletal malformations were observed.

- Skeletal Variations: There was a statistically significant increase in skeletal variations, particularly at sites of ossification.

Summary Of Offspring Parameters, Including Visceral and Skeletal Malformations and Variations

Daily Dose (mg/kg/day)	0 (Control)	5	20	300
Fetal Visceral Malformations:				
Cardiac ventricular septal defect				
No. Fetuses (ratio, %)	0	0	0	3 (2.42)*
No. Litters (ratio)	0	0	0	3

Summary Of Offspring Parameters, Including Visceral and Skeletal Malformations and Variations

Fetal Visceral Variation:				
Thymus: Split				
No. Fetuses (ratio, %)	0	0	0	2 (1.52)
No. Litters (ratio)	0	0	0	1
Thymus: Variation in Shape				
No. Fetuses (ratio, %)	1 (0.60)	1 (0.67)	5 (3.11)	2 (1.89)
No. Litters (ratio)	1	1	3	2
Fetal Skeletal Variation:				
Forepaw, Metacarpal- incompletely ossified:				
No. Fetuses (ratio, %)	0	0	0	3 (2.42)*
No. Litters (ratio)	0	0	0	2
Rib, Knobby:				
No. Fetuses (ratio, %)	0	0	1 (0.54)	7 (7.27)**
No. Litters (ratio)	0	0	1	5
Rib, Wavy:				
No. Fetuses (ratio, %)	0	0	0	3 (2.58)*
No. Litters (ratio)	0	0	0	3
Sternebrea, Not ossified:				
No. Fetuses (ratio, %)	0	0	0	2 (1.52)*
No. Litters (ratio)	0	0	0	2
Thoracic Vertebrae, Centrum incompletely ossified:				
No. Fetuses (ratio, %)	0	3 (1.83)	8 (4.96)**	6 (5.84)*
No. Litters (ratio)	0	3	8	6

*p < 0.05; **p < 0.01; ***p < 0.001

10 Special Toxicology Studies

Study title: GSK2118436B: Evaluation of phototoxicity *in vitro* on Balb/c 3T3 fibroblasts using the Neutral Red Uptake assay.

Study no:	2012N141617
Study report location:	4.2.3.7
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 17, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK2118436B, 111342838, 99.7%

Key study findings:
GSK2118436B was phototoxic in the Neutral Red Uptake assay, under the conditions tested.

Methods:

Strains:	Mouse fibroblasts (Balb/c 3T3 clone A31)
Concentrations in definitive study:	0.316, 1, 3.16, 10, 31.6, 100, 316 and 1000 µg/mL, in the presence and absence of UV-A
Basis of concentration selection:	The maximum concentration of the test article did not exceed 1000 µg/mL, 10 mM, or the limit of solubility, as recommended by regulatory guidelines.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	Chlorpromazine (0.1, 1, 10, 100, 1000 µg/mL)
Formulation/Vehicle:	Phosphate buffered saline (PBS) containing 1% dimethyl sulphoxide (DMSO)

Study Design:

Cells were treated with 100 µL of vehicle, untreated control, test article, or positive control solutions and incubated at 37±1°C in the dark for 60 minutes. Following incubation, one plate for each test article, and negative and positive control was irradiated using the UV-A light source for 97 minutes and 30 seconds, to achieve a UV-A dose of 5 J/cm^2. The remaining plates were wrapped in foil and incubated for the same time period. Following treatment, test solutions were removed from the wells, cells were washed with 150 µL PBS, and 200 µL supplemented DMEM was added to each well. The plates were then incubated for 20±2 hours. At the end of the incubation period, cells were briefly examined microscopically for signs of cytotoxicity. Immediately following the visual assessment, 100 µL of Neutral Red solution (50 µg/mL in DMEM) was added to each well. Following a 3 hour incubation, the Neutral Red solution was removed, cells were washed with 150 µL PBS, and 150 µL of Neutral Red destain

solution (ethanol:acetic acid:distilled water, 50:1:49) was added. Plates were shaken for approximately 40 minutes to allow extraction of Neutral Red from the cells. Optical densities (OD) of each well were read on a VERSAmax™ plate reader, at a wavelength of 540 nm. Neutral Red absorbances were expressed in terms of absolute optical density (OD540).

Analysis:

Where possible, the concentration of the test article inducing a 50% inhibition of Neutral Red uptake (IC_{50} value) was calculated for the test article and the positive control, using a validated software system. The OD540 was measured for each concentration of the test article and positive control and was compared to the OD540 of the negative control(s), as appropriate.

If suitable concentration-response profiles were obtained for the test article in the presence and absence of UV-A light, a Photo-irritation Factor (PIF) was calculated according to the following equation:

$$PIF = \frac{IC_{50} \text{ in the absence of UV-A}}{IC_{50} \text{ in the presence of UV-A}}$$

If a chemical was only cytotoxic after exposure to UV-A light, but was not cytotoxic when tested in its absence, the PIF could not be accurately calculated. In such cases where an IC_{50} value could be calculated in the presence of UV light but not in the absence of UV light, the phototoxic potential of the test article was demonstrated from the determination of the Mean Photo Effect (MPE), as outlined in the OECD Guideline for Testing of Chemicals: 432 In Vitro 3T3 NRU phototoxicity test, April 2004.

Criteria for Positive Results:

Results were considered positive (phototoxic) for test-article if PIF values of > 5 or MPE values of >0.15 were obtained.

Study Validity:

- Irradiated vehicle controls showed a viability of approximately 80% of the non-irradiated vehicle control.
- OD540 in the untreated unirradiated controls > 0.4.
- The positive controls showed a clearly cytotoxic response in the presence of UV-A light, compared to the response seen in the absence of UV-A light, such that the PIF for the positive control was > 6.

Results:

According to the Applicant, the highest concentration (1000 µg/mL) of GSK2118436 was excluded from analysis due to precipitation in the absence and presence of UV-A.

Chlorpromazine induced an acceptable positive response with a PIF value of 61.485. In the untreated un-irradiated controls, OD540 values were greater than 0.4. The irradiated vehicle control showed a viability of approximately 80% of the non-irradiated vehicle control. The assay was therefore considered valid.

Treatment of cultures with GSK2118436 resulted in a decrease in cell survival, both in the absence and in the presence of UV-A light. Cytotoxicity, as indicated by a decrease in Neutral Red uptake, was observed at the highest three concentrations analyzed in the absence of UV-A (31.6 to 316 µg/mL) and all concentrations analyzed in the presence of UV-A (0.316 to 316 µg/mL). The IC_{50} value of GSK2118436 in the absence of UV-A was 26.076 µg/mL. Due to toxicity observed at all concentrations of GSK2118436 in the presence of UV-A, the IC_{50} value could not be reliably calculated and was therefore considered <0.316 µg/mL, the lowest concentration tested. Accordingly, the PIF value was >83, indicating the test article is phototoxic.

IC_{50} and PIF values

Test article	IC_{50} absence of UV-A (µg/mL)[1]	IC_{50} presence of UV-A (µg/mL)[1]	PIF Value
GSK2118436B	26.076	<0.316[2]	>83[3]
Chlorpromazine	48.327	0.786	61.485[4]

1. Concentrations expressed in terms of parent compound.
2. Lowest concentration tested in the assay
3. PIF > 5 indicates a positive phototoxicity response for the test article.
4. PIF > 6 indicates a positive phototoxicity response for the positive control

(table excerpted from Applicant's NDA)

11 Integrated Summary and Safety Evaluation

GlaxoSmithKline submitted a New Drug Application (NDA) for Dabrafenib (Tafinlar™) in patients with unresectable or metastatic melanoma with BRAF V600 mutation as detected by an FDA approved test.

The inhibitory activity of dabrafenib against RAF family kinases was characterized *in vitro* and *in vivo*. Dabrafenib inhibited human wild-type BRAF and CRAF kinases with IC_{50} values of 3.2 and 5.0 nM, respectively, as well as some mutant forms of BRAF kinases. Specifically, dabrafenib inhibited BRAFV600E, BRAFV600K, and BRAFV600D kinases with IC_{50} values of 0.65, 0.5 and 1.84 nM, respectively. Based on additional analyses to characterize the mechanism of action, dabrafenib appeared to be a time-dependent, reversible, ATP-competitive inhibitor of BRAF kinases. Other kinases inhibited at clinically achievable concentrations include CK1, SIK1, NEK11, ALK5, and LIMK1.

The ability of dabrafenib to decrease the phosphorylation of extracellular signal regulated kinase (ERK) and mitogen extracellular signal regulated kinase (MEK), both downstream targets of RAF kinases, was assessed in cell lines containing wild-type

BRAF, BRAFV600E, and RAS mutants. Dabrafenib caused a concentration-dependent decrease in phosphorylated ERK (pERK) and phosphorylated MEK (pMEK) (IC_{50} = 3 nM), with no change in total ERK and total MEK, in ES-2 human ovarian carcinoma cells encoding a BRAFV600E mutation. Dabrafenib-induced inhibition of pERK was also demonstrated in other BRAFV600E containing cell lines. Cell lines carrying wild-type B-Raf were less sensitive to dabrafenib-induced inhibition of pERK, regardless of Ras status, suggesting that BRAFV600E cells are more responsive to BRAF inhibition by dabrafenib. Dabrafenib-induced inhibition of pERK also appeared to correlate with the anti-proliferative activity of dabrafenib in a human melanoma cell line carrying the BRAFV600E mutation, SK-MEL-28 cells.

The anti-proliferative activity of dabrafenib was examined across a panel of 110 human tumor cell lines, each with confirmed BRAF mutational status. Dabrafenib inhibited (gIC_{50} <100 nM) proliferation of 73% of the BRAFV600E containing cell lines and showed little to no activity against all other cancer cell lines tested. Sensitivity to dabrafenib significantly correlated with BRAFV600E presence (p = 3x10-14), but did not extend to other BRAF mutants or cell lines containing activated Ras. A total of four cell lines containing the BRAFV600E mutation (RKO A673, GCT, and NCI-H292) were insensitive to GSK2118436A. These BRAFV600E containing insensitive cell lines were derived from colon carcinoma, sarcoma, and lung tissue, respectively. Based on these data, the patient population most likely to receive clinical efficacy from dabrafenib are patients with melanoma.

The *in vivo* efficacy of dabrafenib was examined in four repeat-dose animal studies utilizing CD-1 *nu/nu* mice bearing A375P F11s tumor xenografts. Mice carrying A375 melanoma xenografts were used since these cells contain BRAFV600E and have shown an *in vitro* sensitivity to dabrafenib. All four studies demonstrated a dose-dependent increase in tumor growth inhibition following dabrafenib administration.

Safety pharmacology studies included an *in-vitro* evaluation of hERG (human ether-á-go-go-related gene) channel interaction, a functional observational battery (FOB) neurological assessment in rats, a respiratory safety pharmacology study in rats, and a cardiovascular safety pharmacology study in dogs. No dabrafenib-induced adverse effects were seen in any of these studies. Dabrafenib was a weak hERG blocker (IC_{25} on hERG potassium current was 11.7 μM), with a low potential to induce QT prolongation.

In regard to pharmacokinetics, dabrafenib exhibited high oral bioavailability in animals (46-82%), moderate- to low-clearance, particularly in dogs (< 12% hepatic blood flow), and strong protein binding (>98% in all species tested, including human). *In vivo* distribution studies indicated that dabrafenib was widely distributed to most major organs. Elimination of dabrafenib in humans occurs predominantly via the fecal route (~71% of administered dose) and urine (~22%). This elimination profile is distinct from both nonclinical species, which exhibited little (~ 1% or less) or no urinary elimination of dabrafenib.

Dabrafenib was a moderate inhibitor of CYP3A4, CYP2C9, and CYP2C19, a weak inhibitor of CYP1A2 and CYP2D6, and a substrate of human P-glycoprotein. The three main dabrafenib metabolites in humans were GSK2285403 (M7; hydroxyl-dabrafenib), GSK2298683 (M4; carboxy-dabrafenib), and GSK2167542 (M8; desmethyl-dabrafenib). Following administration of the recommended twice daily 150 mg dose of dabrafenib, these three metabolites were present at human plasma levels (AUC_{0-24}) of approximately 8, 100, and 6 µg*h/mL, respectively. All three metabolites were also present in rats and dogs during the 13-week repeat-dose toxicity studies. Specifically, animals tolerated GSK2285403 at plasma levels (AUC_{0-24}) ≥ 4 times the level of human exposure following the recommended dose of dabrafenib. At the maximum tolerated dose in animals, GSK2167542 and GSK2298683 plasma exposure levels (AUC_{0-24}) were up to approximately 30% and 50% of human exposure levels, respectively. Based on these data and the indicated advanced cancer patient population, the safety of these metabolites presents a minimal risk.

There are four unique human metabolites denoted M28, M29, M30, and M31 (see appendix). Consequently, both the pharmacological and toxicological properties of these metabolites remain uncharacterized. However, given that these are minor metabolites by virtue of overall exposure level (generally < 1% of administered dose) and the indicated advanced cancer patient population, further evaluation of these metabolites is not required at this time.

The toxicity of repeated daily doses of oral dabrafenib was assessed by conducting 4- and 13-week toxicity studies in rats and dogs. Rats and dogs were considered relevant species for use in toxicity studies since dabrafenib showed similar inhibitory activity against wild-type BRAF in humans, rats, and dogs.

In rats, the main target organs of toxicity were the skin, male reproductive organs, and stomach. In the 13-week toxicity study, rats were orally administered 0, 20, 200, or 400 mg/kg/day of dabrafenib. Plasma exposure levels (AUC_{0-24}) in this study were equivalent to approximately 0, 1, 2, and 2.5 times the exposure level in humans (AUC_{0-24} of 8.7 µg*h/mL) receiving the recommended dose of dabrafenib, respectively. Rats tolerated up to 200 mg/kg/day of dabrafenib. A dose-responsive increase in the incidence of skin lesions (inflamed, dry, red, scabbing, and flaking), was seen on the paws. Skin papules were also observed on the forepaws of one 400 mg/kg/day high-dose male. Skin toxicity corresponded with histopathological hyperkeratosis. Degeneration/depletion in the testis and aspermia in the epididymis was also noted in 100% of dabrafenib-treated male rats, with dose-dependent increases in severity of the findings. Stomach toxicity consisted of histopathological hyperplasia, epithelial down-growth, and infiltration at all doses tested. Epithelial hyperplasia of the forestomach of mice and rats and other tissues, including esophagus, urinary bladder and renal pelvis, has been reported with other RAF inhibitors (Carnahan et al., 2010; Wisler et al., 2011; see appendix). Development of proliferative skin and epithelial forestomach lesions in animals is considered to be pharmacologically-mediated as RAF inhibition can enhance cell growth in wild-type BRAF harboring cells with subsequent paradoxical activation of RAS/RAF/MEK/ERK pathway signaling (Carnahan et al., 2010; see appendix).

In dogs, the main target organs of toxicity were the skin, male reproductive organs, and heart. In the 13-week toxicity study, dogs were orally administered 0, 5, 20, or 60 (males)/100 (females) mg/kg/day of dabrafenib. Plasma exposure levels (AUC_{0-24}) in this study were equivalent to approximately 0, 2, and 11 times (exposure levels for 60/100 mg/kg/day animals were not obtained) the exposure level in humans receiving the recommended dose of dabrafenib, respectively. Dogs tolerated up to 20 mg/kg/day of dabrafenib. Skin lesions and papules were observed at all doses on various areas including the muzzle, pinna, lower jaw, inguinal, scrotum, and ventral thoracic. Histopathological correlates included acanthosis, infiltration, and erosions/crust. Additional toxicities included dose-responsive lymphoid depletion in the thymus and inflammation in the lungs. Degeneration/depletion in the testis and aspermia in the epididymis was noted in 100% of male dogs, irrespective of dose. Partial granular development was also noted in the prostate in dogs administered 20 mg/kg/day of dabrafenib.

Heart toxicity in dogs consisted of marked hypertrophy of the right atrioventricular valve in 1 of 6 dogs administered 50 mg/kg/day for 4 weeks. This adverse change was characterized by focal hemorrhage with fibrin deposition in the valve. Hemorrhage of the atrioventricular value was also noted in the 13-week toxicity study in 1 of 8 dogs at 20 mg/kg/day and 3 of 8 dogs at 60 (males) and 100 (females) mg/kg/day. Toxicities seen in the heart occurred at plasma levels (AUC_{0-24}) ≥ 5 times human exposure following the recommended dose of dabrafenib.

Skin toxicity in rats and dogs was considered clinically relevant since these toxicities occurred in animals at plasma levels (AUC_{0-24}) equivalent to human exposure levels following the recommended human dose of dabrafenib. As expected, based on the results of human clinical trials, the most common adverse reactions to dabrafenib included hyperkeratosis, skin papilloma, and rash. Male reproductive toxicity is also expected to manifest itself in humans at the recommended dose of dabrafenib and was noted in the Tafinlar™ label.

Dabrafenib was not mutagenic *in vitro* in the bacterial reverse mutation assay (Ames test) or the mouse lymphoma assay, and was not clastogenic in an *in vivo* rat bone marrow micronucleus test.

Dabrafenib was evaluated in a combined fertility and embryo-fetal study in Sprague-Dawley rats at daily oral doses of 0, 5, 20, or 300 mg/kg/day. Plasma exposure levels (AUC_{0-24}) were up to 3 times the exposure level in humans receiving the recommended dose of dabrafenib. High-dose dabrafenib exposure was associated with cardiac malformations in developing fetuses (cardiac ventricular septal defects), and a number of visceral and skeletal malformations, including misshapen or split thymuses and decreased skeletal ossification. Additional adverse findings following high-dose dabrafenib included a decrease in the number of corpora lutea, implantations, and live fetuses, an increase in pre- and post-implantation loss, and a reduction in fetal body weights.

In vitro, dabrafenib was phototoxic. However, a low incidence of photosensitivity reactions in clinical studies suggests a minimal risk to cancer patients.

In conclusion, the non-clinical studies support the use of dabrafenib (Tafinlar™) in patients with unresectable or metastatic melanoma with BRAF V600 mutation as detected by an FDA approved test. From a Pharmacology/Toxicology perspective, approval for dabrafenib is recommended.

12 Appendix/Attachments

The Applicant submitted additional data from the published literature relevant to the safety of dabrafenib.

Study title: Carnahan J, et al. Selective and potent Raf inhibitors paradoxically stimulate normal cell proliferation and tumor growth. *Molecular Cancer Therapeutics,* 2010; 9:2399-410.

Summary:
It is well established that B-Raf is an activator of the mitogen-activated-protein kinase (MAPK) pathway, thereby controlling cell proliferation and differentiation. Mutations in B-Raf can lead to aberrant activation of MAPK signaling and result in unregulated cell proliferation and cancer. Cell lines and tumors harboring mutant B-Raf are sensitive to Raf inhibition, as demonstrated by the clinical efficacy of the Raf inhibitors sorafenib, vemurafenib, and dabrafenib. In this study, Carnahan, et al., showed that in contrast to cell lines and tumors harboring mutant B-Raf, exposing those harboring wild-type B-Raf or non-Raf MAPK pathway mutations to Raf inhibitors can result in a dose-dependent and sustained activation of MAPK signaling. In some cell lines, Raf inhibition led to entry into the cell cycle, enhanced proliferation, and significantly stimulated tumor growth in human xenograft models. These paradoxical effects of Raf inhibition were also seen in normal cells *in vivo*. For example, 7-day, twice daily, administration of an oral Raf-inhibitor to mice resulted in treatment-induced hyperplasia of normal epithelial cells in the esophagus and the stomach. This finding was consistent with findings in rats treated with dabrafenib in 4 and 13-week studies. Based on these results, Raf inhibition may induce unexpected proliferation in normal cell and tumor tissue in patients treated with B-Raf inhibitors including dabrafenib.

Study title: Wisler JA et al. Raf inhibition causes extensive multiple tissue hyperplasia and urinary bladder neoplasia in the rat. *Toxicologic Pathology,* 2011; 39:809-22.

Summary:
In this study, seven Raf kinase inhibitors (CI-7) were evaluated in rat toxicity studies. Specifically, rats received vehicle or test article by oral gavage, once daily, for 7

consecutive days; C-1 doses were 30, 100, or 300 mg/kg (five rats/sex/group); C-2 doses were 30, 100, or 300 mg/kg (three rats/group); C-3: 30, 150, or 500 mg/kg (three/group); C-4: 300 mg/kg (two rats); C-5: 80 mg/kg (three rats); C-6: 30 mg/kg (three rats); and C-7: 150 mg (three rats). According to the authors, these dose levels were selected to ensure coverage and inhibition of the B-Raf target and to explore the toxicological profile of each molecule.

As shown in the figure below, all compounds tested induced hyperplasia in multiple tissues. C-1 and C-6 induced hyperplasia at doses ≥ 30 mg/kg/day. Consistently affected was stratified squamous epithelium at a number of sites and transitional epithelium of the urinary bladder and kidney. A 7-day time course study in rats showed morphologic evidence of epithelial proliferation in the nonglandular stomach within four to five hours of a single dose of C-1. Similar indications of cellular proliferation were observed in the urinary bladder by Day 2 and in the heart, kidney, and liver by Day 3. Transcriptional evidence of proliferation in the urinary bladder was detected within four to five hours after a single dose of C-1 consistent with activation of the PI3K/AKT and ERK/MAPK pathways. In a 28-day rat toxicity study of C-1, hyperplasia was observed in the esophagus, nonglandular stomach, skin, urinary bladder, kidney, and heart. Hyperplasia of transitional epithelium of the urinary bladder was particularly severe and, in one female rat, was accompanied by the presence of a transitional cell carcinoma. Though dabrafenib was not included as a test article in these experiments, the results of this study suggest that Raf inhibitors can induce early transcriptional changes driving unregulated cell proliferation, resulting in marked tissue hyperplasia capable of progression to carcinoma.

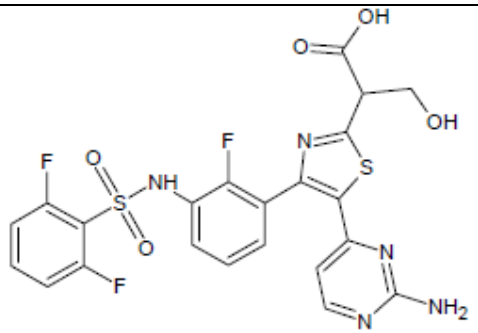
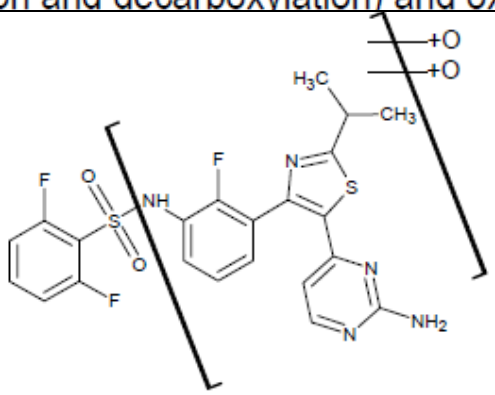
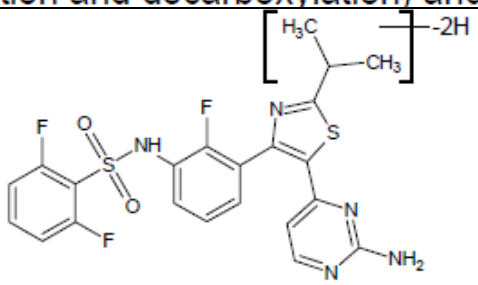
Histological Findings of Cellular Proliferation from Seven-Day Studies of B-Raf Inhibitors in Rats

Compound	C-1			C-2			C-3			C-4	C-5	C-6	C-7
Dose (mg/kg/day)	30	100	300	30	100	300	30	150	500	300	80	30	150
Stomach (nonglandular): hyperplasia/hyperkeratosis, epithelium	+	+	+	—	—	—	—	—	+	+	+	+	+
Esophagus: hyperplasia/hyperkeratosis, epithelium	+	+	+	—	—	—	—	—	+	+	+	—	—
Footpad: hyperplasia, epidermis	NE	NE	—	—	—	+	—	—	—	+	+	+	+
Skin (tail), hyperplasia, epidermis	—	—	—	NC	—	+	—	—	+	—	—	—	—
Urinary bladder: hyperplasia, epithelium	—	+	+	NC	NC	NC	NE	NE	—	+	+	—	+
Kidney (pelvis): hyperplasia, epithelium	—	+	+	NE	NE	—	NE	NE	—	+	+	—	+
Heart: hyperplasia (endocardium and interstitial cells of myocardium)	+	+	+	NE	NE	—	NE	NE	—	+	+	+	—
Sciatic nerve: hyperplasia (fibroblasts or Schwann cells)	+	+	+	NC	NC	NC	NE	NE	—	+	+	+	—

NA, not applicable; NC, not collected; NE, not examined. —, change not present; +, change present.

(table excerpted from literature)

Unique Human Metabolites of Dabrafenib

M30	 (Oxidation and decarboxylation) and oxidation
M28 M29 (co-eluting species)	 (Oxidation and decarboxylation) and oxidation
M31	 (Oxidation-decarboxylation), loss of 2 H

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

/s/

ALEXANDER H PUTMAN
04/11/2013

SHAWNA L WEIS
04/11/2013

WHITNEY S HELMS
04/11/2013

JOHN K LEIGHTON
04/11/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 202806 **Applicant:** GlaxoSmithKline **Stamp Date:** 7/30/2012

Drug Name: Dabrafenib **NDA Type:** Standard

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		Appears to be acceptable.
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		Appears to be acceptable.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		Appears to be acceptable.
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)		X	This is a review issue.
11	Has the applicant addressed any abuse potential issues in the submission?	Not applicable		
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?	Not applicable		

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? YES

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

- None identified or anticipated.

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

/s/

ALEXANDER H PUTMAN
08/31/2012

WHITNEY S HELMS
08/31/2012