CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

204114Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Mekinist (Trametinib)

Date: April 25, 2013
To: File for NDA 204114
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 Mekinist conducted by Drs. Khasar, Weis and Brower and secondary memorandum and labeling provided by Dr. Helms. I concur with Dr. Helms' conclusion that Mekinist may be approved and that no additional studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
04/25/2013

MEMORANDUM

Date: April 19, 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 #204114
Trametinib (MEKINIST)
Re: Approvability of Pharmacology and Toxicology

Non-clinical studies examining the pharmacology and toxicology of trametinib provided to support NDA 204114 for the treatment of patients with BRAFV600E/K mutation positive (as detected by an FDA approved test) unresectable or metastatic melanoma were reviewed in detail by G. Sachia Khasar Ph.D., Shawna L. Weis, Ph.D., and Margaret Brower, Ph.D. The submission included studies of orally administered trametinib in mice, rats, dogs, and rabbits 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 trametinib is an inhibitor of MEK1 and MEK2 activation and downstream activity. MEK1 and MEK2 are ubiquitously expressed proteins that participate in the MAPK/ERK signal transduction cascade. MEK proteins propagate signals between the small GTPase Ras, its downstream immediate effector Raf and the extracellular signal-regulated kinases (ERK1/2). Significant trametinib-mediated inhibition was not detected in a panel of over 40 other kinases. In an *in vitro* experiment with MEK1, trametinib showed a mixed pattern of inhibition with ATP; this pattern is consistent with a trametinib binding site that is distinct from the ATP binding site for MEK. Trametinib inhibited the *in vitro* proliferation of multiple cell lines carrying BRAFV600E or V600K mutations as well as some cell lines with Ras mutations. Trametinib also inhibited tumor growth and levels of phosphorylated ERK in an *in vivo* mouse model implanted with a melanoma line harboring the BRAFV600E mutation.

Trametinib was not highly metabolized by CYP isoenzymes. Trametinib was metabolized primarily by deacylation, demethylation, ketone formation, mono-oxygenation and glucuronidation. One metabolite did demonstrate activity similar to that of the parent compound, however, the clinical exposure to this metabolite was small and it was detectable in animals as well as humans.

Trametinib was negative in assays for genotoxicity. While several potentially genotoxic substances were identified in the [redacted] (b) (4) for trametinib, those substances were controlled during the manufacturing of trametinib and a specification for each of the three impurities was set at [redacted] (b) (4). At the recommended dose of trametinib of 2 mg/day, the specification of [redacted] (b) (4) would result in a clinical dose for each of these impurities of [redacted] (b) (4) At this level the impurities either alone or in combination are below a threshold of toxicological concern. During the course of development two [redacted] (b) (4) impurities were also identified. Levels of these impurities exceeded the specification limit of [redacted] (b) (4) in

(b) (4). Both (b) (4) were screened for genotoxicity using the Derek *in silico* prediction tool. GSK26426258A was positive in this screen, but was negative in a subsequent Ames test. GSK2646257A was not identified as a potential positive in the screen. Derek alone is not considered a sufficient screen for the genotoxic potential of impurities; a second *in silico* screen will be requested for GSK2646257A.

Sprague Dawley rats and Beagle dogs were the primary models used to investigate the toxicology of trametinib. The major target organ for toxicity in both rats and dogs was the skin. Skin toxicities are the most broadly reported adverse events noted clinically as well. Gastrointestinal and hepatic toxicity were noted in both species as well. In rats there were findings of adrenal toxicity and myeloid hyperplasia and degeneration/necrosis in the bone marrow. Bone marrow toxicity correlated with increases in neutrophils and monocytes along with decreases in lymphocytes and red blood cells. Ovarian cysts and decreases in the number of corpora lutea were noted in rats during the 13 week study, including marked decreases in corpora lutea even at doses resulting in exposures less than 0.3 times the human exposure at the recommended dose. The kidney was also identified as a potential target organ in a 4-week rat study with dose dependent increases in serum calcium and increases in urinary protein levels. Renal failure has been reported clinically.

Similarly to the rat, dogs treated with trametinib showed skin, GI, and liver toxicity. In the 13-week dog study, the lungs were also identified as a potential target organ for trametinib-mediated toxicity with findings of pale, raised, or dark areas corresponding with histopathological findings of minimal hemorrhage, mononuclear infiltration, pleural fibrosis, and macrophage accumulation. All findings in the lung were classified as minimal to mild; however, clinically, treatment with trametinib has been associated with cases of interstitial lung disease and pneumonitis.

Ocular toxicity similar to the serous retinopathy and retinal vein occlusion seen clinically has not been well-studied in nonclinical models. Opthalmalogic examinations typically included in general toxicology studies do not appear to predict this toxicity. No clear signs of ocular toxicity were identified in the nonclinical studies submitted to support the trametinib NDA.

Based on the results of *in vitro* hERG testing (IC_{50} values > 1 μM) and *in vivo* ECG monitoring in dogs there does not appear to be a strong potential for trametinib-mediated QT prolongation at clinically relevant exposures; however, other types of cardiac toxicity do appear to be associated with trametinib exposure. In an *in vitro* rabbit heart wedge model there was a decrease in contractility noted at high concentrations of trametinib. Further characterization of potential cardiac toxicity was performed in mice. At trametinib exposures exceeding the clinical exposure at the recommended dose, mice presented with decreases in mean heart rate and mean heart weight along with decreases in left ventricular function. Cardiomyopathy characterized as cardiac failure, left ventricular dysfunction, or decreased ejection fraction has been described clinically in studies of trametinib and is included in the label for the drug.

Reproductive toxicity studies conducted for trametinib consisted of embryofetal development studies conducted in Sprague Dawley rats and Dutch-Belted rabbits. More significant signals for

embyrofetal risk were observed in rabbits. At a 2 mg/m^2 dose level that resulted in maternal exposures of approximately 0.3 the human exposure at the recommended dose there were increases in post-implantation loss, incomplete ossification of bones, cleft palate, and markedly enlarged anterior fonticulus. At the same dose or greater there were increases in total pregnancy loss as well. Decreased fetal body weight was observed at all dose levels tested. Maternal toxicity in rabbits consisted primarily of decreased body weight gain at the 2 mg/m^2 dose level and occurred only after dosing had ended for this study. Based on these studies and its mechanism of action, Pregnancy Category D is recommended for trametinib.

Recommendations: I concur with the conclusion of Drs. Khasar, Weis, and Brower that the pharmacology and toxicology data support the approval of NDA 204114 for MEKINIST. There are no outstanding nonclinical issues that would prevent the approval of MEKINIST for the proposed indication in the BRAF$^{V600E/K}$ positive patient population.

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/s/

WHITNEY S HELMS
04/19/2013

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:	204114
Supporting document/s:	2
Applicant's letter date:	08/02/2012
CDER stamp date:	08/03/2012
Product:	Trametinib (GSK1120212)
Indication:	Patients with unresectable or metastatic melanoma with BRAF V600 mutations
Applicant:	Glaxosmithkline, LLC 1250 South Collegeville Rd Collegeville, PA 19426
Review Divisions:	Division of Hematology Oncology Toxicology (DHOT) Division of Drug Oncology Products 2 (DOP 2)
Reviewers:	G. Sachia Khasar, PhD; Shawna Weis, PhD; Margaret Brower, PhD.
Supervisor/Team Leader:	Whitney Helms, PhD.
Division Directors:	John Leighton, PhD., DABT (DHOT) Patricia Keegan, MD. (DOP 2)
Project Manager:	Norma S. Griffin

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Reference ID: 3293647

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Reference ID: 3293647

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1 Executive Summary

1.1 Introduction

GlaxoSmithKline has submitted this New Drug Application for trametinib (Mekinist™) in support of the treatment of patients with unresectable or metastatic melanoma with a BRAF V600 mutation as detected by an FDA approved test. MEK1 and MEK2 are ubiquitously expressed proteins that participate in the Ras-Raf-MAPK-ERK signal transduction cascade. Mutations resulting in constitutively active BRAF proteins such as BRAF V600E lead in turn to continuous (b) (4) activation of MEK. Trametinib (GSK1120212) is a reversible inhibitor of both MEK activation and the activity of phosphorylated MEK (pMEK; activated MEK). Mekinist is formulated as a tablet that is intended for clinical use by oral administration once daily at a dose of 2 mg.

1.2 Brief Discussion of Nonclinical Findings

GlaxoSmithKline has submitted studies evaluating the pharmacology, pharmacokinetics, and toxicology of trametinib. *In vitro* trametinib was shown to block BRAFV600E catalyzed MEK1/MEK2 activation (IC_{50} values of 0.7 and 0.9 nM, respectively) by binding to unphosphorylated MEK1/MEK2, to inhibit the kinase activity of phosphorylated MEK1/MEK2 (IC_{50} values of 13.2 and 10.7 nM, respectively), and to inhibit the phosphorylation of ERK (IC_{50} values 3.5 to 11 nM). Trametinib also inhibited the growth of BRAF mutant melanoma cell lines *in vitro* and of BRAF mutant tumor xenografts in mice. Inhibition of tumor growth was coupled with a reduction in phosphorylation of ERK.

GSK1120212 was widely distributed in a variety of tissues and exhibited high plasma protein binding in all species tested: 95% in the mouse, 96% in the rat, 97% in the dog, 98% in the monkey, and 97% in humans. Penetration in the brain was low but detectable following even a single dose of trametinib at concentrations of approximately 10% of those found in plasma. Metabolism of GSK1120212 occurred primarily by deacylation, demethylation, ketone formation, mono-oxygenation and glucuronidation. At least one metabolite, designated M5, or GSK1790627, demonstrated pharmacological activity, inhibiting ERK phosphorylation in BRAFV600E mutant cell lines. The predominant route of elimination was via the feces (total 70-93%).

The Applicant used Sprague-Dawley rats and Beagle dogs as the primary models for toxicological assessment of trametinib. In a GLP-compliant 13-week repeat-dose study, male rats were administered trametinib (GSK1120212) at doses of 0, 0.25, 0.5 and 1.0 mg/m^2 while female rats were administered trametinib at doses of 0, 0.125, 0.25, 0.5 mg/m^2, daily by oral gavage. Differences in the dose levels of male and female rats were due to much higher levels of trametinib exposure observed in female rats. The highest exposures measured by AUC during this study in female rats at the 0.5 mg/m^2 dose level and male rats at the 1 mg/m^2 dose level were approximately 287 ng·h/mL, around 0.77 times the human exposure at the recommended daily dose of 2

mg. Target organs of toxicity in rats included the skin, gastrointestinal (GI) tract, lymphoid and hematopoietic organs, liver, and adrenal gland. Male and female animals treated at the high dose (1 mg/m^2 for males, 0.5 mg/m^2 for females) were euthanized early due to severe skin and GI tract toxicities, including acanthosis, ulcerations/erosions, inflammation, and stomach erosion. Serious skin toxicity has been seen in patients treated with trametinib along with gastrointestinal disorders. Dose-dependent increases in neutrophils were present at all dose levels in both male and female rats throughout the treatment period, but were beginning to resolve during the recovery period. Decreases in the number of corpora lutea and the presence of ovarian cysts were observed at all doses, ≥0.125 mg/m^2, suggesting that trametinib has the potential to impair fertility in humans. Finally, in a 4-week study, the kidney was identified as a potential target organ for trametinib with dose dependent increases in calcium in the serum and increases in urinary protein levels at the high dose level of 1 mg/m^2. Renal failure has been reported clinically.

Beagle dogs were administered up to 0.6 mg/m^2 orally daily in a 13-week toxicology study but required dose reduction to 0.45 mg/m^2 by Day 15 due to toxicity. Exposures at the maximum dose level in dogs in this study were all less than half the human exposure at a daily clinical dose of 2 mg measured by AUC (370 ng·h/mL). Similar to the rat, major target organs of toxicity in dogs included the skin and GI tract. In addition the lymph nodes and lungs were target organs in this study. Gross findings of pale, raised, or dark areas in the lungs corresponded with histopathological findings of minimal hemorrhage, mononuclear infiltration, pleural fibrosis, and macrophage accumulation. All findings in the lung were classified as minimal to mild; however, clinically, treatment with trametinib has been associated with cases of interstitial lung disease and pneumonitis.

ECG monitoring was included in the 13-week dog toxicology study and in separate single dose studies in both anesthetized and conscious dogs. No treatement-related effects were observed. *In vitro*, trametinib inhibited hERG channel activity in a concentration dependent manner in assays conducted on CHO-K1 and HEK293 cells but with IC_{50} values of 3.7 µM and 1.54 µM, respectively, suggesting low potential for causing QTc prolongation at physiologically relevant levels. Trametinib had no effect on QT prolongation when tested in the rabbit left ventricular wedge preparation though there was a decrease in contractility in wedges at high concentrations of the drug. In an attempt to further characterize the cardiac toxicity noted during the clinical development of trametinib, the Applicant conducted a study using male mice administered GSK1120212B at dose levels of 0.25 and 0.5 mg/kg given by oral gavage once daily for 21 days. Trametinib-dependent decreases in mean heart rate and lower mean absolute and relative heart weights were observed, regardless of dose level. In addition, trametinib-treated mice had lower left ventricular functional parameters, though the contractility response to dobutamine was preserved in these animals. The decrease in left ventricular function was similar to that reported in humans though mice tolerated trametinib at exposures higher than either humans or other animal species tested and the cardiac findings in this study occurred at exposures 3- to 7-fold higher than the clinical exposure at the recommended dose.

Reproductive toxicology of trametinib was assessed in embryofetal development studies using pregnant Sprague-Dawley rats and pregnant Dutch Belted rabbits. Rats were administered GSK1120212 up to 2.86/1.0 mg/m^2 (loading dose/maintenance dose). Exposure to trametinib in rats at the 2.8.6/1.0 mg/m^2 dose level was approximately 1.9 times the human exposure at the recommended dose. Maternal toxicity at this dose level consisted of skin findings and decreased body weight along with decreased food consumption. Fetal toxicity evidenced by increased post-implantation loss, decreased fetal body weight (approximately 20%) compared to fetuses from both control and mid dose group animals, and increased observations of delayed ossifications and great vessel malformations (missing inominate) were evident at this dose. Mild increases in post-implantation loss in treated dams compared to untreated controls along with decreases in fetal body weight were also evident at lower dose levels in rats in the absence of clear maternal toxicity.

In rabbits at the high dose of 4/2 mg/m^2 which resulted in exposures approximately 0.3 times the exposure in humans at the recommended dose of 2 mg, fetal toxicity was evidenced by increased post-implantation loss, increased incidence of incomplete ossification of bones, increased incidence of cleft palate, and markedly enlarged anterior fonticulus. Doses of 4/2 mg/m^2 or greater also resulted in increases in loss of pregnancy. Reduced fetal body weight was reported at all dose levels. There were no clear signs of maternal toxicity in rabbits. Pregnancy Category D is recommended.

Trametinib was negative for genotoxicity in *in vitro* and *in vivo* assays.

1.3 Recommendations

1.3.1 Approvability

The review of the nonclinical studies (pharmacology, pharmacokinetics, safety pharmacology, toxicology reproductive toxicology, and genetic toxicology) supports a recommendation for approval of the marketing application for trametinib tablets.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

A separate labeling review will be submitted.

Reference ID: 3293647

2 Drug Information

2.1 Drug

CAS Registry Number: 871700-17-3 (for non-solvated parent), 1187431-43-1 (for DMSO solvate)

Code Name: GSK1120212B; The suffix 'B' denotes the dimethyl sulfoxide solvate

GSK1120212A (also referred to as GSK1120212); The suffix 'A' denotes the non-solvated parent.

JTP-74057, JTP-78296, JTP-75303 (Japan Tobacco reference numbers)

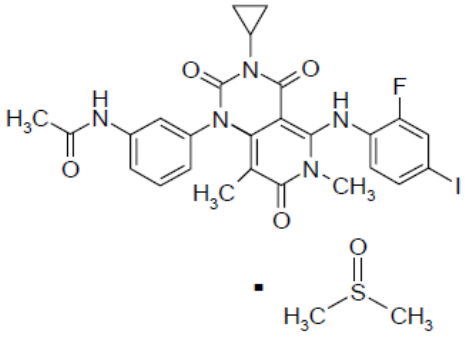
Chemical Name: Acetamide, N-[3-[3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-3,4,6,7-tetrahydro-6,8-dimethyl-2,4,7-trioxopyrido[4,3-*d*]pyrimidin-1(2*H*)-yl]phenyl]-, compound with 1,1'-sulfinylbis[methane] (1:1)

IUPAC: equimolecular combination of N-(3-{3-cyclopropyl-5-[(2-fluoro-4-iodophenyl)amino]-6,8-dimethyl-2,4,7-trioxo-3,4,6,7-tetrahydropyrido[4,3-*d*]pyrimidin-1(2*H*)-yl}phenyl)acetamide with (methylsulfinyl)methane

Molecular Formula/Molecular Weight

$C_{26}H_{23}FIN_5O_4.C_2H_6OS$/ 693.53 (DMSO solvate of parent); [redacted] (b) (4) (non-solvated parent)

Structure or Biochemical Description



An alternative representation of the chemical structure used by GlaxoSmithKline is given below:

Pharmacologic Class: kinase inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 102,175; IND 113,557

2.3 Drug Formulation

The drug product is an immediate release tablet for oral administration containing trametinib dimethyl sulfoxide equivalent to 0.5 mg, 1 mg or 2 mg of trametinib (nonsolvated parent). The composition of trametinib tablets is given in Table 1.

Table 1: Composition of Trametinib tablets

Component	Quantity (mg/tablet)			Function	Reference to Standard[1]
Strength	0.5	1	2		
Trametinib Dimethyl Sulfoxide[2]	(b) (4)			Active	GSK
Mannitol				(b) (4)	USP
Microcrystalline Cellulose					NF
Hypromellose (b) (4)					USP
Croscarmellose Sodium					NF
Sodium Lauryl Sulfate					NF
Colloidal Silicon Dioxide					NF
Magnesium Stearate[3]					NF
(b) (4)					
Yellow (b) (4)					Supplier
White (b) (4)					Supplier
Pink (b) (4)					Supplier
(b) (					USP
Total Tablet Weight	149.35	159.65	169.95	-	-

(b) (4)

(Excerpted from the Applicant's submission)

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

Three potential genotoxic impurities were identified: the genotoxic [redacted], and 2 impurities of [redacted]. The Applicant set the specification for each impurity at [redacted] for the drug substance and does not expect further formation of the impurities beyond this stage. Given the recommended daily dose of 2 mg of Mekinist, the level of exposure of the impurities in humans is [redacted]. At this dose, the level of either individual or the combined impurities does not exceed the threshold of toxicological concern and the proposed specifications are acceptable from a pharmacologic/toxicologic safety perspective.

[redacted]

During development the Applicant reported increased levels of two [redacted] impurities, [redacted], in the 0.5 mg and 1 mg tablets of Mekinist during a [redacted] test (Table 2).

Table 2: [redacted] Impurity levels

Impurity	Proposed Specification	Dose mg/day*	Reported Impurity Level		Dose mg/day‡
			0.5 mg	1 mg	
[redacted]					

*Impurity dose based a specification of [redacted] and a daily dose of 2 mg of mekinist
‡ Impurity dose based on actual levels of impurities and a daily dose of 2 mg of Mekinist using 0.5 mg or 1 mg tablets.

Both [redacted] were screened for genotoxicity using an in silico prediction tool (Derek v.13). [redacted] was identified as a potential genotoxin in this screen but was negative the Ames test. Though these [redacted] do not appear to be genotoxic, the [redacted] test showed that the specification could not be met. [redacted] The CMC review team has confirmed that the Applicant [redacted]. At a specification of [redacted] these degradant are within the ICHQ3B limits for drug products and do not require qualification in animal studies.

2.6 Proposed Clinical Population and Dosing Regimen

Mekinist is intended for the treatment of patients with unresectable or metastatic melanoma with $BRAF^{V600}$ mutation as detected by an FDA approved test. The recommended dose is 2 mg orally once daily, to be administered one hour before or two hours after a meal.

2.7 Regulatory Background

07/30/2010 – End of Phase 2 meeting
12/20/2010 - Orphan drug designation
05/09/2012 - Pre-NDA Meeting
06/29/2012 - Fast Track designation
08/03/2012 – NDA received

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

❖ A Fixed Dosing Combination Study using the MEK Inhibitor GSK1120212B and AKT inhibitor GSK2141795B on a panel of Colon, Lung and Pancreatic Cell Lines. (2010N105273_00)
❖ Efficacy of the oral AKT inhibitor GSK2141795 and the oral MEK inhibitor GSK1120212, administered separately or in combination, in two KRAS mutant pancreatic tumor xenograft models (2010N106611_00)
❖ Anti-tumor effect of BRAF and MEK inhibitors in combination with pazopanib (GW786034B) in BRAF mutant human melanoma xenograft in mice (2011N111685_00)
❖ Effect of GSK2110183 on multiple myeloma cell lines alone and in combination with proteasome inhibitors (bortezomib, carfilzomib), dexamethasone, immunomodulator (lenalidomide) or MEK inhibitor (GSK1120212). (2011N113163_01)
❖ Cellular assays with GSK2118436A, GSK1120212B, and GSK2126458A as a single agent and in combination in$BRAF^{v600}$ mutant melanoma cell lines that acquired resistance to GSK2118436A mediated by NRAS or MEK mutations (2011N116394_00)
❖ Cellular assays with GSK2118436A, GSK1120212B, and GSK2126458A as single agents and in combination with each other in $^{BRAFV600E/K/D}$ mutant melanoma cell lines (2011N116395_00)
❖ Evaluation of GSK2118436A (KN59) and GSKl120212B (KN45) in the A375P F11S Human Melanoma Xenograft Model (2011N120928_00)
❖ Evaluation of GSK2118436A (KN59) Combined with GSKl120212B (KN45) in the A375P F11S Human Melanoma Xenograft Model (2012N132871_01)
❖ Efficacy study ofGSK2141795C, GSKI363089G, GW786034B, and GSK1 1202128 in LIX014-FP1+5 human primary HCC cancer xenograft model (CPB-P10-2201) (2012N137997_00)
❖ MTD Studies of GSK1120212B and GSK2636771B in nu/nu mice (2012N138879_00)
❖ Estimating the minimum biologically active target concentration of GSK1120212B for in vivo activity in sensitive BRAF mutant lines (melanoma and CRC) (2012N139008_00)
❖ Comparative in vitro activity of GSK1790627 and GSK1120212 (2012N139081_00)
❖ Efficacy of MEK inhibitor GSK1120212B and BRAF inhibitor GSK2118436A, administered separately or in combination, in mouse tumor xenograft models of human melanoma (2012N139280_00)
❖ Cell Lines Response to GSK1120212B MEK Inhibitor (UH2007/00097/00)
❖ Biochemical Characterization of a Potent and Selective MEK Inhibitor, GSK1120212

(UH2008/00021/00)
❖ An acquired point mutation in MEK2 in HCT116 cells causes resistance to MEK inhibitor GSK1120212 (UH2008/00029/00)
❖ Use of Mitogen-stimulated PBMCs as a Surrogate Tissue to Measure MEK Inhibition (UH2008/00030/00)
❖ Molecular Targets and Mode-of-Actions of 15 Inducers (UH2008/00032/00)
❖ Comparative Study of JTP-74057 and Existing Anticancer Drugs - (in vitro) Combination Effect of JTP-74057 with Existing Anticancer Drugs (UH2008/00044/00)
❖ Analysis of the Effects of JTP-74057 on the Proteins Involved in Cell Cycle (UH2008/00045/00)
❖ A Study of Binding of JTP-74057 to Unphosphorylated MEKl (UH2008/00046/00)
❖ Pharmacodynamic and Efficacy Effects of GSK1120212B in Mice (UH2008/00051/02)
❖ In vitro Activity of GSK1120212B, a MEK inhibitor, Against Cancer Cell Lines from Haematological Origin (UH2009/00041/00)
❖ In vitro activity of GSK1120212B, a MEK inhibitor, against human melanoma, pancreatic cancer and colon cancer cell lines in combination with clinical standard of care drugs. (UH2009/00046/00)
❖ Combination Studies Between CENP-E Inhibitor GSK923295A and MEK Inhibitor GSK1120212B (UH2009/00093/00)
❖ In vitro Combination Studies of MEK (GSK1120212) and PI3K (GSK1059615) Inhibitors in Cancer Cell Lines (UH2010/00026/00)
❖ A Fixed Dosing Combination Study using the MEK Inhibitor GSK1120212B and Lapatinib in a Panel of Breast Cell Lines (UH2010/00040/00)
❖ Discovery of Candidate Circulating Metabolic PD Biomarkers for Raf/MEK Inhibition in Melanoma (UH2010/00041/00)
❖ Effect on Apoptosis for Two Colon Cancer Cell Lines Treated with CENP-E Inhibitor GSK923295A, MEK Inhibitor GSK1120212B or Both (UH2010/00044/00)
❖ A Fixed Dosing Combination Study using the MEK inhibitor GSK1120212B and PI3K inhibitor GSK2126458A on a panel of 10 Breast Cancer Cell Lines (UH2010/00051/00)
❖ A Fixed Dosing Combination Study using the MEKi inhibitor GSK1120212B and PI3K inhibitor GSK2126458A on a panel of Colon, Lung and Pancreatic Cell Lines (UH2010/00052/00)

PK/ADME

Absorption
❖ The Pharmacokinetics of GSK1120212 in Plasma Following Oral Administration ofGSK1120212B at Nominal Doses of 0.l, 0.3 or 1 mg/kg/day to Female Athymic Nude Mice (nu/nu) for 14 Days Followed by a Washout Period (2011N121723 00)
❖ An Evaluation of Systemic Exposure of GSK1120212 Following 22 Days of Repeat Oral Administration ofGSK1120212B in CD-1 Female Nude Mice Bearing a A375P F11s-Human Melanoma Tumor Xenograft (Bioanalysis and Pharmacokinetic Support for A375P Flls-GSK-e202) (2011N125777_00)
❖ Pharmacokinetics of GSK1120212 Following Oral Administrations of (b) (4) Suspension ofGSK1120212B to Male Sprague-Dawley Rats Under Fed Conditions (CD2007 /00787100)
❖ GSK1 120212B: Single-Dose Oral Toxicokinetic Study in Beagle Dogs (RD2007/00991/00)

❖ Pharmacokinetic study of JTP-74057 in Mice, Rats and Dogs (UH2007/00035/00)
❖ GSKl120212: Preliminary Investigation of the Preclinical Pharmacokinetics, In Vitro Blood Cell Partitioning, Stability and In Vitro Plasma Protein Binding (UH2007100095/02)
Distribution
❖ An In Vitro Investigation of the Transport via Heterologously Expressed Human Breast Cancer Resistance Protein of [14C]GSK1120212 in MDCKII-BCRP cells (RD2008/00032/01)
❖ An In Vitro Investigation into the Inhibition by GSKI120212 of Xenobiotic Transport via Human Breast Cancer Resistance Protein Heterologously Expressed in MDCKII Cells. (RD2007/01466/00)
❖ Elimination of Radioactivity in Male and Female Intact and Male Bile Duct-Cannulated Sprague-Dawley Rats and Quantitative Tissue Distribution of Radioactivity in Partially Pigmented Male Rats Following a Single Oral Administration of [14C]GSK1120212 (1 mg/kg) (CD2008/00024/00)
❖ An In Vitro Investigation of the Passive and Absorptive Membrane Permeability of C4CJGSKII20212 in MDCKII-MDRI Cells (CD2007/01031/00)
❖ An ln Vitro Investigation into the Inhibition by GSKI120212 of Xenobiotic Transport via Human OATPIBI and OATPI83 (CD2007/01007/00)
❖ An In Vitro Investigation of the Inhibition by GSK1120212 ofXenobiotic Transport Via Human P-Glycoprotein, Heterologously Expressed in MDCKII Cells (CD2007/00975/00)
❖ An In Vitro Study of Blood Cell Association of [14C]GSK1120212 in Healthy and Disease State (Cancer) Humans (2012N133368_00)
❖ In Vitro Permeability Categorization of GSK 1120212 According to the Biopharmaceutics Classification System. (2010N104737_00)
Metabolism
❖ Protein Binding, Metabolic Stability, and CYP Inhibitory Activity of JTP-74057 in Vitro (UH2007/00036/00)
❖ An In Vitro Evaluation of the Effect ofGSK1120212 on PXR/CAR Mediated CYP3A4, CYP2B6, and CYP2C8 induction in Cultured Primary Human Hepatocytes (2012N131823_00)
❖ An in vitro investigation into the enzyme(s) responsible for hydrolytic cleavage of [14C]GSK1120212 using appropriate in vitro systems (2012N135962 01)
❖ An In Vitro Investigation of the Potential for GSKll20212 Bioactivation Following Incubation of [14C]GSK1120212 with Human Liver Microsomes (CD2007/00194/00)
❖ GSK1120212: Preliminary investigation of the in vitro drug metabolism (UH2007/00111/00)
❖ In Vitro Cell Based Evaluation of GSK1120212B as an Activator of the Nuclear Receptor Rat PXR (RR2007 /00034/00)
❖ In Vitro Cell Based Evaluation ofGSK1120212B as an Activator of the Nuclear Receptor PXR [human] (RR2007/00033/00)
❖ Metabolism of GSK1120212 in Male and Female Intact and Male Bile Duct-Cannulated Sprague-Dawley Rats following a Single Oral Administration of [14C]GSK1120212 at a Dose Level of 1 mg/kg (CD2010/0029/00)
❖ The in vivo metabolism of GSK1120212 was investigated in male and female intact dogs following a single oral administration of [^{14}C]GSK1120212 (DMSO solvate) at a dose level of 0.5 mg/kg (CD2008/01199/00)
❖ An investigation of hepatic metabolism of GSK1120212 following administration of [^{14}C]GSK1120212 to the isolated perfused liver of the male Sprague-Dawley rat

(CD2008/01198/00)
❖ An in vitro investigation of the hepatic metabolism of [^{14}C]GSK1120212 in mouse, rat, female rabbit, dog, cynomolgus monkey and human (CD2008/00819/00)
❖ An In Vitro Evaluation of the Induction Effect of GSK 1120212 on Cytochrome P450 Expression in Cultured Primary Human Hepatocytes (CD2007/01330/00)
❖ An In Vitro Investigation into the Inhibition of Human Cytochrome P450 Enzymes by GSK 1120212 (CD2008/00124/00)
❖ NRII2 Human (Pregnane X Receptor isoform 1) Luciferase Reporter %Activation- pEC50- RTP Agonist 384-well Frozen Cells: Assay Protocol. (2012N135051 00)
❖ NR1I2 Rat (Pregnane X Receptor isoform 1) Luciferase Reporter % Activation- pEC50- RTP Agonist 384-well Frozen Cells: Assay Protocol (2012N135053_00)

Safety Pharmacology

Study Number	Study Title
CD2007/01303/00 (05BINKGP01)	Effect of p15 Inducer (JTP-78296) on general condition and behavior in rats (neurobehavioral study)
CD2007/00963/00 (G07310)	GSK1120212B: Acute effects on respiratory function following oral administration in the conscious CD(SD) rat
CD2008/00116/00 (04BP15P01)	Evaluation of the effects of p15 inducer R2 compounds on the hERG channel using the whole cell patch clamp method
FD2007/00151/00 (V27613)	GSK1120212B:Effect on hERG tail current recorded from stably transfected HEK-293 cells
UH2007/00108/00	GSK1120212B:Acute effect on QT interval, T_{p-e} interval and genesis of early after-depolarization in the isolated rabbit left ventricular wedge preparation
CD2007/01301/00 (05BCSAAD01)	Effect of p15 inducer related compound on QT prolongation in Isoflurane-anesthetized dogs
CD2007/00962/00 (G07309)	GSK1120212B: Acute effects on cardiovascular function following oral administration in the conscious Beagle dog
2011N128277_00	GSK1120212B: 3-Week Once Daily Oral Investigative Study in Male Mice

Toxicology

Study Number	Study Title
CD2010/00178/00	GSK1120212B: A 13 Week Oral Gavage Toxicity Study in the Rat with a 4 Week Recovery
CD2010/00179/00	GSK1120212B: A 13 Week Oral Gavage Toxicity Study in the Beagle Dog with a 4 Week Recovery
2010N109544 00/G1 0218	GSK1120212B: Oral Embryo-Fetal Development Study in Rats (GlaxoSmithKline Study No. G 1 0218)
2011N117363_01/D11104	GSK1120212B: Oral Dose Range Study in Female Rabbits (GlaxoSmithKline Study No. D11104)
2011N124059_00/G11166	GSK1120212B: Oral Embryo Fetal Development Study in Rabbits (GlaxoSmithKline Study No. G11166)
2011N123720 00/ Ames-1121	(b) (4) Bacterial Mutation Assay (Ames Test) With *Salmonella typhimurium* and *Escherichia coli* (screening study) (GlaxoSmithKline Study Number Ames-1121) (Atlas number:16373)
201 ON1 05201_00/ Ames-851	GSK2646258A: Bacterial Mutation Assay (Ames Test) With *Salmonella typhimurium* and *Escherichia coli* (screening study). (GlaxoSmithKline

	Study Number Ames-851) (Atlas no: 16373)
2011N128277_00/I11228	GSK1120212B: 3-Week Once Daily Oral Investigative Study in Male Mice

3.2 Studies Not Reviewed

Study Number	Study Title
CD2008/00117/00/04106	Preliminary Single Oral Dose Toxicokinetic Study of JTP-74057 (GSK1120212) Acetic Acid Solvate and JTP-75201 in Rats (Polyethylene Glycol 400 Solution)
CD2006/01539/00/006330	GSK1120212B: 10-Day Oral Dose Range Toxicity Study in Beagle Dogs (GlaxoSmithKline Study No. D06330)
2012n131888_00	Local Lymph Node Assay in the Mouse
2012N139087 00	Weight of Evidence Review oflmmunotoxicity Potential for GSK1120212 (MEK inhibitor; oncology),,
2012N135966_00/112021	GSK1120212B: Investigation of effects on viability and mitochondrial function in cultured rat cardiomyocytes
CD2008/00084/00/05002	Follow-Up Two-Week Oral Dose Study of JTP-74057 Acetic Acid Solvate and JTP-75201 in Male Rats - Sequential Analyses of Urinary Parameters
CD2007/01411/00	GSK1120212B: Investigative Urinalysis Daily Oral Gavage Study in Male Rats
CD2008/00083/00/04157	Two-Week Oral Dose Study of JTP-74057 Acetic Acid Solvate and JTP-75201 in Male Rats - Sequential Analyses of Urinary Parameters
CD2009/00139/00/G09010	GSK2126458A and GSK1120212B: 7-Day Oral Toxicity Study in Female Rats (GlaxoSmithKline Study No.: G090 1 0)
2011N112335_00/G10260	GSK1120212B and GSK2118436B: 4-Week Twice Daily Oral Toxicity Study in Dogs

4 Pharmacology

4.1 Primary Pharmacology

Molecular Mechanism of Action (including PD):

Cell Lines Response to GSK1120212B MEK Inhibitor (UH2007/00097/00)

Certain BRAF mutations appear to confer sensitivity to GSK2110212B. By surveying a large number of cell lines with varying sensitivities to GSK2110212B and ranking them by sensitivity, the Sponsor has shown that many of the most sensitive cell lines carry mutations in ras or c-raf. While not a perfect correlation, there is a preponderance of ras or raf mutants among the highly sensitive lines, and a relative paucity of these mutants among the highly resistant (UH2007-00097).

Similarly, the authors attempted to correlate basal levels of pERK with sensitivity to GSK2110212B. The correlation was not conclusive; however, the Sponsor argues that at the extremities of pERK expression, a pattern of sensitivity to GSK2110212B could

be detected. Susceptible cells all expressed high levels of baseline pERK, whereas resistant cells expressed low levels (UH2007-00097).

Biochemical Characterization of a Potent and Selective MEK Inhibitor, GSK1120212 (UH2008/00021/00)

GSK1120212B was demonstrated to preferentially inhibit (about 10-20 fold) the activation of MEK1 by BRAFV600E over inhibition of the activity of pMEK, as measured by the decreased phosphorylation of its direct (b) (4) target extracellular regulated kinase (ERK) (Table 3). GSK1120212 also demonstrated the ability to inhibit cellular proliferation of the BRAFV600E+ SK-MEL-28 cell line and showed a direct effect of trametinib on inhibition of MEK-mediated activation of ERK in the same cell line.

(adapted from Applicant's submission—study 2012N139081)

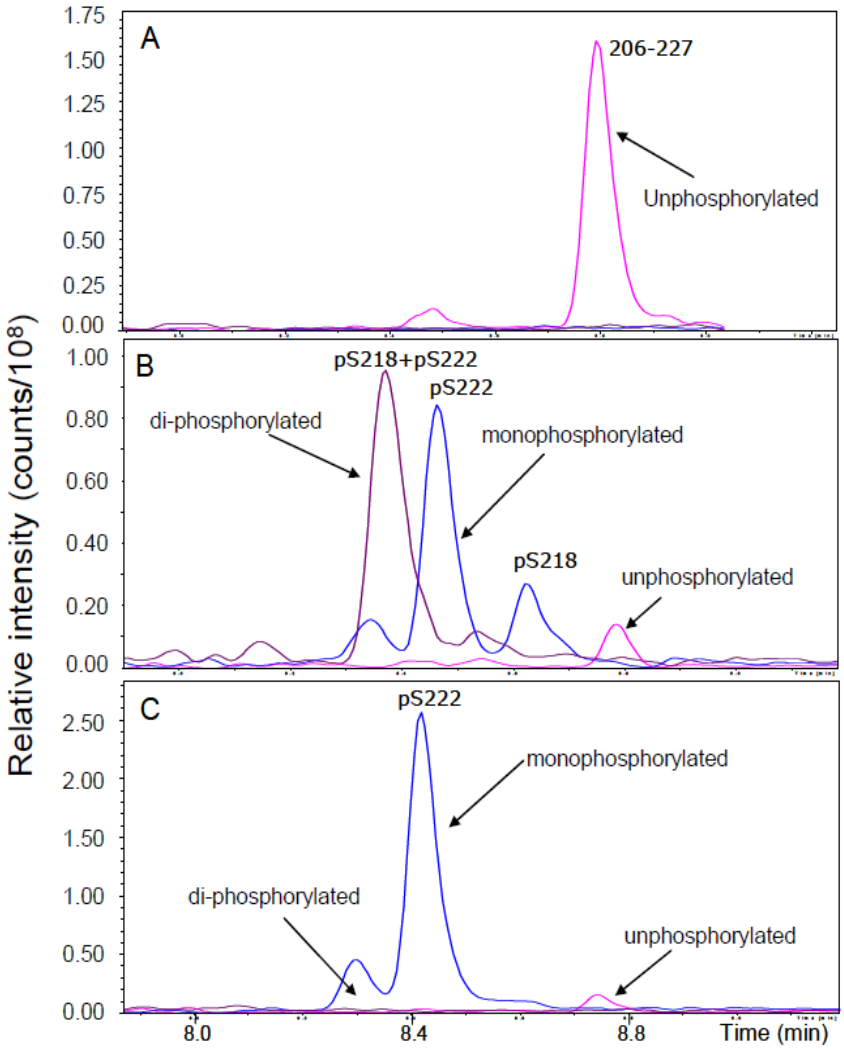
Table 3: GSK1120212 Inhibitory Potency in Kinase and Cell-Based Assays

	Biochemical Enzyme Inhibition IC_{50} (nM)		**Cellular Inhibition (SK-MEL-28: BRAFV600E+ cell line) IC_{50} (nM)**	
	Inhibition of unactivated MEK1	**Inhibition of phospho-MEK-1 (b) (4) activity**	**pERK**	**Proliferation**
Trametinib (GSK1120212)	**0.72±0.01**	**14.1±0.35**	**1.6±0.6**	**0.9±0.3**
M5 Metabolite (GSK1790627)	**1.01±0.05**	**34.55±6.15**	**2.0±0.1**	**1.7±0.1**

The mode of MEK inhibition was evaluated in studies of MEK1-catalyzed ERK2 phosphorylation at multiple concentrations of ATP and GSK1120212B.

To further elucidate the mechanism of GSK1120212 inhibition of MEK, the ability of GSK1120212 to affect phosphorylation of the MEK1 peptide 206-227 (which contains the two key activating residues on MEK1) was examined by LC/MS. MEK1 was incubated with B-RAFV600E in the presence or absence of GSK1120212B. The presence of GSK1120212B in the culture prevented significant phosphorylation of the critical S218 residue of the MEK1 protein (Figure 1).

Figure 1: Patterns of phosphorylation of MEK1 peptides in the absence and presence of GSK1120212



A. MEK1 peptide (206-227) alone (Negative control), B. MEK1 peptide+BRAFV600E (Positive control), C. MEK1 peptide+BRAFV600E in the presence of GSK1120212

The selectivity of GSK1120212 was demonstrated in *in vitro* potency assays. GSK1120212 was tested against a panel of over 40 kinases and found to have little off target activity (Table 4)

Table 4: GSK1120212 Kinase Screen

GSK1120212ATarget	N	pIC50	Notebook Reference	Protocol	Assay Format
AKT1	2	<5	U22937/187, N1441/48	AP2736v2, AP2736v3	LS-SPA
AKT2	2	<5	U22937/187, N1441/49	AP3599v1, AP3599v2	LS-SPA
ALK5	2	<5	U22480/109, N1434-26	AP769v4, AP769v5	FP
Aurora A/TPX2	1	<4.91	U22982/172	AP3634v1	LS-SPA
Aurora A/TPX2	1	<5	N1447-24	AP6183v1	IMAP
Aurora B/INCENP	1	<4.91	U22982/172	AP3637v1	LS-SPA
Aurora B/INCENP	1	<5	N1448-24	AP5932v2	IMAP
B-Raf (V600E)[1]	1	7.2	N1426/25	AP6875v2	MEK ATPase
B-Raf (V600E)[1]	1	<4.91	U22461/138	AP4972v1	FP
CAMKK2	1	<5	N1413-18	AP6838v2	IMAP
CDK2/Cyclin A	1	<4.91	U21761/195	AP2886v2	LS-SPA
EGFR	2	<5	U23280/108, N1363-25	AP2029v5, AP2029v6	TR-FRET
ERBB2	2	<5	U23280/105, N1363-25	AP2043v5, AP2043v6	TR-FRET
ERBB4	1	<4.56	N2756-14	AP7718v3	TR-FRET
ERK2	1	<4.91	U22961/45	AP2535v1	FP
GSK3B	1	<4.56	N1670-42	AP7032v1	FP
IGF1R	2	<5	U23284/119, N1367-17	AP5088v1, AP5088v2	TR-FRET
IKK2	1	<4.56	N1401-79	AP157v7	TR-FRET
INSR	2	<5	U23284/129, N1367-17	AP4833v2, AP4833v3	TR-FRET
ITK	1	<4.56	N1606-43	AP3023v4	TR-FRET
JNK1	1	<5	N1411-22	AP2354v3	TR-FRET
JNK2	1	<5	N2223-7	AP8033v1	TR-FRET
JNK3	1	<5	N1493-20	AP7490v1	FP
LCK	1	<4.56	N1670-41	AP4162v2	IMAP
MAPKAPK2	1	<4.91	U23340/3	AP2883v2	LS-SPA
MAPKAPK2	1	<4.56	N1457-50	AP5279v2	IMAP
MET	1	<4.91	U22909/47	AP2502v2	TR-FRET
P38alpha	1	<4.56	N1606-44	AP2113v3	TR-FRET
p70S6K	1	<4.91	U23331/062	AP2861v1	LS-SPA
PAK1	1	<5	N2224-19	AP7073v1	IMAP
PDK1	1	<5	N1329-48	AP7863v1	LS-SPA
PI3Kalpha	1	<5	N2525-10	AP5316v3	LS-SPA
PI3Kbeta	1	<5	N2525-11	AP5644v2	LS-SPA
PI3Kgamma	1	<5	N1420-20	AP5404v2	LS-SPA
PI3Kgamma	1	<4.91	U23338/30	AP3310v1	FP
PLK1	1	<4.91	U23448/7	AP2887v1	LS-SPA
ROCK1	1	<4.91	U23310/68	AP2858v1	LS-SPA
ROCK1	1	<5	N1329-50	AP5818v1	IMAP
RSK1 (rat)	1	<4.91	U23331/063	AP2862v1	LS-SPA
SGK1	1	<4.91	U23232/46	AP4657v2	IMAP
SRC	1	<4.91	U21968/126	AP779v3	TR-FRET
SYK	1	<4.56	N1457-51	AP424v8	TR-FRET
VEGFR2	1	<5	U22852/56	AP355v5	TR-FRET
WEE1	1	<4.91	U23310/73	AP3906v2	TR-FRET
YAK3	1	<4.91	U22274/136	AP2890v1	LS-SPA
ZAP70	1	<4.56	N1457-68	AP2254v3	TR-FRET

[1]B-Raf (V600E) is referred to as B-Raf (V599E) by GSK Screening and Compound Profiling
FP- Fluorescence polarization assay, LS-SPA- LEADseeker scintillation proximity assay (GE Heathcare Life sciences, Piscataway, NJ), IMAP - IMAP technology (Molecular Devices, Sunnyvale, CA) fluorescence depolarization assay, TR-FRET- Time resolved- fluorescence resonance energy transfer.

Estimating the minimum biologically active target concentration of GSK1120212B for *in vivo* activity in sensitive BRAF mutant lines (melanoma and CRC) (2012N139008_00)

In report 2012N139008-00, the Applicant conducted a meta-analysis of former *in vitro* studies conducted with GSK1120212. The purpose of this review was to utilize earlier *in vitro* datasets that the Sponsor felt were not comparable due to differences in experimental design, to estimate a minimum concentration necessary to inhibit growth of highly sensitive BRAF-mutant melanoma cell lines. By comparing results of 15

BRAF mutant lines, the Applicant concluded that meaningful growth suppression could be expected at concentrations of 15-20 nM (10-14 ng/mL) for BRAF-mutant cell lines.

Cellular assays with GSK2118436A, GSK1120212B, and GSK2126458A as single agents and in combination with each other in BRAFV600E/K/D mutant melanoma cell lines (2011N116395_00)

In Study 2011N116395_00, 17 cell lines homozygous or heterozygous for one of the $BRAF^{V600}$ mutations were examined to evaluate their sensitivity to GSK1120212B in the context of their genetic mutation status. The following melanoma cell lines were used to characterize the genetic mutations most responsive to GSK1120212B: A101D, A2058, A375, COLO-829, SK-MEL-1, SK-MEL-24, SK-MEL-28, SK-MEL-3, SK-MEL-5, UACC-257, UACC-62, WM-115, YULAC, YUMAC, YUSIT1, WW165, A375PF11, and IGR-1. Cultures were seeded in 96-well plates and exposed to serial dilutions of test compound (range: 0.05 nM to 1 µM). At the end of a 24 or 72 hour incubation period, apoptosis and ATP levels, respectively, were assessed by chemiluminescence.

As a single agent, GSK1120212B exhibited growth inhibitory IC_{50} values in the range of 1.4-9.5 nM in 6/8 cell lines heterozygous for the V600E BRAF mutation (V600V/E). All three homozygous V600E mutant cell lines were highly sensitive to GSK2110212B, with IC_{50} values of between 1.4-6.3 nM. Similarly, heterozygous V600V/K mutants and homozygous V600K mutants were highly sensitive to GSK2110212B, with IC_{50} values of 0.3-45 nM (the majority were <3 nM).

Discovery of Candidate Circulating Metabolic PD Biomarkers for Raf/MEK Inhibition in Melanoma (UH2010/00041/00)

In Study UH2010/00041/00, the Applicant attempted to identify pharmacodynamic biomarkers for Raf/MEK inhibition from the plasma of female CD-1 nude mice that hosted A375F11 xenografts. Tumor homogenates were also evaluated. Samples were evaluated by ESI LC-MS for changes in carnitines, catabolites of leucine, and hydroxyisovalerate, and TCA cycle intermediates. Although changes expected from pathway inhibition were detected in plasma, none was sufficiently robust to warrant further development.

Pharmacodynamic and Efficacy Effects of GSK1120212B in Mice (UH2008/00051/02)

Study UH2008/00051/02 evaluated the pharmacodynamic activity of GSK1120212B in nude mice hosting an array of xenografted tumor types (breast, melanoma, NSLC, colon, prostate, and pancreas). Tumor growth inhibition was evaluated and tumor and brain samples were collected for pharmacodynamic (PD) assessment (pERK inhibition). The study evaluated both daily and intermittent dosing schedules. 3 mg/kg PO was found to be the MTD when administered daily. The pharmacodynamic effect of GSK1120212B (pERK inhibition) was demonstrated in xenografted tumor samples collected at the end of the dosing phase. pERK inhibition was demonstrated in

xenografts by immunocytochemical staining. The study evaluated known PD endpoints (pERK) as well as markers of general cell signaling (p16, p27 and Ki-67). GSK1120212B reduced pERK staining at doses of 1 and 3 mg/kg/day, whereas PD effects on p-27-, caspase-3- and Ki67-staining were modest and variable. GSK1120212B was found to cross the blood brain barrier. Measured concentrations were ~10% of plasma following a single dose; ~ 20% of plasma following multiple dosing. Peak CNS concentrations were achieved quickly (within 2-4 hours) and measurable concentrations were detectable for at least 8 hours, suggesting relatively slow clearance from that compartment.

Molecular Targets and Mode-of-Actions of 15 Inducers (UH2008/00032/00)

Study UH2008/00032/00 was undertaken to clarify the mechanism of action of JTP-74057 (GSK1120212B). GSK1120212B inhibited phosphorylation of MEK and ERK in B-Raf-mutant HT29 cells; however, there was no effect of GSK1120212 on Raf activity, as indicated by c-Raf- or B-Raf- mediated MPB phosphorylation. The timecourse of pMEK inhibition is rapid. Suppression of phosphorylation (as assessed by p-MEK and p-ERK Western blot analysis) was evident within 30 minutes of exposure and persisted through 24 hours while in the presence of drug. After washout, levels of pMEK and pERK began to recover within 2 hours, though levels of pERK remained low even at 24 hours post-washout. Levels of pMEK returned to approximately baseline levels within 4-24 hours. Culturing cells in the presence of GSK1120212B resulted in suppression of c-Myc protein levels, and induction of p15 and p27 in cultured cells, in a concentration-dependent fashion. Concentrations of 10-100 nM were associated with complete suppression of c-Myc, maximally suppressed Cyclin D1 (100 nM) and maximally induced p15 and p27, as evaluated by Western blotting. Suppression of c-Myc was noted at 2 hr post-exposure. Suppression of CyclinD1 began by 4 hours, indicating that these are later-occurring phenomena than the effects on pMEK and pERK.

Based upon these data, the Applicant proposes the following model of tumor growth inhibition: GSK1120212B binds to MEK and blocks MEK and ERK phosphorylation. As a result of pERK suppression, c-Myc expression is reduced, which induces p15 and p27. Upregulation of p15 and p27 then reduce cyclin D1 and arrest cells in G1.

Analysis of the Effects of JTP-74057 on the Proteins Involved in Cell Cycle (UH2008/00045/00)

Study UH2008/00045/00 examined the effect of GSK1120212B on cell cycle proteins in cultured HT29 and COLO205 cells. The effects of GSK1120212 were complex and time-dependent. Short-term activities (0.25-4 hours) included: suppression of pMEK and pERK, reduction of cyclin D1 and D2 (>4 hours), and suppression of c-Myc (> 2 hours). Later effects included suppression of cyclins D1, D2, A, and E (>48 hours), suppression of pRb (Ser795 and 807/811), and induction of p15 and p27 (both > 48 hours). GSK1120212B also induced PARP cleavage and caspase activation within 4 days at 100 nM.

A Study of Binding of JTP-74057 to Unphosphorylated MEKl (UH2008/00046/00)

Study UH2008/00046/00 evaluated the binding of GSK1120212B to unphosphorylated MEK1 using Biacore S51. The Applicant compared the activity of GSK1120212B to Pfizer's PD0325901, which they claim is confirmed to be an (b) (4) inhibitor of MEK1. The measured Kd of GSK1120212B for unphosphorylated MEK1 was $1.9X10^{11}$M. The Applicant claims that GSK1120212B did not compete for ATP in this assay; however, they tested only one concentration of ATP. Interpretation of the binding model is complicated by multiphasic responses across the concentration range.

Mechanism of Resistance to GKS1120212B: An acquired point mutation in MEK2 in HCT116 cells causes resistance to MEK inhibitor GSK1120212 (UH2008/00029/00)

In Report UH2008/00029/00, the Applicant evaluated the mechanism of resistance to GSK1120212B by evaluating a colon cancer cell line, HCT116, k-ras mutant cell line deficient in mismatch-repair. GSK1120212B produced a number of drug-resistant colonies following exposure to high drug concentrations (1 μM). Isolated clones were sequenced for changes in the MAPK pathway genes. They identified a clone in which a single missense point mutation (L119P) conferred resistance to GSK1120212B. Knockdown with siRNA re-sensitized cells to the drug.

Cellular assays with GSK2118436A, GSK1120212B, and GSK2126458A as a single agent and in combination inBRAFv600 mutant melanoma cell lines that acquired resistance to GSK2118436A mediated by NRAS or MEK mutations (2011N116394_00)

In Report 2011N116394_00 the Applicant investigated the mechanism of tumor cell resistance using a panel of 9 $BRAF^{V600E}$ mutant A375 and YUSIT1 cell lines. Cells were selected for drug resistance by growth in cultured medium containing increasing concentrations of GSK2118436A (dabrafenib), an inhibitor of RAF kinases. The results indicated that NRAS and MEK mutations contribute to BRAF inhibitor resistance in cells selected with GSK2118436A. In the BRAF inhibitor resistant selected cell lines evaluated, addition of GSK2110212B to GSK2118436A was able to overcome the resistance.

Combination Chemotherapy Studies:

Effect of GSK2110183 on multiple myeloma cell lines alone and in combination with proteasome inhibitors (bortezomib, carfilzomib), dexamethasone, immunomodulator (lenalidomide) or MEK inhibitor (GSK1120212). (2011N113163_01)

GSK1120212B was not active in multiple myeloma cell lines, either alone or in combination with AKT inhibitor, GSK2110183B (2011N113163_01)

Efficacy of MEK inhibitor GSK1120212B and BRAF inhibitor GSK2118436A, administered separately or in combination, in mouse tumor xenograft models of human melanoma (2012N139280_00)

Study 2012N139280_00 evaluated the effects of GSK1120212B in combination with the BRAF inhibitor, GSK2118436A. By some regimens, the combination of BRAF and MEK inhibition yielded additive growth inhibition compared to the same doses of each agent when administered alone. It should be noted, however, that high doses (10-30 mg/kg) of GSK1120212B were no better, and were sometimes worse than lower doses (1-3 mg/kg).

A Fixed Dosing Combination Study using the MEK Inhibitor GSK1120212B and AKT inhibitor GSK2141795B on a panel of Colon, Lung and Pancreatic Cell Lines. (2010N105273_00)

Study 2010N105273_00 evaluated the effect of GSK1120212B plus the AKT inhibitor GSK2141795B on colon, lung, and pancreatic cancer cell lines. Cells were genotyped for various mutations, including KRAS, NRAS, BRAF, PIK3CA, and PTEN. Each cell line was tested with both agents at an array of concentrations. Evidence for both synergy and antagonism was obtained in these experiments. While in some cell lines, a pattern of increasing or decreasing activity was evident across the concentration gradients for the two test articles, in many others, antagonistic and synergistic responses alternated across the concentration range with little evidence of a dose-relationship. The Applicant did not interpret the cytostatic activity obtained from these combinatorial studies with GSK1120212B and GSK2141795B in the context of the genetic modifications observed.

Anti-tumor effect of BRAF and MEK inhibitors in combination with pazopanib (GW786034B) in BRAF mutant human melanoma xenograft in mice (2011N111685_00)

Study 2011N111685_00 evaluated the effect of GSK2118436 (a BRAF inhibitor), GSK1120212B (a MEK inhibitor), and pazopanib (a multi-kinase and angiogenesis inhibitor) in human melanoma xenografts. When used in combination, GSK1120212B (0.1-0.3 mg/kg qd) and pazopanib (100 mg/kg bid) completely suppressed tumor

growth for the 40-day duration of the study. GSK1120212B alone at a dose of 0.1 mg/kg QD was ineffective (comparable to vehicle control) in suppressing growth of A375PF11s melanoma tumors in nude mice. All regimens were reasonably well-tolerated as evident from an evaluation of body weights. A similar result was obtained when the BRAF inhibitor, GSK2118436A (10-30 mg/kg qd) was combined with pazopanib (100 mg/kg bid). It should be noted, however, that none of the treatments evaluated in this study led to regression of the tumors relative to baseline, as evident from measurements of tumor volume.

Evaluation of GSK2118436A (KN59) and GSKl120212B (KN45) in the A375P F11S Human Melanoma Xenograft Model (2011N120928_00)

In Study 2011N120928_00 two agents, GSK1120212B and GSK2118436A (2 lots), were compared using human melanoma xenograft models. Daily oral doses of 0.3 mg/kg GSK1120212B or 30 mg/kg GSK2118436 (qd, po) were comparably effective in suppressing tumor growth in nude mice.

Cell Lines Response to GSK1120212B MEK Inhibitor (UH2007/00097/00)

In Study UH2007/00097/00 the Applicant characterized an array of cell lines for sensitivity, partial resistance or insensitivity to GSK2110212B, then evaluated the genetic mutations associated with sensitivity or resistance. They measured the effects of GSK1120212B on the expression and kinetics pERK and on cell proliferation. While the majority of the highly sensitive cells were BRAF or RAS mutant, the correlation between mutation status and resistance is not perfect. WT cells were generally less sensitive to GSK1120212B; however, 20% of BRAF mutant and 28% of RAS mutant cells were also resistant or insensitive to GSK1120212B. Conversely, 28% of RAS WT cells were sensitive to GSK1120212B. Normal proliferating HUVECs (human umbilical vein endothelial cells) were highly sensitive to GSK1120212B and non-proliferating HUVECs were partially or mostly resistant to GSK1120212B.
The Applicant correlated levels of pERK to drug sensitivity in a screen of several cell lines that were demonstrably sensitive or resistant. In general, resistant cell lines expressed lower basal levels of pERK (Thr 202 / Tyr 204); however, there were resistant cell lines that expressed measurable basal levels of pERK and sensitive cell lines that did not. In general, treatment with 100 nM GSK1120212B decreased expression of pERK; however, the effect was of short-duration following washout (<1 hour).

MTD Studies of GSK1120212B and GSK2636771B in nu/nu mice (2012N138879_00)

Study 2012N138879_00 evaluated the MTD of GSK1120212B when administered in combination with GSK2636771B in nu/nu mice. Oral doses of GSK1120212B (0.3 mg/kg) and GSK2636771B (30 mg/kg) were considered the maximum tolerable doses on the qdX15 schedule. A 3-fold decrease in GSK2636771B dose (from 30 to 10

mg/kg) did little to affect the tolerability of the combination as determined by changes in body weight, suggesting that the dose of GSK1120212B was dose-limiting.

Efficacy of the oral AKT inhibitor GSK2141795 and the oral MEK inhibitor GSK1120212, administered separately or in combination, in two KRAS mutant pancreatic tumor xenograft models (2010N106611_00)

Study 2010N106611_00 evaluated the effect of GSK2141795 (AKT inhibitor) in combination with GSK1120212B in human pancreatic xenografts. Daily oral doses of 30 mg/kg GSK2141795 and 0.3 mg/kg GSK1120212B were most efficacious in reducing the rate of tumor volume growth over the 90 day dosing interval; however, none of the doses was associated with tumor regression.

Efficacy study ofGSK2141795C, GSKI363089G, GW786034B, and GSK1 1202128 in LIX014-FP1+5 human primary HCC cancer xenograft model (CPB-P10-2201) (2012N137997_00)

In Study 2012N137997_00 the Applicant evaluated the effect of GSK1120212B in LIX014-FPI+5 primary human HCC cancer xenografts. LIX014 overxpresses c-MET. The combination of GSK1120212B (0.5 mg/kg) and GSK786034B (100 mg/kg) was able to slow the progression of HCC in xenografts when administered by daily oral gavage.

Evaluation of GSK2118436A (KN59) Combined with GSKl120212B (KN45) in the A375P F11S Human Melanoma Xenograft Model (2012N132871_01)

The purpose of study 2012N132871_01 was to evaluate the effect of GSK1120212B and GSK2118436A on tumor growth in mouse xenografts implanted with A375PF11S human melanoma cell tumors selected for resistance to GSK2118436A. When administered alone at a daily oral dose level of 0.3 mg/kg, GSK1120212B exhibited little activity in xenografts. At doses of 1 mg/kg (monotherapy) or at doses of 0.3 or 1 mg/kg in combination with either 30 or 100 mg/kg GSK2118436A, tumor growth was suppressed for the 60-day duration of dosing.

Comparative Study of JTP-74057 and Existing Anticancer Drugs - (in vitro) Combination Effect of JTP-74057 with Existing Anticancer Drugs (UH2008/00044/00)

Study UH2008/00044/00 was conducted to evaluate the effect of GSK1120212B alone or in combination with various chemotherapeutics, in cultured colorectal carcinoma cells (HT-29). GSK1120212B was active against HT-29 cells when used in monotherapy and exhibited additive activity or synergism when assessed in combination. Synergistic cell growth inhibition was observed with 5-FU, Iressa, and oxaliplatin. Conversely, in combination with vincristine, incubation with GSK1120212B resulted in increased cell growth (antagonism).

Use of Mitogen-stimulated PBMCs as a Surrogate Tissue to Measure MEK Inhibition (UH2008/00030/00)

In Study UH2008/00030/00 the Applicant describes the method for an assay to evaluate the *in vivo* activity of GSK1120212B patient hPBMCs stimulated with PMA. hPBMCs were used as a pharmacodynamic surrogate tissue for monitoring systemic GSK1120212B-mediated MEK suppression (as indicated by a reduction in pERK production). The utility of the method was found to perform satisfactorily in mPBMCs isolated from nude mice treated with GSK1120212B.

A Fixed Dosing Combination Study using the MEK inhibitor GSK1120212B and PI3K inhibitor GSK2126458A on a panel of 10 Breast Cancer Cell Lines (UH2010/00051/00)

Study UH2010/00051/00 evaluated the effect of a fixed dose combination of GSK1120212B with the PI3K inhibitor, GSK2126458A on a panel of 10 breast cancer cell lines. They also evaluated each cell line for expression of hormone receptors. Across cell lines, the combination treatment produced variable degrees of additive activity or synergy using the EOHSA and Bliss measures.

Effect on Apoptosis for Two Colon Cancer Cell Lines Treated with CENP-E Inhibitor GSK923295A, MEK Inhibitor GSK1120212B or Both (UH2010/00044/00)

In Study UH2010/00044/00, the Applicant evaluated GSK1120212B and CENP-E Inhibitor, GSK923295A in RKO and SW48 cells (colon cancer), to assess the potential of the agents to induce apoptosis when administered separately and in combination. When exposed to GSK923295A (200 nM) and GSK1120212B (100 nM) for 24 hours the combination treatment produced an apoptotic response that was greater than the contribution of each agent alone, suggesting that the two agents may act synergistically in these cells.

A Fixed Dosing Combination Study using the MEK Inhibitor GSK1120212B and Lapatinib in a Panel of Breast Cell Lines (UH2010/00040/00)

Study UH2010/00040/00 evaluated the combination effect of GSK1120212B plus lapatinib in a panel of breast cancer cell lines, which had a range of genetic defects. The responses were highly variable, and most showed little evidence of combination activity; however in a small subset of experiments, IC_{50}s were obtained for the combination.

In vitro Combination Studies of MEK (GSK1120212) and PI3K (GSK1059615) Inhibitors in Cancer Cell Lines (UH2010/00026/00)

Study UH2010/00026/00 evaluated the combination of GSK1120212 plus the PI3K inhibitor, GSK1059615 in a panel of cancer cell lines. The combination was reportedly

more effective than either agent alone in over half of the cell lines tested; however, attempts to correlate efficacy with the presumed genetic defects were uninformative. Toxicity endpoints were not evaluated in this study.

A Fixed Dosing Combination Study using the MEK inhibitor GSK1120212B and PI3K inhibitor GSK2126458A on a panel of Colon, Lung and Pancreatic Cell Lines (UH2010/00052/00)

Study UH2010/00052/00 evaluated the combination of GSK1120212 plus the PI3K inhibitor, GSK2126458A in a panel of colon, lung and pancreatic cancer cell lines. GSK1120212 and GSK2126458A increased cell death when administered in combination versus alone, though with significant variability between cell lines and between replicate experiments with the same cell line.

Combination Studies Between CENP-E Inhibitor GSK923295A and MEK Inhibitor GSK1120212B (UH2009/00093/00)

Study UH2009/00093/00 evaluated the combination of GSK1120212B with the CENP-E inhibitor, GSK923295A in a panel of pancreatic, colon and lung cancer cell lines. The combination showed increased activity over individual agents in a subset of cell lines tested.

In vitro activity of GSK1120212B, a MEK inhibitor, against human melanoma, pancreatic cancer and colon cancer cell lines in combination with clinical standard of care drugs. (UH2009/00046/00)

Study UH2009/00046/00 evaluated GSK1120212B against and in combination with the standard of care (SoC) in a series of melanoma, pancreatic and colon cancer cell lines of known genotypic background. Neither the SoC nor GSK1120212B was active in the melanoma cell lines tested. The combination was also inactive. In pancreatic cell lines, additive activity of GSK1120212 with gemcitabine or erlotinib was only observed in the PL45 cell line (a KRAS mutant line). In colon cancer cells, 5-FU and irinotecan were additive with GSK1120212 in BRAF mutants and some KRAS mutants.

Comparative in vitro activity of GSK1790627 and GSK1120212 (2012N139081_00)

GSK1120212B is hydrolyzed at the amide to produce GSK1790627. The cellular activities of GSK1120212B and GSK1790627 were compared in assays to evaluate their potencies against activated and unactivated MEK as well as their ability to inhibit ERK phosphorylation and cell proliferation in $BRAF^{V600E}$ mutant SK-MEL-28 cells. Both compounds were similarly potent in these assays, indicating that the avtivity of GSK1120212 may be at least partially attributable to its pharmacologically-active metabolite, GSK1790627 (2012N139081_00).

In vitro Activity of GSK1120212B, a MEK inhibitor, Against Cancer Cell Lines from Haematological Origin (UH2009/00041/00)

Study UH2009/00041/00 evaluated the activity of GSK1120212B in a panel of hematological cancer cell lines. AML and CML cell lines were found to be sensitive to GSK1120212B; increased sensitivity of the AML cell lines to the drug was demonstrated in combination with rapamycin, ara-C, bexarotene and sorafenib. There was no demonstrable GSK11202112B activity against B cell leukemia, B cell lymphoma or Burkitt's lymphoma.

4.2 Secondary Pharmacology

In Vitro Pharmacology - Study of JTP-74048, JTP-74057 (GSK1120212B), JTP-74059, JTP-74077 and JTP-75201 (CD2007/01300/00)

In Study CD2007/01300/00, GSK1120212B (JTP-74057) exhibited a lack of off-target activity as demonstrated by failure to inhibit binding or enzymatic activity in a panel of over 45 receptors and enzymes.

Activity of MEK Inhibitors Against Multiple Purified Kinases (UH2008/00047/00)

GSK1120212B was evaluated for off-target enzyme inhibition in an array of isolated enzymes. The compound was found to be positive for MEK1 inhibition, but negative for inhibition of all other enzymes tested at concentrations of up to 10 µM.

4.3 Safety Pharmacology

Study title: Effect of p15 Inducer (JTP-78296) on general condition and behavior in rats (neurobehavioral study)

Study no.:	CD2007/01303/00 (05BINKGP01)
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study report:	June 13, 2005
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	JTP-78296, lot C, JTP-74057 dimethyl sulfoxide solvate; purity not provided

Note: Drug lots tested in (b) (4)

Key Study Findings:

- Depressed motor activity and general body condition within 24 hours of 100 mg/kg JTP-78296 were a result of extreme dose level. Neurotoxic effects were not noted in separate studies conducted with tolerated drug doses.

Methods and Results:
A single oral dose of JTP-78296 (JTP-74057 dimethyl sulfoxide solvate suspended in 0.5% methylcellulose [MC]) was administered to 4 SD (IGS) male rats at 100 mg/kg (600 mg/m^2). Chloropromazine was used as the positive control. Diarrhea (1st noted at 4 hours post dose) and depressed body weight gain (33% of control) were observed in all animals within 24 hours of dosing, and reduced spontaneous locomotion, prone position, blepharoptosis, mydriasis, and piloerection were exhibited in 1 to 3 animals within this same time period. A GLP study assessing neurobehavioral parameters was not conducted.

Findings were considered to be a result of the intolerable dose level (600 mg/m^2). Mortality and early study termination were observed at 0.5 and 1.0 mg/m^2/day in rats dosed for 13 weeks. Neurotoxic findings were not observed at tolerated doses of trametinib

Study title: GSK1120212B: Acute effects on respiratory function following oral administration in the conscious CD(SD) rat

Study no.:	CD2007/00963/00 (G07310)
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	GlaxoSmithKline, Hertfordshire, UK
Date of study initiation:	October 1, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK1120212B, lot #: E169079, purity: 99.4%

Key Study Findings:

- Transient decrease in body temperature (0.8ºC decrease relative to concurrent control) at 1mg/m^2. There were no changes in respiratory parameters.

Results:
Male CD (SD) rats were administered single oral doses of 0.125, 0.5 and 1 mg/m^2 GSK1120212. There were no changes in tidal volume, respiratory rate, minute volume, or total pulmonary resistance up to 5 days following dosing. A transient decrease in body temperature was observed 1 hour following dosing of 1 mg/m^2.

Study title: Evaluation of the effects of p15 inducer R2 compounds on the hERG channel using the whole cell patch clamp method

Study no.:	CD2008/00116/00 (04BP15P01)
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not provided
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	JTP-74048B, JTP-74057C, JTP-74059D, JTP-74077B) lot # and purity not provided

Key Study Findings:

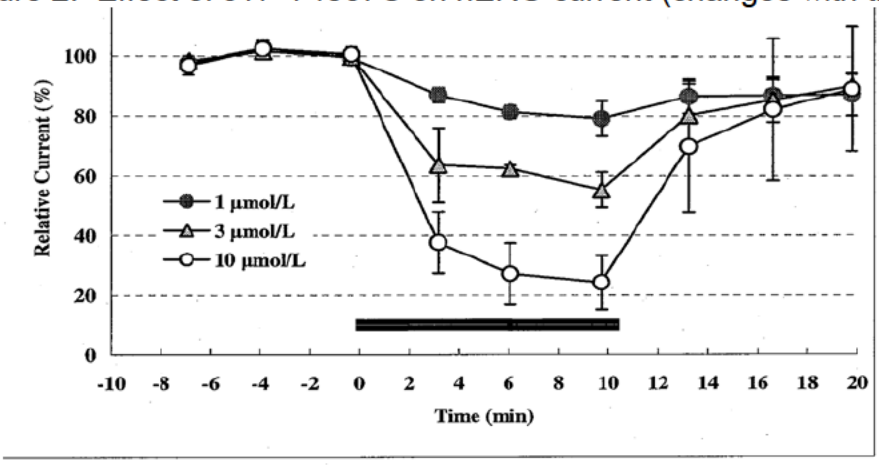
- Trametinib inhibited hERG current in a concentration dependent manner with an IC_{50} of 3.7 µM (2276 ng/mL).

Methods and Results:

hERG potassium channel inhibition was evaluated by manual patch-clamp electrophysiology using Chinese hamster ovary (CHO)-K1 cells expressing hERG channels. Compounds tested included JTP-74048B, JTP-74057C, JTP-74059D, and JTP-74077B; code number JTP-74057C was indicated to be the parent compound trametinib.

JRP-74057C exhibited a concentration dependent inhibition of hERG current and IC_{50} of 3.7 µM. The inhibition was reversible following washout (see Figure 2).

Figure 2: Effect of JTP-74057C on hERG current (changes with time)



(Excerpted from Applicant's submission)

Study title: GSK1120212B: Effect on hERG tail current recorded from stably transfected HEK-293 cells

Study no.:	FD2007/00151/00 (V27613)
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	GlaxoSmithKline, Hertfordshire, UK
Date of study initiation:	April 19, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK1120212 prepared in DMSO; drug lot and purity not provided

Key Study Findings:

- Trametinib inhibited hERG channel tail current in a concentration dependent manner with IC_{25}, IC_{50}, and IC_{75} values of 0.448, 1.54, and 5.37 µM (0.2757, 0.9477 and 3.2617 ng/mL, respectively.

Methods and Results:

hERG potassium channel inhibition was evaluated by the effect of parent compound GSK 1120212 (batch # PAD1000107) on the ion channel in the human embryonic kidney cell (HEK-293) transfected with hERG cDNA. Peak hERG tail current amplitude was measured prior to and following exposure to GSK 1120212, vehicle 0.3% DMSO) or positive control (E-4031).

Mean hERG tail current was decreased by 11.8% in DMSO vehicle exposure compared to 93.4% inhibition following E-4031 exposure. When tail currents were corrected for the mean vehicle effect, the maximum soluble concentration of 10.91 µM GSK 1120212 (6.71 µg/mL) produced 94.4% inhibition of hERG tail current. A number of additional concentrations (0.033, 0.109, 0.327, 1.09, and 3.27 µM) were studied, resulting in a concentration-dependent inhibition: IC_{25}, IC_{50}, and IC_{75} values of 0.448, 1.54 and 5.30 µM (0.275, 0.947 and 3.26 µg/mL), respectively.

Study title: GSK1120212B: Acute effect on QT interval, T_{p-e} interval and genesis of early after-depolarization in the isolated rabbit left ventricular wedge preparation

Study no.:	UH2007/00108/00
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study report:	June 18, 2006
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	GSK1120212, batch #NMA19-024A; purity not provided

Key Study Findings:

- Trametinib produced a significant dose related decrease in isometric contractile force at concentrations of 10 and 30 µM, and decreased the Tp-e interval at 30 µM. QT prolongation was not observed.

Methods and Results:

The arterially perfused rabbit ventricular wedge preparation demonstrates the propensity of agents to cause Torsades de Points (TdP) with relatively high sensitivity and specificity.

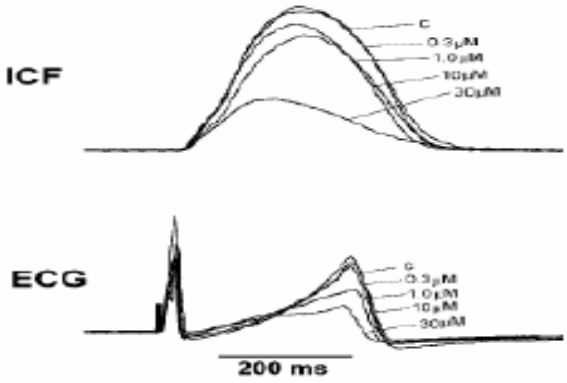
GSK1120212B (DMSO solvate form of GSK1120212) was tested at concentrations of 0.3, 1, 10 and 30 µM, and had no effect on QT prolongation. GSK1120212B produced significant decreases in isometric contractile force (ICF) at concentrations of 1, 10 and 30 µM (10 µM = ~6150 ng/mL), and significantly decreased Tp-e interval at 30 µM (see Table 5 and Figure 3). These effects occurred at concentrations higher than would be expected at the proposed clinical dose (2 mg/day: C_{max} = 22 ng/mL), although decreased LVEF has been observed clinically. Note: ICF findings were similar at 1 and 10 µM.

Table 5: Effects of GSK1120212 on the QT, Tp-e intervals, EAD, TdP, QRS duration and isometric contractile force

		Control	0.3 µM	1 µM	10 µM	30 µM
1120212	QT (ms)	331.0±6.2	331.3±8.0	324.3±8.3	318.5±6.7	324.0±11.5
	T_{p-e} (ms)	55.3±2.1	54.0±2.5	53.0±3.4	50.8±4.8	41.0±2.3**
	EAD/TdP	0	0	0	0	0
	TdP Score	0.0	0.0	0.0	-0.5	-1
	QRS (ms)	38.0±2.3	38.0±2.3	38.5±1.7	39.5±1.8	41.8±1.3
	ICF(%)	0.0±0.0	2.8±3.3	-14.0±6.5	-16.3±4.5*	-64.8±0.4**

ICF: isometric contractile force; *: p<0.05; **:p<0.01.

Figure 3: ECG and isometric contractile force (ICF) tracings recorded in control conditions and in the presence of GSK1120212 (BCL = 2000ms)



(Excerpted from Applicant's submission)

Based on these data, the drug is not expected to exert a TdP risk *in vivo* when free plasma concentrations are less than 30 µM. Sodium current may be inhibited at high

drug concentrations. The observed decrease in isometric contractile force and Tp-e interval without a marked increase in QRS duration was interpreted to possibly be associated with a potential inhibitory effect on calcium current.

Study title: Effect of p15 inducer related compound on QT prolongation in Isoflurane- anesthetized dogs

Study no.:	CD2007/01301/00 (05BCSAAD01)
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study report:	June 13, 2005
GLP compliance:	Yes
QA statement:	No
Drug, lot #, and % purity:	JTP-74057, Lot E, purity not provided

Key Study Findings:

- No changes in cardiovascular parameters (QT interval, QTc, PR interval, QRS duration, BP, or heart rate) following administration of 1 mg/kg JTP-74057 to anesthetized dogs

Methods and Results:
Following the continuous 10 minute infusion of JTP-74057 to isoflurane-anesthetized male beagle dogs (n=3) at 1 mg/kg (20 mg/m^2; plasma concentration of ~ 3-fold effective dose), cardiovascular monitoring was conducted prior to and 1, 3, 5, 10, 15 and 30 minutes following dosing. Plasma trametinib concentration increased with time up to 10 minutes following dosing initiation, with a peak concentration of 2.5 µM (1500ng/mL). There were no effects on QT prolongation, QTc, PR interval, QRS duration, BP, or heart rate.

Notes: As a result of the solubility limit of the drug in DMSO, 1 mg/kg was considered the maximum feasible dose. The plasma concentration estimation was based on the plasma concentration of 0.81 µmol/L at the effective dose of 3 mg/kg in mice.

Study title: GSK1120212B: Acute effects on cardiovascular function following oral administration in the conscious Beagle dog

Study no.:	CD2007/00962/00 (G07309)
Study report location:	Electronic submission, M4.2.1.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 27, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK1120212B (b) (4) DMSO solvate form); batch # EE169079, 99.4% pure

Key Study Findings:

- No changes in cardiovascular parameters, arterial blood pressure, heart rate, ECG intervals, or body temperature following oral administration of 0.5, 0.75 or 1.5 mg/m^2 to conscious dogs

Methods

Doses:	0.5, 0.75, 1.5 mg/m^2
Frequency of dosing:	Single doses
Route of administration:	Oral gavage
Dose volume:	Not indicated
Formulation/Vehicle:	Sodium lauryl sulfate/D-Mannitol
Species/Strain:	Beagle dog
Number/Sex/Group:	4M/dose
Age:	22-32 months
Weight:	9.4-12.4kg
Satellite groups:	None
Unique study design:	▲Study was performed in conscious dogs dosed on separate days with 7 days between each dose according to a modified 4 x 4 Latin square crossover paradigm (see below). ▲Two additional dogs administered vehicle or 0.75 mg/m^2 on D29 due to technical problems associated with acquisition of telemetry signal on D8 and D22 with Dog 103. (Data from Dog 103 collected on D8 and 22 excluded from analysis). ▲Cardiovascular and ECG measurements obtained in conscious, non-restrained dogs using surgically implanted telemetry device (Data Sciences International). ▲Parameters measured: -Systolic, diastolic, and mean arterial BP -Heart rate -Pulse pressure -Body temperature -ECG intervals (RR, QRS, PR, QT, calculated QT_c) ▲QTc calculated from QT data according to equation $QTc=QT + a(e^{b700} - e^{bRR})$ ▲Data recorded 5 days following dosing

Reference ID: 3293647

Dosing Schedule (0.5, 0.75 or 1.5 mg/m² of Test Article or Vehicle)*					
Animal Number	Day 1	Day 8	Day 15	Day 22	Day 29
101	Vehicle	0.5	0.75	1.5	NA
102	0.75	Vehicle	1.5	0.5	NA
103	1.5	0.75@	0.5	Vehicle@	NA
105	0.5	1.5	Vehicle	0.75	NA
106	NA	NA	NA	NA	0.75
107	NA	NA	NA	NA	Vehicle

* Dose is expressed as mg/m² of the parent compound and the first day of dosing was designated as Day 1.
@ Due to a technical problem, these doses were repeated at the end of the study.
NA Animals were not dosed on these days.

(Excerpted from Applicant's submission

Study Title: GSK1120212B: 3-Week Once Daily Oral Investigative Study in Male Mice
Study #: 2011N128277_00
This study was conducted at GlaxoSmithKline, King of Prussia, PA. It was not monitored for GLP compliance.
The objective of this study was to determine the effects of GSK1120212 on cardiac structure and function using histopathology and echocardiography following daily, oral, repeat dosing in male Crl:CD1(ICR) mice for approximately 3 weeks

Mice were selected for the *in vivo* study of cardiac toxicity of trametinib as this species tolerated higher systemic exposures than those observed in rats in repeat dose toxicology studies.

Fifteen male mice/group were administered 0.25 and 0.5 mg/kg trametinib daily by oral gavage for 21 days, with an additional 15 males at each dose level included for toxicokinetics evaluation. On Days 21/22 of the study echocardiography was conducted for 10 mice (anesthetized with isoflurane) at each dose level prior to and following challenge with the sympathomimetic drug dobutamine to assess cardiac contractile reserve.. Histopathology was evaluated using the remaining 5 mice in each group. Clinical observations, body weights, echocardiography, heart and lung weights, and macroscopic and microscopic observations were recorded.

Decreased mean absolute and relative heart weights were observed in mice given ≥0.25 mg/kg/day (~10% less than controls at each dose level). There were no trametinib-related macroscopic or microscopic findings in these mice. Based on AUC trametinib exposure in mice at doses of 0.25 and 0.5 mg/kg/day was ~3- and 7-fold (Table 6), the clinical exposure at the recommended dose of 2 mg/day in humans, respectively.

Table 6: A summary of plasma toxicokinetic values in mice

Male Composite Toxicokinetic Parameters:			
Parameter n=3/group/timepoint	Period	Dose (mg/kg/day)	
		0.25	0.50
AUC_{0-t} (ng.h/mL)[a]	Day 7	1110	2640
C_{max} (ng/mL)	Day 7	89.9	328
T_{max} (h)	Day 7	2.00	2.00

a. For the purpose of calculating AUC_{0-t}, the concentrations at time zero were assigned with the composite concentrations at 24 hours

Echocardiographic assessments showed no differences in left ventricular wall thickness or dimensions at end diastole in trametinib treated mice, however, there were effects on left ventricular function as evidenced by decreases in cardiac output (CO), stroke volume (SV), ejection fraction (EF), fractional area change (FAC) and fractional shortening (FS). Additionally, heart rate was lower in mice given 0.5 mg/kg/day as compared to vehicle controls. Importantly, dobutamine-induced contractility was preserved in trametinib-treated mice and was slightly enhanced in mice given 0.5 mg/kg/day.

These *in vivo* results demonstrate decreased left ventricular function performance in mice administered trametinib, similar to that seen in human subjects, although at higher exposures.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The Pharmacokinetics of GSK1120212 in Plasma Following Oral Administration of GSK1120212B at Nominal Doses of 0.1, 0.3 or 1 mg/kg/day to Female Athymic Nude Mice (nu/nu) for 14 Days Followed by a Washout Period (2011N121723 00)

Exposure (AUC_T) was essentially dose-proportional (Table 7); however, there was evidence of accumulation from Day 1 to 7, but by Day 14, steady state appeared to have been reached as there was no further accumulation. Peak (C_{max}) exposures were supraportional over the dose range from 0.1-1.0 mg/kg/day; a 10-fold increase in dose yielded up to 40-fold increase in peak exposure. T_{max} generally occurred within 2-4 hours of dose administration. Half-life and clearance were not estimated due to volume limitations.

Table 7: The PK Profile of GSK1120212 in Female Athymic Nude Mice Following Oral Dosing for 14 Days

Parameter	Day	Dose (mg/kg/Day)		
		0.1	0.3	1.0
AUC_T	1	122	705	1604
	7	349	1747	6911
	14	347	1431	6785
C_{max}	1	18.9	98.5	310
	7	29.6	165	786
	14	23.3	155	944
T_{max}	1	0.50	2.00	2.00
	7	2.00	4.00	4.00
	14	2.00	4.00	2.00

An Evaluation of Systemic Exposure of GSK1120212 Following 22 Days of Repeat Oral Administration ofGSK1120212B in CD-1 Female Nude Mice Bearing a A375P F11s-Human Melanoma Tumor Xenograft (Bioanalysis and Pharmacokinetic Support for A375P Flls-GSK-e202) (2011N125777_00)

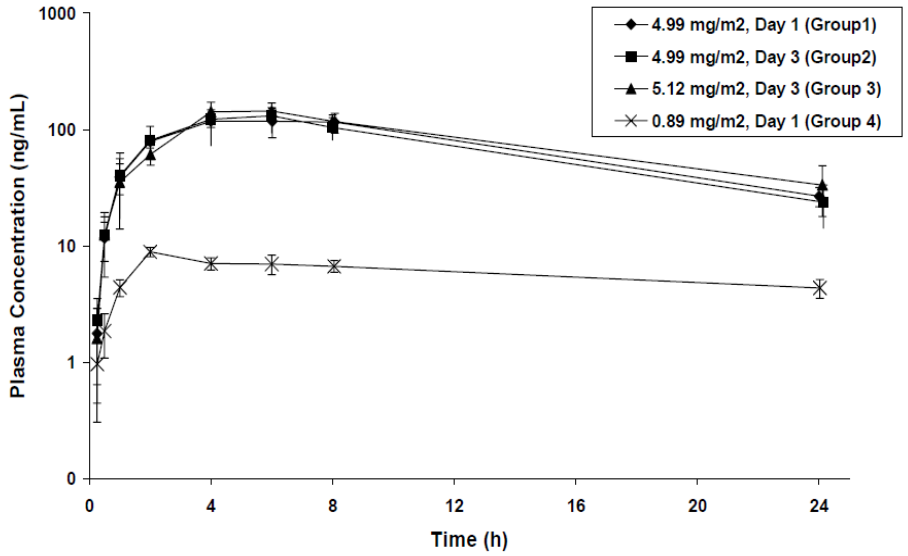
The pharmacokinetic profiles of female CD-1 nu/nu mice (N = 2-3/timepoint) hosting A375PF11S xenografts were assessed following 22 days of repeat-dose exposure at a dose of 0.3 mg/kg. Plasma samples were collected at 0 (pre-dose), 2, 4, or 8 hours post-dose. Trough (pre-dose) concentrations ranged from ~25-80 ng/mL. Peak circulating post-dose exposures were in the range of ~180-330 ng/mL. Concentrations remained high (~200 ng/mL) for at least 8 hours following dose administration.

Pharmacokinetics of GSK1120212 Following Oral Administrations of (b) (4) Suspension ofGSK1120212B to Male Sprague-Dawley Rats Under Fed Conditions (CD2007 /00787100)

The pharmacokinetic profile of GSK1120212 was evaluated in SD rats, following oral gavage of different suspension formulations (GSK1120212-DMSO solvate; 5% mannitol, 1.5% HPMC, 0.2% SDS and (b) (4); particle sizes of GSK1120212 ranged from (b) (4)). The purpose of this study was to evaluate different (b) (4) formulations for day-to day variability within formulations, and to compare and evaluate the effect of storage on formulation stability. A secondary objective was to set doses for subsequent toxicology studies. To that end, doses (nominal doses of 1 or 5 mg/m^2; 5 mL/kg) were administered on Days 1 and 3. Groups 1 and 2 received the same formulation administered (dose = 5 mg/m^2) on Days 1 and 3, respectively, to assess the stability of the formulation. Group 3 received a freshly prepared formulation

(dose = 5 mg/m^2). Group 4 received a dose of 1 mg/m^2 on Day 1 to assess dose-linearity of the formulation. There was no demonstrable difference between freshly prepared or stored formulations over the 3 day period (Applicant-Figure 4). Doses were supraportional, as AUC and C_{max} increased 12-15-fold over the 5-fold increase in dose.

Figure 4: Mean (n=3/group) Concentration-Time Profiles of GSK1120212 administered on Days 1 or 3 at dose levels of 1 or 5 mg/m^2 in male SD rats (Applicant-Derived)



Pharmacokinetic study of JTP-74057 in Mice, Rats and Dogs (UH2007/00035/00)

The Applicant evaluated the single-dose oral bioavailability and pharmacokinetic profile of GSK1120212 (JTP-74057) after oral or IV administration, using various dosage forms (acetic acid solvate, DMSO solvate, or free form) in mice, rats and dogs. A summary of these results is provided in Table 8.

The IV forms were dissolved in DMSO and either administered directly (rat) or mixed in 100% PEG400 plus MiliQ water at a ratio of 1:2:7 (dog). Oral suspensions of the free form of GSK1120212 were prepared in 0.5% (w/v) MC. The acetic acid solvate oral dosing solution was weighed and dissolved in 100% PEG400. The DMSO solvate was weighed and mixed into 0.5%(w/v) MC solution, then ground and sonicated. Plasma samples were evaluated by LC-MS/MS or LC/PDA/MS.

Peak and overall exposures were generally dose-proportional in the mouse and supraportional the rat. The dog did not receive multiple dose levels, so no conclusions about dose proportionality were made.

Table 8: Pharmacokinetics of GSK1120212 across multiple species by the oral and IV routes of administration with multiple dosage forms

Species (dose: mg/kg)	Route	%F	AUC (μM*hr)	Cmax (μM)	T1/2 (hr)	Vdss (L/kg)
Mouse	PO	111[‡]	23.5[‡]	2.7[‡]	3.8	--
(1 IV and 3 PO)	IV		7.7[§]	0.57		0.9[§]
Rat	PO	42±7[†]	6.1±1.1[†]	0.47±0.14[†]	5.5±0.7	--
(1 IV and 3 PO)	IV		4.9±0.5[§]	0.62±0.05[§]		2.9±0.4[§]
Dog	PO	86±22[‡]	2.8±0.7[‡]	0.13±0.02	13.3±3.2‡	--
(0.3 IV and PO)	IV		3.3±0.1[§,]	0.17±0.03[§]		3.0±0.8[§]

†DMSO solvate

‡Acetic acid solvate

§Free form

*for IV = concentration at 5 minutes post-dose

GSKI120212: Preliminary Investigation of the Preclinical Pharmacokinetics, In Vitro Blood Cell Partitioning, Stability and In Vitro Plasma Protein Binding (UH2007100095/02)

This report summarizes data from a number of PK and bioanalytical studies. Details of the methodology, including dose, dosage form, species and route, are summarized in Applicant-Table 9 and Applicant-Table 10.

Table 9: Studies summarized in Report UH2007/00095/02) (Applicant-derived)

DMPK Study Number	Study Type
06MCD1393	Rat po PK on (b) (4) suspension of free form (3 mg/kg)
07CDUP0641	Rat po PK on suspension of free form (10 mg/kg)
06MCD1771	Rat po PK on DMSO solvate (3 mg/kg)
06MCD1285	Monkey iv/po PK (3 mg/kg; 5 mg/kg)
06MCD1222	Blood:Plasma concentration ratio (mouse, rat, dog, monkey, human)
06MCD1223	Blood stability (human)
06MCD1408, 06MCD1409	Plasma protein binding (rat, mouse, dog, monkey and human)
06MCD1681	Allometric scaling of human PK
06MCD1921	MEK and PI3K combination study in mouse

Table 10: Dosing regimens used in rat, monkey and mouse PK studies (Applicant-derived)

Study Type	Study ID	Formulation	Dose mg/kg	Dose Concentration mg/mL	Dose volume mL/kg	Time points min
Rat PO suspension of free form	07CDUP0641	(b) (4)	10	1	10	Blood: 0 (prior to dosing) and 20, 40, 60, 90, 120, 240, 360, 480, 720, 1440, 1800 and 2160
Rat PO suspension of (b) (4) free form	06MCD1393	(b) (4) sodium lauryl sulfate)	3	0.3	10	Plasma: 0 (prior to dosing) and 20, 40, 60, 90, 120, 240, 360, 480, 720, and 1440
Rat PO suspension of DMSO solvate (Fed vs Fasted)	06MCD1771	(b) (4)	3	0.6	5	Plasma: 0 (prior to dosing) and 20, 40, 60, 90, 120, 240, 360, 480, 720, and 1440
Monkey IV/PO solution of DMSO solvate	06MCD1285	(b) (4) propylene glycol (b) (4) propylene glycol (b) (4)	0.3 (IV) 0.3 (PO)	0.1 (IV) 0.1 (PO)	3 (IV) 3 (PO)	Blood: 0 (prior to dosing) and 15, 30, 45, 60, , 65, 75, 90, 120, 180, 240, 360, 480, 720 and 1440 (IV dosing) Blood 0 (prior to dosing) and 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 720 and 1440 (PO dosing) Urine: -18h-0h (predose), 0h-8h, and 8h-24h
Mouse PO suspension of DMSO solvate either alone or in combination with GSK1059615	06MCD1921	(b) (4)	5 (GSK1120212) 40 (GSK1059615) 5 + 40 (GSK1120212 + GSK1059615)	0.5 (GSK1120212) 4 (GSK1059615_ 0.5 + 4 (GSK1120212 +GSK1059615)	10 (GSK1120212) 10 (GSK1059615) 10 + 10 (GSK1120212 + GSK1059615)	0 (prior to dosing) and 15, 30, 60, 120, 240, 360,480 and 1440 min

Exposures in the rat were greatly affected by the formulation; administration of the free form produced an approximately 30-fold lower exposure than the DMSO solvate. Exposures were also reduced under fed conditions (2-3X). Exposures in the mouse following oral administration were supraportional, as an 8-fold increase in dose yielded an approximately 40-fold increase in exposure. The kinetic results are summarized in Table 11.

Table 11: Summary of Pharmacokinetic Parameters in Mice and Rats administered GSK1120212

Species	Formulation	Dose	C_{max}	AUC
Rat	Free form suspension[1]	10	5.7 ± 1.5	102.5
	Free form suspension[2]	3	22.0 ± 6.0	422.5 ± 122.5
	DMSO solvate suspension[3]	3 (fed)	65 ± 24	1016.6 ± 404.4
	DMSO solvate suspension[3]	3 (fasted)	319	3334.2
Mouse	Not stated	5	88 ± 14	1065.8 ± 113.9
	Not stated	40	7705 ± 1463	41984.2 ± 10350.2

(b) (4)

(b) (4) in 1% SDS

[3]DMSO solvate suspension in 0.5% w/v MC

The ratio of whole blood to plasma was <1, suggesting that blood cell association was low in all species tested (Applicant-Table 12).

Table 12: Summary of the Blood to Plasma Partitioning of GSK1120212 in Rat, Mouse, Dog, Monkey and Human Plasma (Applicant-derived)

Species	Nominal Conc. (ng/mL)	Cb/Cp	Nominal Conc. (ng/mL)	Cb/Cp
Rat	500	0.88 ± 0.01	5000	0.89 ± 0.05
Mouse	500	0.70 ± 0.02	5000	0.75 ± 0.10
Dog	500	0.54 ± 0.01	5000	0.59 ± 0.03
Monkey	500	0.72 ± 0.05	5000	0.63 ± 0.04
Human	500	0.50 ± 0.04	2000	0.56 ± 0.04

Cb/Cp = ratio of concentration in blood to the concentration in plasma
Raw Data listed in Appendix 13

Plasma protein binding was assessed by equilibrium dialysis. *In vitro* plasma protein binding was high in all species tested at 500 ng/mL. Binding was 95% in the mouse, 96% in the rat, 97% in the dog, 98% in the monkey, and 97% in humans.

Distribution

An In Vitro Investigation of the Transport via Heterologously Expressed Human Breast Cancer Resistance Protein of [^{14}C]GSK1120212 in MDCKII-BCRP cells (RD2008/00032/01)

[^{14}C]GSK1120212 was evaluated as a potential substrate of the breast cancer resistance protein (BCRP) in cultures of MDCKII cells transfected with human BCRP. Confluent cultures of polarized MDCKII-BCRP cells were incubated in medium containing 3 µM of [^{14}C]GSK1120212, for 15-30 minutes. Medium was tested for the presence of test article. As indicated in Table 13, there was no net transport of GSK1120212 in either the apical to basolateral (a→b), or in the basolateral to apical (b→a) directions. Cimetidine, a BCRP substrate, was used as a positive control. GF120918, a specific inhibitor of BCRP was added to replicate test and control cultures as a negative control. GSK1120212 was negative for BCRP-mediated transport in this study (Applicant-Table 13).

Table 13: BCRP-Mediated Transport Studies in MDCKII-BCRP Monolayers

Compound	Rate A→B (pmoles/min/cm²) [1]	Rate B→A (pmoles/min/cm²)	Efflux Ratio [2]	BCRP Substrate	A→B Mass Balance (%)	B→A Mass Balance (%)
[¹⁴C]GSK1120212	1.1 ± 0.04	1.4 ± 0.01	1.3	N	99 ± 6.3	101 ± 2.8
[¹⁴C]GSK1120212 + GF120918 [3]	1.3 ± 0.14	0.88 ± 0.03	0.69		97 ± 5.7	100 ± 2.8
[³H]Cimetidine [4]	0.28 ± 0.03	1.6 ± 0.02	5.6	Y	99 ± 1.6	101 ± 1.6
[³H]Cimetidine + GF120918	0.30 ± 0.05	0.27 ± 0.02	0.88		99 ± 2.8	101 ± 1.5

1. Data are the mean ± standard deviation from three monolayers. All donor compartments contained Lucifer yellow to determine monolayer integrity (pass criterion P7.4 ≤50 nm/s).
2. Compounds classified as a BCRP substrate if apical efflux ratio ≥2.
3. GF120918 was used at ca. 2 µM in both donor and receiver compartments.
4. Cimetidine (3 µM) was used as positive control (criterion for assay acceptability: efflux ratio >2).

An In Vitro Investigation into the Inhibition by GSKI120212 of Xenobiotic Transport via Human Breast Cancer Resistance Protein Heterologously Expressed in MDCKII Cells. (RD2007/01466/00)

GSK1120212 was evaluated as a potential inhibitor of the of [^{3}H]cimetidine transport in polarized cultures of MDCKII cells that exogenously express hBCRP. GSK1120212 was found to be an inhibitor of BCRP-mediated transport of [^{3}H]cimedine with an IC_{50} of 1.1 µM (Applicant-Figure 5 and Applicant-Table 14).

Figure 5: Inhibition of [^{3}H]Cimetidine Transport by GSK1120212

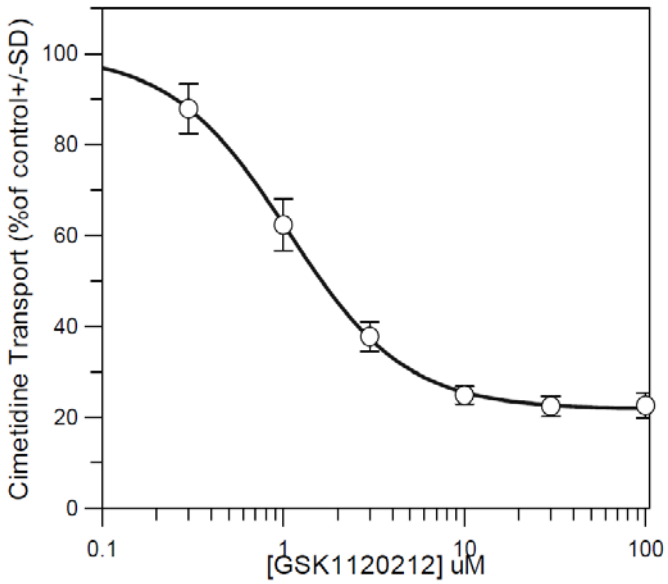


Table 14: Effect of GSK1120212 on hBCRP-Mediated Transport of 80 nM [^{3}H]Cimetidine in MDCKII BCRP Cells

Compound	Conc. (μM)	[^{3}H]-Cimetidine transport rate (pmole/cm^2/h) ± SD	[^{3}H]-Cimetidine transport rate (% control) ± SD
GSK1120212	0.3	3.5 ± 0.22	88 ± 5.4
	1	2.5 ± 0.23	62 ± 5.6
	3	1.5 ± 0.13	38 ± 3.3
	10	1.0 ± 0.07	25 ± 1.9
	30	0.90 ± 0.09	22 ± 2.2
	100	0.91 ± 0.11	23 ± 2.7
[^{3}H]-Cimetidine only		4.0 ± 0.15	100 ± 3.8
GF120918	2	0.79 ± 0.15	20 ± 3.8

1. Data are the mean and standard deviation from three monolayers.
2. SD is the standard deviation

Elimination of Radioactivity in Male and Female Intact and Male Bile Duct-Cannulated Sprague-Dawley Rats and Quantitative Tissue Distribution of Radioactivity in Partially Pigmented Male Rats Following a Single Oral Administration of [^{14}C]GSK1120212 (1 mg/kg) (CD2008/00024/00)

The mode and kinetics of [^{14}C]GSK1120212 hepatic elimination was evaluated in 3 male and 3 female intact SD rats, and 3 male bile-duct cannulated (BDC) SD rats. In addition, quantitative whole body autoradiography (QWBA) was performed in 7 male partially-pigmented (Long-Evans) rats to assess the tissue distribution of radiolabel following a single oral dose. The radiochemical purity and gravimetric specific activity of [^{14}C]GSK1120212 used in this study, were 98.0% and 73.4 μCi/mg, respectively, and mass balance was achieved, as total recoveries ranged from 90.6-101% of administered dose (Applicant-Table 15).

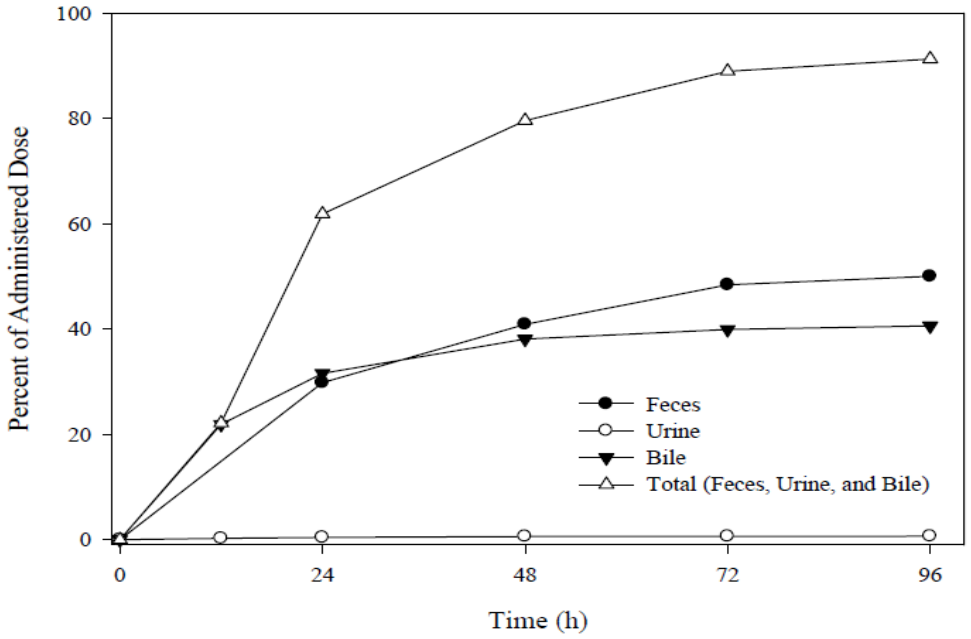
Table 15: Total mean recovery of radioactivity following oral administration of 1 mg/kg [14C]GSK1120212

Matrix	Percent of Administered Dose Group Intact Males	Intact Females	BDC Males
Feces	97.6 ± 2.96	82.8 ± 22.3	50.0 ± 3.40
Bile	-	-	40.6 ± 2.42
Urine	0.63 ± 0.06	0.91 ± 0.77	0.67 ± 0.04
Cage Rinse	0.02 ± 0.01	0.77 ± 1.31	0.01 ± 0.00
Cage Wash	0.01[a]	1.81[a]	0.11 ± 0.07
Cage Wipe	0.03[a]	1.06[a]	0.19 ± 0.08
Carcass	2.45 ± 0.29	3.16 ± 0.04	5.82 ± 0.78
Bile Cannula	-	-	0.00[a]
Jacket Rinse	-	-	0.00[a]
Total	101 ± 2.80	90.6 ± 15.3	97.4 ± 1.97

Note: Values are the mean ± standard deviation (n=3), where applicable.
- Not determined
BDC Bile duct-cannulated
a: At least one value was below the limit of quantitation; therefore, the standard deviation was not reported.

The majority of GSK1120212 was eliminated in the feces. A smaller amount was eliminated in urine. Elimination by both routes was most rapid during the first 24-48 hours in males and females (Figure 6; males only), and biliary content accounted for ~40% of the radioactivity eliminated in feces.

Figure 6: Mean Cumulative Elimination of Radioactivity in Male BDC SD Rats Following Administration of 1 mg/kg [^{14}C]GSK1120212



At early time points, tissue concentrations were highest in the liver, intestines, kidney, adrenals, harderian glands, preputial glands, stomach mucosa, pituitary glands, pancreas, salivary glands, and thyroid. Penetration was low in the brain. As compared with blood levels, the compound exhibited modest accumulation in pigmented skin and the uveal tract, indicating that the compound may have a potential for phototoxicity; however, the affinity was evidently low, as radioactivity levels were BLQ (<11.0 ng equiv/g) by 72 hours in these tissues (Table 16).

Table 16: Concentrations of Radioactivity in Melanin-Containing Tissues vs. Blood as Determined by QWBA

	2	**4**	**8**	**24**	**72**	**168**
Blood (cardiac)	143	102	56.4	13.9	BLQ	BLQ
Skin (unpigmented)	169	208	113	48.1	BLQ	BLQ
Skin (pigmented)	253	247	134	42.6	BLQ	BLQ
Uveal tract	400	393	263	99.9	BLQ	BLQ

An In Vitro Investigation of the Passive and Absorptive Membrane Permeability of C4CJGSKII20212 in MDCKII-MDR1 Cells (CD2007/01031/00)

The passive membrane permeability (pH 7.4) and the directional membrane permeability of [^{14}C]GSK1120212 were assessed in cultures of polarized epithelial cells expressing the MDR1 gene (MDCKII-MDR1). GSK1120212 exhibited high permeability and high absorption in this assay when evaluated at pH 7.4. At pH 5.5, the absorption of GSK1120212 was decreased, as indicated in Applicant-Table 17. Only apical to basolateral transport was evaluated in the absorption model, which is most relevant for assessing absorption from the GI tract. Amprenivir, a known MDR1 substrate, was used as a positive control in this model.

Table 17: Passive and Absorptive Membrane Permeability of [^{14}C]GSK1120212 in MDCKII-MDR1 Cell Monloayers

Compound		pH	Transport medium		P_x (nm/s)	Permeability Class
			Apical well	Basolateral well		
3 μM [^{3}H]amprenivir	Passive	7.4	DMEM	DMEM	174 ± 13	High
3 μM [^{14}C]GSK1120212	Passive	7.4	DMEM	DMEM	111 ± 28	High
3 μM [^{14}C]GSK1120212	Absorptive	7.4	FaSSIF	DMEM + 1% HSA	105 ± 13	High
3 μM [^{14}C]GSK1120212	Absorptive	5.5	FaSSIF	DMEM + 1% HSA	32 ± 6.4	Moderate

Data are the mean ± standard deviation from three monolayers
The pH of DMEM in all investigations is pH7.4
Permeability classification was as low (< 10 nm/s), moderate (10 - 100 nm/s) or high (> 100 nm/s).
FaSSIF: Fasted State Simulated Intestinal Fluid
DMEM: Dulbecco's Modified Eagle Medium
HSA: Human serum albumin

An In Vitro Investigation into the Inhibition by GSK1120212 of Xenobiotic Transport via Human OATPIBI and OATPIB3 (CD2007/01007/00)

GSK1120212 was evaluated for inhibition of the human hepatic uptake transporters, OATP1B1 and OATP1B3 in cells stably transfected to over-express these genes. (CHO-OATP1B1 and Human Embryonic Kidney MSRII for B1 and B3, respectively). Inhibition was measured by a decrease in the uptake of probe substrate [^{3}H]-Estradiol 17βD-glucuronide ([^{3}H]-EG).

[3H]GSK1120212 was found to inhibit [^{3}H]-EG uptake by both pathways at concentrations of ~ 1µM (IC_{50} for inhibition of OATP1B1 is 1.3±0.12 µM; the IC_{50} for inhibition of OATP1B3 is 0.94 ± 0.35 µM) – see Applicant-Figure 7 and Applicant-Figure 8**)**. Positive ([3H]-EG+10 µM rifamycin) and negative inhibition controls ([^{3}H]-EG) were employed in both assays and the rate and extent of transport were within acceptable limits based historical data.

Figure 7: Inhibition of [3H]-EG Uptake via the Human OATP1B3 Transporter by GSK1120212 in CHO-OATP1B1 Cells

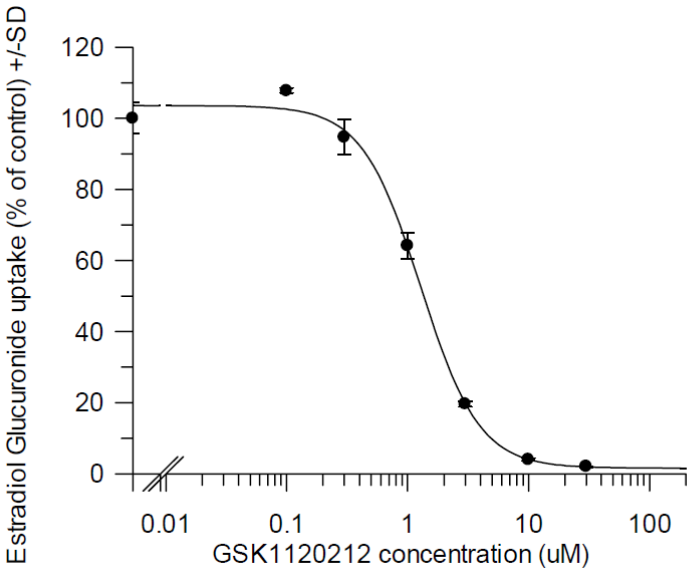
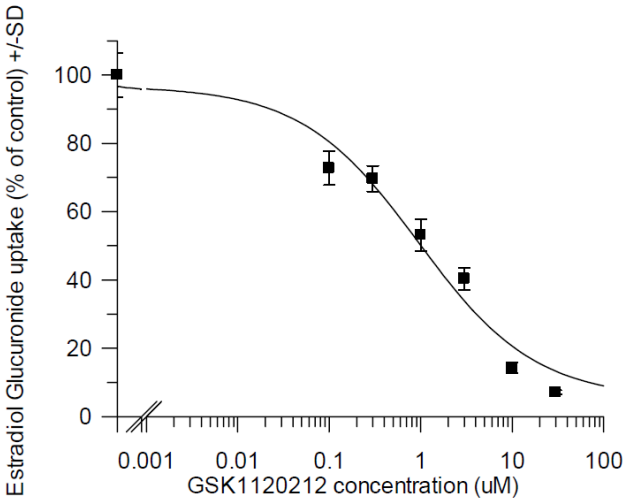


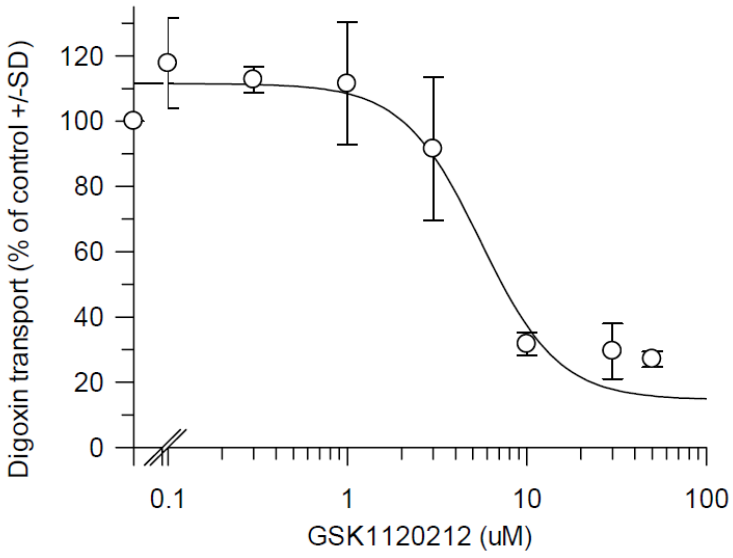
Figure 8: Inhibition of [^{3}H]-EG Uptake via the Human OATP1B3 Transporter by GSK1120212 in HEK-MRSII Cells Expressing OATPB3



An In Vitro Investigation of the Inhibition by GSK1120212 of Xenobiotic Transport Via Human P-Glycoprotein, Heterologously Expressed in MDCKII Cells (CD2007/00975/00)

GSK1120212 was tested for inhibition of human P-glycoprotein (PGP) in cultured cells overexpressing the PGP gene (MDR1). Inhibition was evaluated by monitoring uptake of digoxin, a probe substrate for the PGP transporter. GSK1120212 was found to inhibit PGP-mediated [^{3}H]-digoxin uptake by MDCKII-MDR1 cells, with an IC_{50} of 5.5 µM (Applicant-Figure 9). Positive (GF120918) and negative (digoxin only) controls for inhibition were within acceptable limits based on historical data.

Figure 9: Inhibition of Human PGP transport of 30 nM [^{3}H]-Digoxin by GSK1120212 in MDCKII-MDR1 Cells



An In Vitro Study of Blood Cell Association of [^{14}C]GSK1120212 in Healthy and Disease State (Cancer) Humans (2012N133368_00)

The plasma to blood cell partitioning of GSK1120212 was evaluated in blood samples collected from healthy and cancer patients. Samples were spiked with concentrations of 1, 10 or 50 ng/mL and incubated for 120 minutes, which was previously demonstrated to be the time at which equilibrium is achieved under these conditions.

There was no effect of disease state on the blood to plasma ratio; however, as the concentration of spiked drug increased, the percent found in plasma also increased, suggesting that binding in the blood cell compartment is concentration-dependent (or perhaps saturable). No concentration-dependency was observed at concentrations of 1 or 10 ng/mL; however, at higher concentrations, blood cell association decreased by about 2X (from 82-92% to 48-49%; Applicant-Table 18). GSK1120212 was found to be stable in human blood.

Table 18: Mean Blood Cell Association for GSK1120212 in Healthy Volunteer Blood and Blood from Human Donors Who Have Cancer

Species (sex)	Concentration (ng/mL)	Mean Hematocrit Value (%)[a]	Mean Blood/Plasma Concentration Ratio	Mean % Associated with Blood Cells
Healthy Human (male)	1	42.5	3.38	83
	10		3.20	82
	50		1.10	48
Disease State (cancer) Human (male)	1	36.5	7.92	92
	10		4.49	86
	50		1.25	49

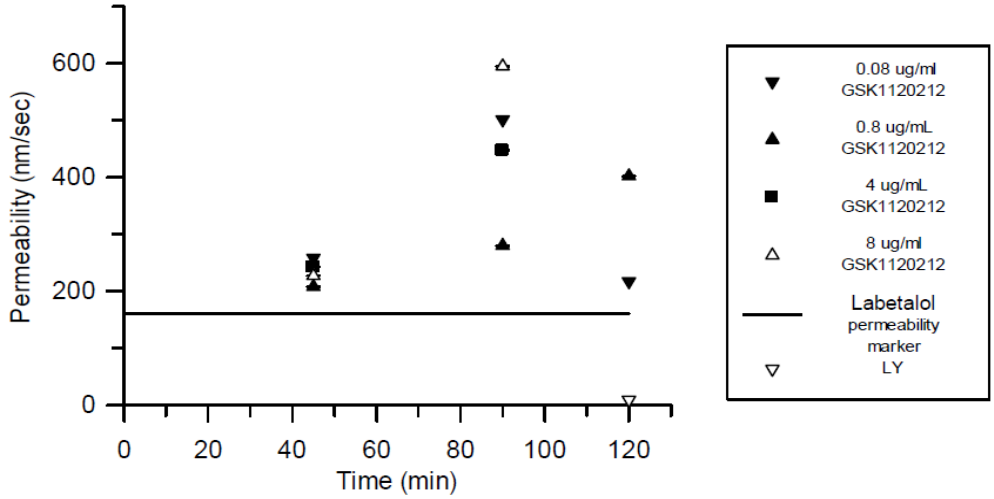
a. Hematocrit range for healthy male human whole blood: 40-52% [Zuckerman, 2007].

In Vitro Permeability Categorization of GSK 1120212 According to the Biopharmaceutics Classification System. (2010N104737_00)

The permeability category for GSK1120212 was assessed using the MDCKII-MDR1 cell line, a polarized canine epithelial kidney cell line stably transfected with the human MDR1 gene. Concentrations tested in this assay ranged from 0.08-8 µg/mL. Two pH ranges were evaluated, and all values were compared to those of the positive control, labetalol, a compound that exhibits high permeability. Only apical to basolateral permeability was assessed.

At pH 7.4, the permeability of GSK1120212 was 162-595 nm/sec. At pH 5.5, the permeability ranged from 186-611 nm/sec. These values exceeded those of the high-permeability positive control (labetalol); thus GSK1120212 was considered highly permeable in this assay (Applicant-Figure 10).

Figure 10: In vitro permeability of GSK1120212 over Time at pH 7.4 in MDCK-MDR1 cells



Lucifer yellow (LY) was measured at the 120 minute time point for all incubations. LY values are considered acceptable if ≤ 50 nm/sec.
GSK1120212 was assayed at the highest dose strength (2 mg) in 250mL of water: 8, 4, 0.8 and 0.08 µg/mL.
Labetalol is the reference marker for high permeability. A compound is considered a high permeant if the permeability value is equal to or greater than labetalol.

Metabolism

An In Vitro Evaluation of the Effect of GSK1120212 on PXR/CAR Mediated CYP3A4, CYP2B6, and CYP2C8 induction in Cultured Primary Human Hepatocytes (2012N131823_00)

GSK1120212 was evaluated in isolated human hepatocytes for its potential to inhibit CYP induction, as measured by upregulation of mRNA, in response to phenytoin and rifampicin, two canonical PXR-mediated CYP-inducers. GSK1120212 did not block the upregulation of CYPs 3A4, 2C8, or 2B6 in response to phenytoin or rifampicin exposure. Consistent with its known effects on CYP2C8 enzyme activity, however, increasing concentrations of GSK1120212 did reduce CYP2C8 catalytic activity both in the presence and absence of inducers (Table 19).

Table 19: Effect of Treating Cultured Human Hepatocytes with GSK1120212 on CYP2C8 mRNA and Catalytic activity (3 individual donors) in the Presence of Inducers or DMSO

a. **CYP2C8:**

Treatment	0.1% DMSO						50 μM Phenytoin						10 μM Rifampicin					
	mRNA treated/control			Catalytic % control			mRNA treated/control			Catalytic % control			mRNA treated/control			Catalytic % control		
Donor No.	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
0.0 μM GSK1120212	1.0	1.0	ND	100	100	100	3.5	4.0	ND	112	349	172	9.7	6.4	ND	NA	261	122
0.01 μM GSK1120212	0.89	1.1	ND	60	90	62	8.0	5.2	ND	103	287	131	13	10	ND	NA	270	118
0.05 μM GSK1120212	1.0	1.4	ND	44	91	50	6.3	5.5	ND	68	168	117	13	12	ND	NA	183	114
0.5 μM GSK1120212	1.2	2.1	ND	42	77	38	4.4	6.1	ND	68	109	42	13	9.2	ND	NA	108	49
1.0 μM GSK1120212	0.94	1.6	ND	57	59	22	5.0	6.4	ND	86	121	24	20	6.9	ND	NA	103	17
25 μM Sulforaphane	0.93	0.82	ND	77	133	58	1.4	1.1	ND	53	80	56	1.1	0.3	ND	NA	105	97

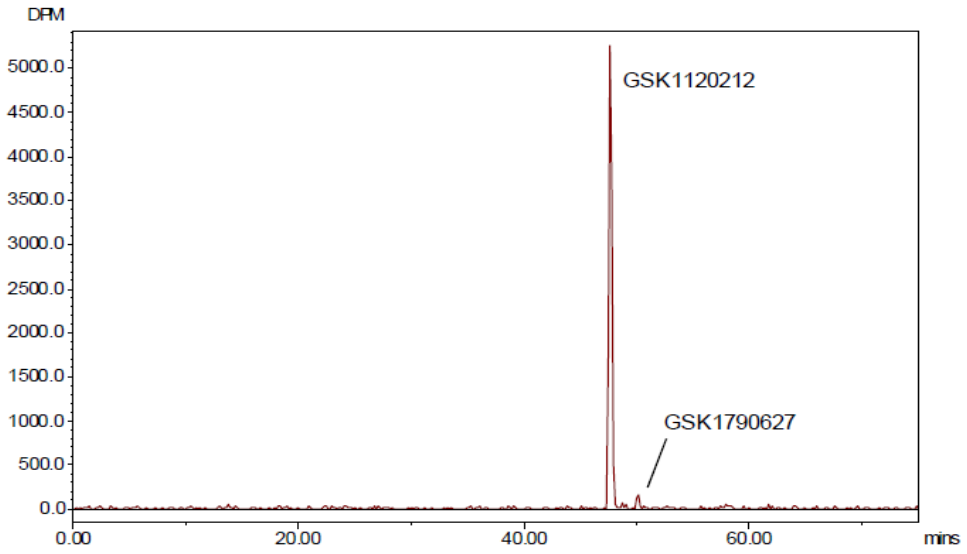
Values are the mean of 2 wells.
Data are presented to 2 significant figures.
ND: not determined.
NA: Not available due to the technical error

An in vitro investigation into the enzyme(s) responsible for hydrolytic cleavage of [^{14}C]GSK1120212 using appropriate in vitro systems (2012N135962 01)

GSK1120212 undergoes hydrolytic cleavage to produce GSK1790627. The Applicant evaluated cleavage of ^{14}C-GSK1120212 (specific activity 54.7 μCi) in incubations with human recombinant carboxylesterases 1b, 1c and 2 (hECS1b, hCES1c or hCES2); with acetylcholinesterases (AChE or BChE; human erythrocyte-derived), and human liver microsomes. The results were analyzed by radio-HPLC.

GSK1120212 was not a substrate of hECS1b, hCES1c or hCES2 or AChE or BChE, as no cleavage product was produced following incubation with these enzymes. GSK1120212 underwent metabolic cleavage in the presence of human liver microsomes (Applicant-Figure 11); however, the levels were very low (near the LOD, and below the LOQ).

Figure 11: Radiogram of [^{14}C]GSK1120212 Incubations with Human Liver Microsomes



An In Vitro Investigation of the Potential for GSK1120212 Bioactivation Following Incubation of [^{14}C]GSK1120212 with Human Liver Microsomes (CD2007/00194/00)

GSK1120212 was assessed for the potential to produce reactive metabolites via oxidative bioactivation, following incubation of [^{14}C]GSK1120212 with human liver microsomes. Following incubation, protein precipitation, and vacuum filtration of the reaction mixture, quantification of filter-associated radioactivity was measured by liquid scintillation. Reactions were performed in the presence and absence of NADPH. In the presence of NADPH, GSK1120212 produced 36±2.9 pmol/mg of retained radioactivity (data are expressed as the average pmol/mg of protein in one experiment preformed in triplicate), whereas acetaminophen produced 129±11 (Applicant-Table 20). The Applicant did not provide the LOD or LOQ for this assay. Compared with the positive control (acetaminophen), GSK1120212 is considered to have low potential for oxidative bioactivation.

Table 20: Retained [^{14}C]GSK1120212-Related Material Following Incubation in the presence of Liver Microsomes

	Binding in the absence of NADPH (pmol/mg)		Binding in the presence of NADPH (pmol/mg)		NADPH-Dependent binding (pmol/mg)	
Incubation Time (min)	30	60	30	60	30	60
GSK1120212	7.5±16	4.8±4.3	21±4.0	41±2.6	13±13	36±2.9
Acetaminophen Control	NA[1]	12±3.2	NA[1]	141±9.7	NA[1]	129±11

1. Acetaminophen control incubations were performed for 60 minutes only.

GSK1120212: Preliminary investigation of the in vitro drug metabolism (UH2007/00111/00)

The metabolite profile of GSK1120212 was investigated in a series of *in vitro* experiments intended to identify human metabolites and to identify appropriate animal models to characterize the preclinical safety profiles. In addition, the potential for CYP inhibition and induction by GSK1120212 and/or its metabolites was characterized using microsomes and recombinant human CYP isoenzymes (rhCYP). Metabolic stability was assessed following incubation with a variety of rhCYPs. Finally, intrinsic clearance in animals and humans was determined in hepatocytes and microsomal preparations obtained from multiple species. Permeability was also assessed in PGP-expressing MDKC cells.

Metabolic profiling was assessed in a series of *in vitro* studies using hepatocytes, microsomes, and rhCYPs from the rat, mouse, monkey, dog, and human. All *in vitro* human metabolites appear to be represented in at least one nonclinical species (Applicant-Table 21). Of the ten metabolites identified *in vitro* in the dog, rat, mouse, monkey, and human, three metabolites (PC2, PC3 and PC5) were not detected in subsequent hepatocyte studies in the rat or dog; however, all three are produced in the mouse.

Table 21: Summary of GSK1120212 metabolites identified from in vitro incubations with dog, rat, mouse, monkey and human liver microsomes, hepatocytes and rhCYP isoenzymes

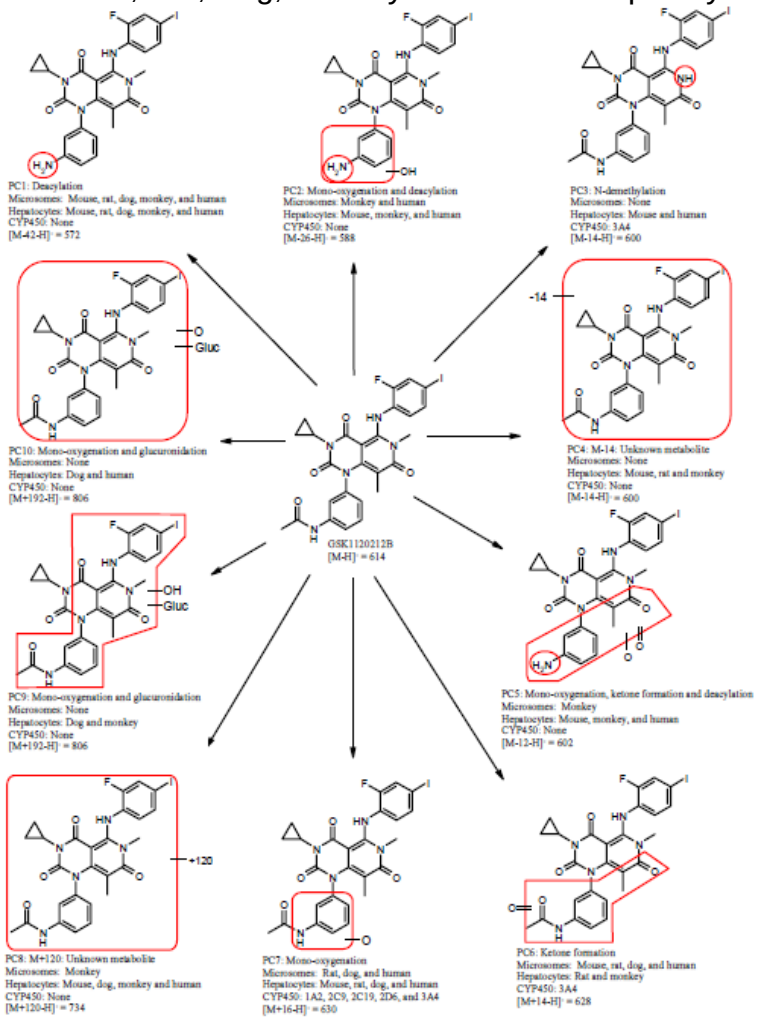
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Mouse	√	√	√	√	√	√	√	√		
Rat	√	(-)	(-)	√	(-)	√	√			
Dog	√	(-)	(-)		(-)	√	√	√	√	√
Monkey	√	√		√	√	√		√	√	
Human	√	√	√		√	√	√	√		√
1A2							√			
2C9							√			
2C19							√			
2D6							√			
3A4			√			√	√			

PC1: Deacylation; PC2: Monooxygenation and deacylation; PC3: N-demethylation; PC4: Unknown metabolite M-14; PC5: Mono-oxygenation, ketone formation and deacylation; PC6: Ketone formation; PC7: Mono-oxygenation; PC8: Unknown metabolite M + 120; PC9-10: Mono-oxygenation and glucuronidation
(-) indicates metabolite present in human but not in rat or dog incubations.

Reference ID: 3293647

GSK1120212 was found to be metabolized primarily by deacylation, demethylation, ketone formation, mono-oxygenation and glucuronidation. The proposed metabolic pathway for GSK1120212 is given in Figure 12 (Applicant-Derived).

Figure 12: Proposed Metabolic Pathway for GSK1120212 Following Incubation with Mouse, Rat, Dog, Monkey and Human Hepatocytes



GSK1120212 was a substrate of CYPs 1A2, 2C9, 2D6, 2C19, and 3A4; however, the extent of metabolic clearance by these pathways appears to be relatively low (Applicant-Figure 13). Although the different assay formats varied numerically in the extent of metabolic inhibition by GSK1120212, the compound was generally considered a concentration-dependent inhibitor of CYPs 2C9 (IC_{50} = 2.2-5 µM), and 3A4 (IC_{50} = 3.2 µM). Less inhibitory potential was observed for CYPs 2C19 and 2D6 (IC_{50} ~ ≥10 µM; Applicant-Table 22**)**. CYP1A2 activity was increased (activation) in the presence of GSK1120212. There was no metabolism-dependent inhibition observed. Finally, the Applicant's evaluation of permeability indicates that GSK1120212 is not a PGP substrate, and that the drug is passively permeable across MDCKII-MDR1

monolayers; however, permeability has been evaluated separately in other studies conducted with GSK1120212.

Figure 13 Metabolic Stability of GSK1120212 Following Incubation with rhCYPs 1A2, 2C9, 2C19, 2D6 and 3A4

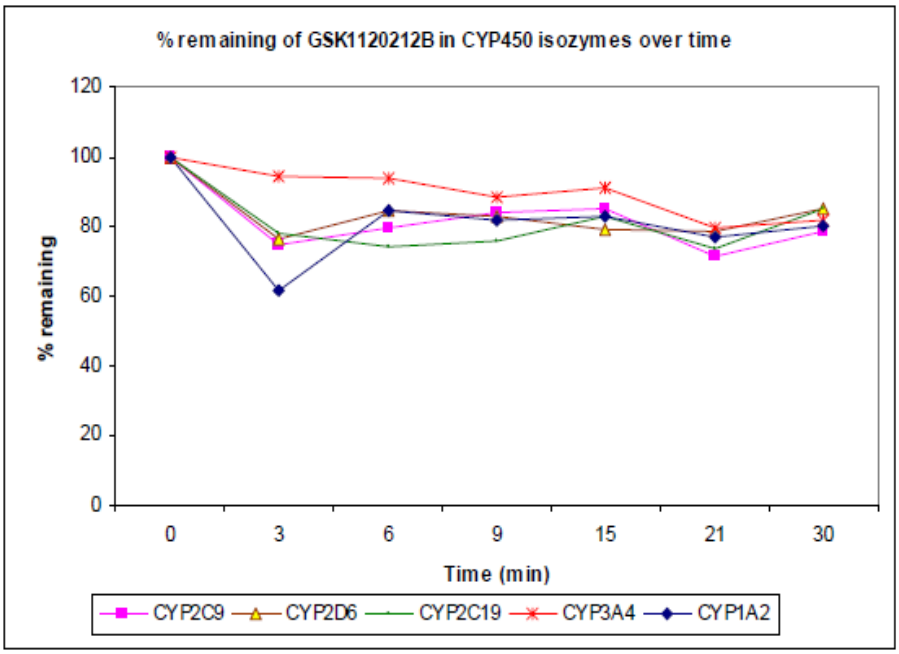


Table 22: CYP inhibition: Percentage Control Activity in Human Liver Microsomes Incubated in the Presence of GSK1120212

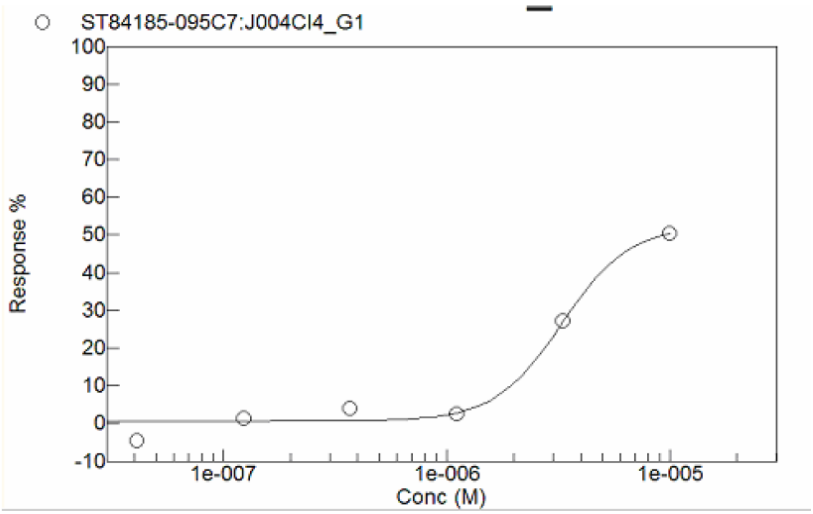
Study Number	GSK1120212 Concentration (μM)	Percentage Control Activity (%)					
		1A2	2C9	2C19	2D6	3A4 (Nif)	3A4 (mid)
06MCD1117	0	100.000	100.000	100.000	100.000	100.000	100.000
	0.02	96.600	97.100	101.000	94.400	103.300	102.200
	0.05	110.000	101.000	101.900	93.700	100.200	106.300
	0.170	102.900	98.200	93.400	88.400	106.300	55.300[1]
	0.540	111.900	95.200	92.600	85.800	107.200	128.300
	1.650	103.800	80.500	83.400	78.200	95.000	153.600
	5.450	98.200	48.100	47.000	54.300	91.500	210.800
	16.50	100.000	14.700	13.500	30.200	78.000	200.600

[1] Not included in calculation

In Vitro Cell Based Evaluation ofGSK1120212B as an Activator of the Nuclear Receptor PXR (RR2007/00033/00)

The potential for GSK1120212 to induce CYPs via the pregnane X receptor (PXR) pathway was investigated in HepG2 cells that were stably transfected to express the luciferase reporter gene under the control of the human PXR promoter. GSK1120212B exposure of HepG2 cells stably transfected with a luciferase reporter gene under the control of the human PXR promoter, resulted in a maximum response that was 33.6-50.4% of the efficacious human PXR activator, rifampicin. Figure 14 provides one of the two response curves generated in this study. GSK1120212B is therefore considered a moderate inducer of PXR target genes, such as CYP3A4, in humans.

Figure 14: Concentration-Response Curve Following Exposure of hPXR-Luc HepG2 cells to GSK1120212B



Concentration response curve for GSK1120212B. Data represents n = 1 calculated from 11 point, three fold, serial dilution curve in 100% DMSO with concentrations ranging from 0.2 nM to 10 μM. Data pictured shows 6 data points ranging from concentration 4.1 E -8 to 10 μM for clarity.

In Vitro Cell Based Evaluation of GSK1120212B as an Activator of the Nuclear Receptor Rat PXR (RR2007/00034/00)

The potential for GSK1120212 to induce CYPs via the pregnane X receptor (PXR) pathway was investigated in HepG2 cells that were stably transfected to express the luciferase reporter gene under the control of the rat PXR promoter. Luciferase levels achieved following stimulation of cells with the rat PXR activator PCN (5-pregnan-3β-OL-20-ONE-16α carbonitrile), used as a positive control in this assay, were within expected levels. Exposure of cells to increasing concentrations of GSK1120212 did not induce luciferase activity in this assay (< 5% of positive control); thus, under the conditions of this assay, GSK1120212 is considered unlikely to induce PXR-related genes, such as CYP3A4, in rats. These results did not support the findings of Study RR2007/0033/00, in which GSK1120212 was found to induce PXR transactivation in

comparison to the rifampicin positive control; thus, the ability of GSK1120212 to transactivate PXR-promoter elements is equivocal.

Metabolism of GSK1120212 in Male and Female Intact and Male Bile Duct-Cannulated Sprague-Dawley Rats following a Single Oral Administration of [^{14}C]GSK1120212 at a Dose Level of 1 mg/kg (CD2010/0029/00)

The metabolic profile of GSK1120212 was investigated in male and female intact SD rats and in male bile duct-cannulated (BDC) SD rats following a single oral dose of 1 mg/kg [^{14}C]GSK1120212. Plasma samples were collected at 2, 4, 8, and 24 hours from intact animals. Bile was collected for 48 and 72 hours post-dose. Feces and urine were collected and pooled (by matrix) for up to 144 hours post-dose. Samples were extracted from their respective matrices and analyzed by quantitative radio-HPLC. Metabolites were analyzed by LC/MS.

The major circulating species in this study was parent, which was maintained through 24 hours post-dose in males and females. The following metabolites were observed in plasma from males and females: M5, M7, M12, M13; however, their levels were low (< 10%). A Summary of the metabolites and the matrices in which they appeared, is given in Table 23**.** In addition, liver exposure was measured for parent (P), M13, M15, and M16.

Table 23: Qualitative Metabolite Detection by Matrix

	Plasma	**Bile**	**Feces**
P	+++ (> 24h)	+	+++
M2	ND	+	ND
M4	ND	++	ND
M5	+	+	ND
M6	ND	++	ND
M7	+	+	+
M12	+	ND	+
M13	+	ND	+
M16	ND	+	+
M17	ND	+	+
M18	ND	++	ND
M19	ND	+	+
+ = LOD – 10% of presumed administered dose ++ = 11 – 50% of presumed administered dose +++ = 51-90% of presumed administered dose			

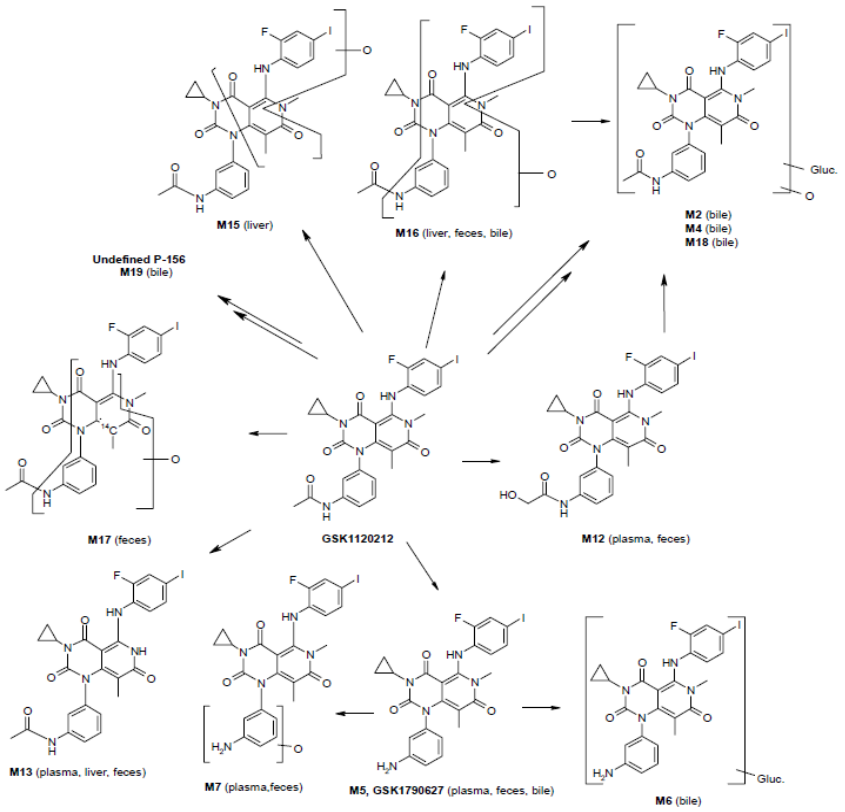
Reference ID: 3293647

The proposed metabolic pathway is provided in Applicant-Figure 15. The predominant route of elimination was via the feces (total 70-93%). Total parent levels in the feces were between 50-85% in this study. Of that, unabsorbed parent presumably accounted for the bulk of fecal parent elimination, as biliary excretion of parent was low (< 10%). Overall, biliary elimination (obtained in BDC males) accounted for approximately 51% of the total radioactivity eliminated. The majority (30%) of radioactivity eliminated in the bile was accounted for by the aggregate of the M4, M6 and M18 metabolites.

Urinary excretion was <1% of the presumed administered dose; therefore, urinary metabolites were not evaluated in this study.

It should be noted that metabolite data are expressed as a percentage of the total radioactive dose; however, the specific activity (µCi/mg) of the administered compound was not provided in the report. Moreover, the study does not claim to have achieved mass balance, nor was it evidently designed to do so, since the carcass, cage contents and cage wash were not evaluated for radioactivity. For these reasons, the study cannot be used to draw quantitative conclusions about metabolite exposure levels in the rodent.

Figure 15: Proposed Metabolic Pathway of GSK1120212 in the Rat



The in vivo metabolism of GSK1120212 investigated in male and female intact dogs following a single oral administration of [^{14}C]GSK1120212 (DMSO solvate) at a dose level of 0.5 mg/kg (CD2008/01199/00)

The metabolism of [^{14}C]GSK1120212 was evaluated following a single oral dose of 0.5 mg/kg in intact male and female dogs. According to the Applicant, the specific activity and radiochemical purity of the dose suspension prepared at the Test Facility (b) (4) was determined to be 84.4 µCi/mg and >99.0%,respectively. Plasma samples were collected at 2, 4, 8, and 24, post-dose. Feces were collected at 24, 48, 72, 96, and 120 hours. Urine was collected predose, at 12 and 24 hours, then every 24 hours thereafter (up to 168 hours). Quantification of radioactivity was performed by liquid scintillation counting (LSC). Identification of radiolabeled species was conducted by LC/MS.

Preterm euthanasia of 2 females was necessitated by poor clinical condition at approximately 48 hours post-dose; thus, sample analysis was performed for one male and one female dog. The proposed metabolic pathway is given in Sponsor-Figure 16. The largest circulating species in this study was unchanged parent, which accounted for ~60-80% of the administered dose. Exposure to GSK1120212 was robust through 24 hours post-dose. The majority of the radioactivity was excreted unchanged in the feces (9-12%). Unchanged parent was also detected at a low level in urine (<1%). Overall urinary elimination accounted for approximately 4% of the dose.

The major route of metabolic clearance was oxidation (M12, M24), N-demethylation (M13), deacylation plus mono-oxidation (M7), and deionization plus mono-oxygenation (M23). Oxidation, with or without deacetylation and deionization, contributed to over 20-30% of metabolic clearance.

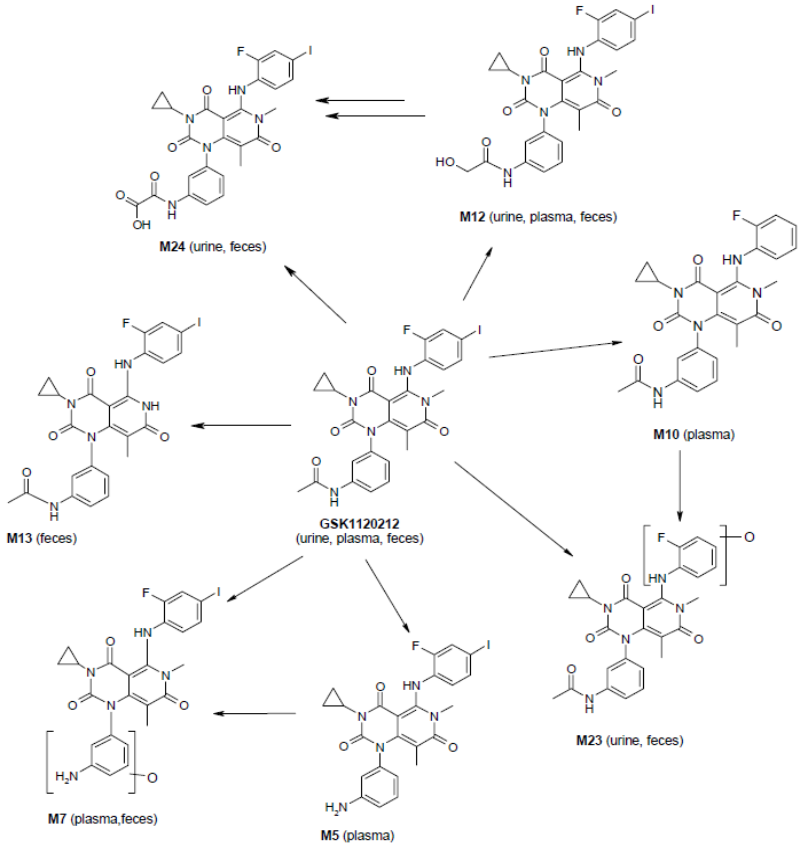
It should be noted that metabolite data are expressed as a percentage of the total radioactive dose; however, the study did not achieve mass balance (50-70% of administered dose detected); thus the study cannot be used to draw quantitative conclusions about metabolite exposure levels in the dog.

Table 24: Presence of Metabolites in Biological Compartments of Dog

	Plasma	**Feces**	**Urine**
Parent	+++	++	+
M5	+	ND	ND
M7	+	+	ND
M10	+	ND	ND
M12	+	+	+
M13	ND	+	ND
M23	ND	+	+
M24	ND	+	+

+ = LOD – 10% of presumed administered dose
++ = 11 – 50% of presumed administered dose
+++ = 51-90% of presumed administered dose

Figure 16: Proposed Metabolic Pathway for GSK1120212 in the Dog



An investigation of hepatic metabolism of GSK1120212 following administration of [^{14}C]GSK1120212 to the isolated perfused liver of the male Sprague-Dawley rat (CD2008/01198/00)

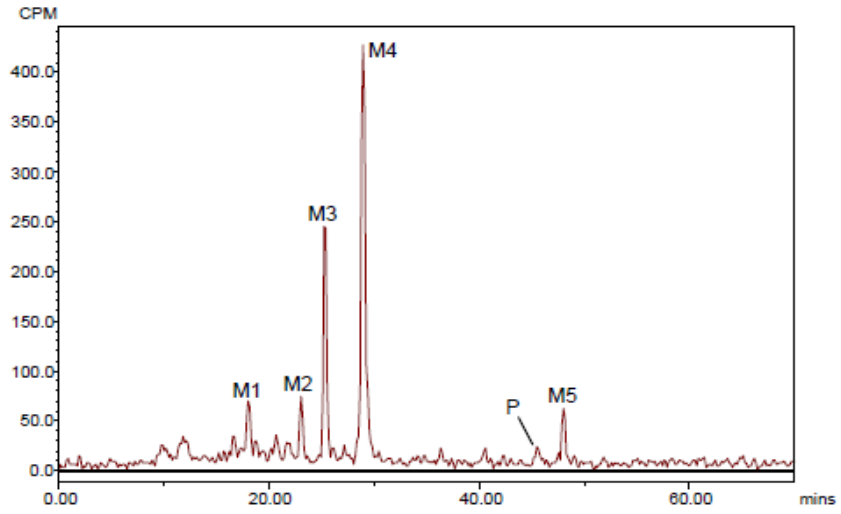
Bile duct-cannulated rats were euthanized and livers were perfused in situ at a nominal dose level of 30 mg/kg (50 µCi/animal) [^{14}C]GSK1120212 (plus cold GSK1120212) formulated into a solution of unspecified composition.

Bile was collected predose, and during the perfusion process. Predose bile samples from all five animals were pooled. Livers were flushed and weighed but not analyzed.

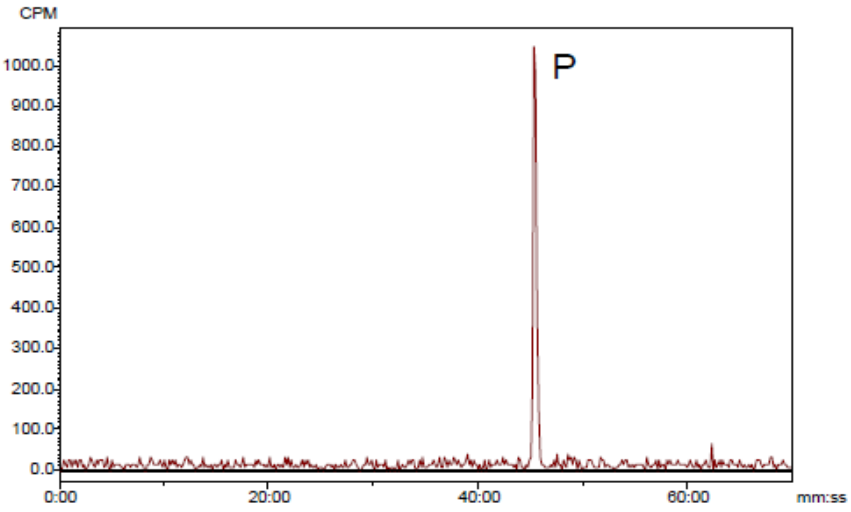
Metabolism of GSK1120212 was predominately via mono-oxygenation plus glucuronidation (denoted M2 and M4), and deacetylation (denoted M5). In addition, two metabolites, with masses corresponding to parent+303 (denoted M1) and parent+305 (denoted M3) were identified. A sample radiochromatogram showing the

resolution by retention time and relative peak intensities of these individual metabolites is provided in Sponsor-Figure 17**.**

Figure 17: Radiochromatogram of pooled post-dose IPRL bile (top) and pooled perfusate supernatant after dosing with [14C]GSK1120212 at a dose of 30 mg/kg in perfusate



Retention time (min)



Retention time (min)

An In Vitro Investigation into the Human Oxidative Enzymology of [^{14}C]GSK1120212 (CD2008/00864/00)

The biotransformation of GSK1120212 was investigated in human liver microsomes and recombinant cytochrome P450 (CYP) enzymes. Incubations with human microsomal preparations evaluated the effect of selective CYP3A4 inhibition (azamulin)

Reference ID: 3293647

and dependence on oxidative cofactors (NADPH) to confirm the metabolic route (Table 25). Microsomal preparations produced 11 metabolites, of which 6 were dependent on the presence of oxidative cofactor (NADPH): M7, M12, M13, M16, M17, M22. Each metabolite was present at very low levels by LC/MS, such that the aggregate accounted for < 1% of [^{14}C]GSK1120212. The remaining 5 metabolites (M5, M10, M15, M20, M21) were not dependent on NADPH. These are likely not the result of CYP-mediated processes. Because the levels of metabolite formation were so low, the effects of selective CYP inhibitors could not be reliably assessed; however, inhibition of CYP3A4 by azamulin led to loss of four of the 5 NADPH-dependent metabolites (M7, M12, M17, M22), suggesting that CYP3A4 is involved in their formation.

These data suggest that CYP3A4 is involved in the oxidative metabolism of GSK1120212. Contribution by other enzymes involved in non-NADPH-dependent pathways is also suggested.

Table 25: Summary of in Vitro [^{14}C] GSK1120212 Biotransformation in Incubations with Human Microsomes and CYP3A4 Isoenzymes

	Microsomes			**CYP3A4**	
ID	**+NADPH**	**-NADPH**	**+Azamulin**	**+NADPH**	**-NADPH**
P	X	X	X	X	X
M5	X	X	X	X	X
M7	X	--	--	X	--
M10	X	X	X	X	X
M12	X	--	--	X	--
M13	X	--	X	--	X
M15	--	X	X	X	X
M16	--	--	--	X	--
M17	X	--	--	X	--
M20	X	X	X	X	X
M21	X	X	X	X	X
M22	X	--	--	X	--

P= parent; M5 = deacetylation (P-42); M7 = deaceylation+monooxygenation (P-42+6); M10 = deionization (P-126); M12 = monooxygenation (P+16); M13 = monooxygenation (P+16); M16 = = monooxygenation (P+16); m17 = = monooxygenation (P+16); M20 = Undefined (P+34); M21 = Undefined (P+30); M22 = P-14

An *in vitro* investigation of the hepatic metabolism of [^{14}C]GSK1120212 in mouse, rat, female rabbit, dog, cynomolgus monkey and human (CD2008/00819/00)

[^{14}C]GSK1120212 was incubated in cultures of primary hepatocytes isolated from the CD-1 mouse (1♂), SD rat (1♂), Beagle dog (1♂), Dutch-belted rabbit (1♀), cynomolgus monkey (1♂), or two human livers.
Cells were cultured for 24 hours in the presence of 12.5 µM [^{14}C]GSK1120212, after which, lysates were prepared for radio-HPLC metabolite profiling and LC/MS analysis. The rate of ethoxycoumarin (25 µM) disappearance was within historical ranges for each species, indicating that the cultures were metabolically active.
Three metabolites formed by deacetylation with or without glucuronidation or monooxygenation, were identified in primary human hepatocyte cultures. Of these, all were present in at least three nonclinical species tested, as summarized in Table 26

Table 26: Interspecies Comparison of Metabolite Formation in Cultures of Primary Human, Mouse, Rat, Rabbit, Dog and Monkey Hepatocytes

	Human	Mouse	Rat	Rabbit	Dog	Monkey
M5	X	X	X	X	X	X
M6	X	X	X	X	--	X
M7	X	X	ND	X	ND	X

M5 = deacetylation; M6 = deacetylation+glucuronidation; M7 = deacetylation+monooxygenation

An In Vitro Investigation into the Inhibition of Human Cytochrome P450 Enzymes by GSK 1120212 (CD2008/00124/00)

The potential to inhibit CYP-mediated metabolism of canonical substrates, was evaluated following incubation of GSK1120212 with isolated CYP isoenzymes (CYPs 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4). Moderate to strong inhibition (< 10 µM) was observed for CYPs 2C8, 2C9, 2C19. Weak inhibition (> 10 µM) was observed for CYPs 1A2, 2A6, 2B6, 2D6, and 3A4. Inhibition was not metabolism-dependent for CYPs 2C8 and 2C9, as indicated in Applicant-.Table 27.

Inhibition of CYP2C8 by GSK1120212 occurs in the nM range, and could therefore occur clinically. Inhibition of other isozymes (2D6, 2C19, 2C9, 2C19), all occur at high exposures (µM concentrations) suggesting that these are less likely to precipitate DDIs with other co-administered substrates of these isoenzymes.

Table 27: Inhibition of Cytochrome P450 by GSK1120212

CYP	Substrate	Direct Inhibition IC_{50} (μM)	Metabolism-Dependent Inhibition		
			Control pre-inc[1] IC_{50} (μM)	NADPH pre-inc[2] IC_{50} (μM)	Fold Change[3] in IC_{50}
1A2	Phenacetin	>10	>10	>10	1.0
2A6	Coumarin	>10	>10	>10	1.0
2B6	Bupropion	>10	>10	>10	1.0
2C8	Rosiglitazone	0.34	0.24	0.26	0.92
2C9	Diclofenac	4.1	5.5	4.6	1.2
2C19	S-mephenytoin	5.0	5.4	5.4	1.0
2D6	Bufuralol	>10	7.7	6.7	1.1
3A4	Atorvastatin	>10	>10	9.8	1.0
3A4	Midazolam	activation	activation	activation	ND
3A4	Nifedipine	>10	>10	>10	1.0

1. Microsomes, buffer and GSK1120212 pre-incubated for 20 minutes with probe substrate prior to initiation of reaction with NADPH.
2. Microsomes, buffer and GSK1120212 pre-incubated for 20 minutes with NADPH prior to initiation of reaction with probe substrate.
3. ND = Not determined

An In Vitro Evaluation of the Induction Effect of GSK 1120212 on Cytochrome P450 Expression in Cultured Primary Human Hepatocytes (CD2007/01330/00)

The ability of GSK1120212 to induce CYP450 enzyme expression was evaluated in primary cultured human hepatocytes. In comparison to canonical inducers, little meaningful induction was observed for most CYP isoenzymes. High concentrations of GSK1120212 (3-10 μM), induce CYPs 2B6 and 3A4 at levels of up to 75% of the canonical inducers of those enzymes (Applicant-Table 28). The fold-multiples associated with these levels of induction were between 12-33X.

These data suggest that at relatively high concentrations, trametinib-mediated CYP induction can promote enhanced drug clearance and increased metabolite formation; however, given the relatively low plasma levels expected clinically, it is unclear whether these effects would manifest given the low exposure of trametinib in patients compared to concentrations required for CYP induction.

Table 28: Induction of CYP isoenzymes relative to canonical inducers (%)

Treatment	CYP1A2	CYP2B6	CYP3A4
	%of 50 μM Omeprazole	%of 50 μM Phenytoin	%of 10 μM Rifampcin
0.5% DMSO	0.00	0.00	0.00
0.01 μM GSK1120212	1.7	9.4	13
0.03 μM GSK1120212	1.1 ± 0.28	2.8 ± 3.1	7.7 ± 6.9
0.1 μM GSK1120212	1.2 ± 1.0	4.7 ± 8.2	7.9 ± 7.2
0.3 μM GSK1120212	2.2 ± 0.32	18 ± 11	23 ± 18
1 μM GSK1120212	2.4 ± 0.92	21 ± 7.1	33 ± 18
3 μM GSK1120212	2.1 ± 1.9	44 ± 12	61 ± 36
10 μM GSK1120212	2.6 ± 1.3	76 ± 54	67 ± 57
50 μM Omeprazole	100	ND	ND
50 μM Phenytoin	ND	100	ND
10 μM Rifampicin	ND	ND	100

Values are mean ± standard deviation from 3 human hepatocyte donors except the data for 0.01 μM are the mean of 2 human hepatocyte donors.
Data are presented in 2 significant figures.
ND: not determined.

5.2 Toxicokinetics

GSK1 120212B: Single-Dose Oral Toxicokinetic Study in Beagle Dogs (RD2007/00991/00) (Sponsor-Derived)

The toxicokinetic profile of GSK1120212 was evaluated in beagle dogs (1/sex/group) for the purposes of characterizing dose-linearity. Each animal received two doses of test article separated by a 14-day washout period. Administered doses were 0.25, 0.5, 1.0, and 2.0 in males and 0.25, 0.325, 0.425, and 0.5 mg/ m^2 in females. Exposures were essentially dose-proportional over the range from 1-2 mg/m^2 in males, but markedly supraportional over the range from 0.325-0.5 mg/m^2 in females (Applicant-Table 29). The compound exhibited a long half-life as indicated by slow rate of clearance depicted in Applicant-Figure 18. Exposures appear to be higher in females than males (Applicant-Table 29 – compare exposures at 0.5 mg/m^2).

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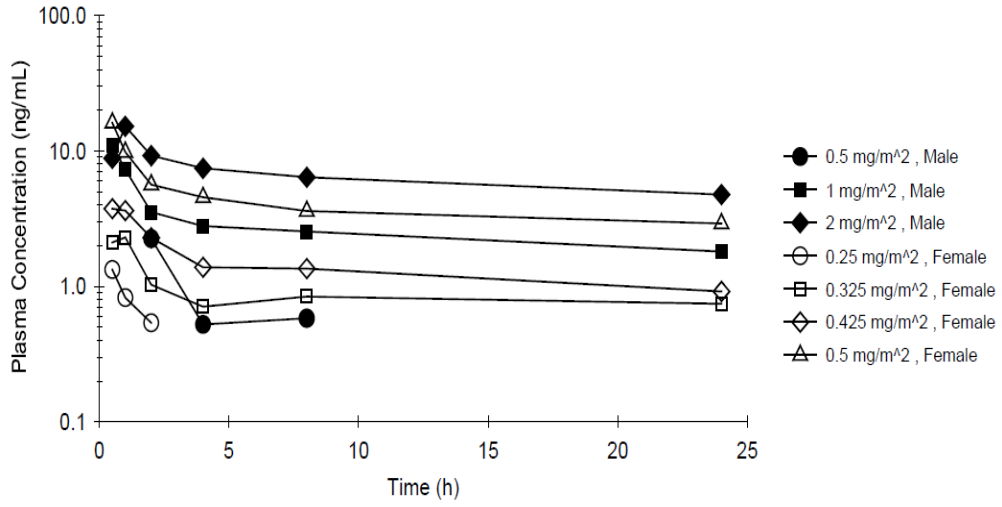
Table 29: TK Parameters in the Beagle Dog administered GSK1120212 on Days 1 and 14 (Sponsor-Derived)

Toxicokinetic Parameters:				
Parameter	Dose (mg/m^2)			
	0.25	0.5	1	2
Male (n=1)				
AUC_{0-t} (ng.h/mL)	NC	5.71	64.0	153
C_{max} (ng/mL)	NC	2.26	11.1	15.2
T_{max} (h)	NC	2.00	0.50	1.00
Parameter	Dose (mg/m^2)			
	0.25	0.325	0.425	0.50
Female (n=1)				
AUC_{0-t} (ng.h/mL)	1.53	20.7	32.7	96.2
C_{max} (ng/mL)	1.33	2.30	3.75	16.1
T_{max} (h)	0.50	1.00	0.50	0.50

NC = Not Calculated.

Figure 18: Individual single-dose concentration-time profiles for GSK1120212 in Beagle dogs (Applicant-Derived)



6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: GSK1120212B: A 13-Week Oral Gavage Toxicity Study in the Rat with a 4 Week Recovery

Study no.:	804013
Study report location:	eCTD: 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 19, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK1120212, Lot # 091216933, and Purity 99.4%

Key Study Findings

- 10 main group animals (≥0.25 mg/m^2), were found dead or euthanized early,
- Target organs of toxicity included skin, GI tact, lymphoid and hematopoietic organs, liver, adrenals, and female reproductive organs
- Male and female animals treated at the high dose (1 mg/m^2 for males, 0.5 mg/m^2 for females) were euthanized early due to severe skin and GI tract toxicities, including acanthosis, ulcerations/erosions, inflammation, and stomach erosion
- Bone marrow hypercellularity and degeneration/necrosis at mid and high dose
- Decreased corpora lutea and presence of ovarian cyst at all doses ≥0.125 mg/m^2

Methods

Doses:	Males: 0, 0.25, 0.5, 1.0 mg/m^2; Females: 0, 0.125, 0.25, 0.5 mg/m^2
Frequency of dosing:	Daily x 13 weeks
Route of administration:	Oral (Gavage)
Dose volume:	40 mL/m^2
Formulation/Vehicle:	Suspension in 1.5% (w/v) Hydroxypropylmethylcellulose (HPMC; Pharmacoat 603, 5% (w/v) Mannitol P60, 0.2% (w/v) Sodium Lauryl Sulfate (SLS)
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	12/sex/group main study; 6/sex/group control, MD and HD for recovery
Age:	10 to 12 weeks old
Weight:	Males: 353 to 410 g; Females: 225 to 265 g
Satellite groups:	3/sex/group Toxicokinetics

Table 30: Summary of experimental design

Group Number	Dose (mg/m2/day)#	Number of Animal			
		Main Study		Recovery Study	
		Males	Females	Males	Females
Toxicology Animals					
1/ Vehicle Control	0	12	12	6	6
2/ GSK1120212	0.25	12	—	—	—
3/ GSK1120212	0.5	12	—	6	—
4/ GSK1120212	1.0	12&	—	6&	—
5/ GSK1120212	0.125	—	12	—	—
6/ GSK1120212	0.25	—	12	—	6
7/ GSK1120212	0.5	—	12&	—	6&

All doses and concentrations are expressed in terms of parent compound, GSK1120212. Individual dose volumes were determined in mL based upon body surface area (BSA in m^2) calculated from the most recently collected body weight; BSA (m^2) = 10.5 x (BW)$^{2/3}$/10^4 where BW is in grams [Harkness, 1977]. Doses determined by body surface area (mg/m^2) calculated from the most recent body weight. The dose volume was 40 mL/m^2 (which corresponds to ~ 6 mL/kg for a 0.3 kg rat).

& As of Day 49 until the end of the treatment period, dose administration was discontinued for the surviving high dose animals.

Male and female rats were administered different low and high doses (Table 30) based on results from a 21-day study submitted to support the initiation of clinical trials and previously reviewed under IND 102175 by Dr. Robeena Aziz and summarized here. In this study GSK1120212-related skin lesions (acanthosis, ulceration, inflammation, and exudation) that were dose-dependent in both incidence and severity were observed at the end of treatment in males and females given ≥0.5 mg/m^2/day and contributed to morbidity in a female given 1 mg/m^2/day. Following a 2-week recovery period, microscopic findings in skin were still evident in rats given ≥0.5 mg/m^2/day but were less prevalent and/or severe than at the end of the treatment. Microscopic findings in the glandular stomach (mineralization) were seen at the end of the treatment period in males given ≥0.25 mg/m^2/day and females given ≥0.125 mg/m^2/day and were

Reference ID: 3293647

associated with increases in serum phosphorus and calcium-phosphorus product in males given 1 mg/m^2/day and females given ≥0.5 mg/m2/day. At the end of the 2 week recovery period, gastric changes (mineralization) were reduced in incidence in males given ≥0.5 mg/m^2/day and females given 1 mg/m^2/day and were absent in females given 0.5 mg/m^2/day. A dose of 1 mg/m^2/day was not tolerated in females based on skin lesions. For similar doses, systemic exposures in females were approximately two-fold higher than in males.

Observations and Results

Mortality

Altogether, 10 main study group rats (5 males and 5 females) were found dead or preterminally euthanized during the course of the study. Cause of death was most frequently attributed to skin toxicity (Table 31). Due to observations of adverse clinical signs in male and female rats at the high dose levels (1.0 and 0.5 mg/m^2, respectively) these animals were dosed for 48 days only. All the surviving high dose group animals were necropsied on Day 50.

Table 31: Day and causes of mortality for main study group rats

Animal Number	Sex	Group Number Identification/ Dose Level	Fate/ Day	Noteworthy Pathology Findings	Cause of Death
3004	M	3/Mid dose/Main (0.5 mg/m^2/day)	US/ Day 72	Skin ulcer/erosion; Stomach erosion, hyperplasia, inflammation.	Stomach inflammation
3014	M	3/Mid dose/Rec (0.5 mg/m^2/day)	US/ Day 55	Skin ulcer/erosion; Stomach erosion, hyperplasia, inflammation, mineralization.	Skin ulcer/erosion
4004	M	4/High dose/Main (1.0 mg/m^2/day)	US/ Day 37	Skin ulcer/erosion; Stomach erosion, hyperplasia, mineralization.	Skin ulcer/erosion
4006	M	4/High dose/Main (1.0 mg/m^2/day)	US/ Day 44	Skin ulcer/erosion; Stomach hyperplasia, mineralization.	Skin ulcer/erosion
4011	M	4/High dose/Main (1.0 mg/m^2/day)	US/ Day 44	Skin ulcer/erosion; Stomach dilatation, mineralization.	Skin ulcer/erosion
6509	F	6/Mid dose/Main (0.25 mg/m^2/day)	FD/ Day 86	Skin ulcer/erosion.	Bleeding accident (hemorrhage, skeletal muscle, ventral thoracic region)
7503	F	7/High dose/Main (0.5 mg/m^2/day)	US/ Day 37	Skin ulcer/erosion; Stomach erosion, hyperplasia, inflammation, mineralization.	Skin ulcer/erosion
7506	F	7/High dose/Main (0.5 mg/m^2/day)	FD/ Day 22	Skin ulcer/erosion; Stomach hyperplasia, inflammation.	Bleeding accident (hemorrhage, skeletal muscle, ventral thoracic region)
7512	F	7/High dose/Main (0.5 mg/m^2/day)	US/ Day 44	Skin ulcer/erosion; Stomach erosion, hyperplasia, inflammation, mineralization.	Skin ulcer/erosion
7517	F	7/High dose/Rec (0.5 mg/m^2/day)	US/ Day 44	Skin ulcer/erosion; Stomach mineralization.	Skin ulcer/erosion

Sex: M = Male; F = Female.
Fate: US = Unscheduled sacrifice; FD = Found dead.
Identification: Main = main study; Rec = recovery study

In addition, 3 toxicokinetic rats were found dead or preterminally euthanized during the course of the study (Table 32).

Table 32: Day and Causes of Mortality for Toxicokinetic Rats

Animal Number	Sex	Group Number Identification/ Dose Level	Fate/ Day	Noteworthy Pathology Findings	Cause of Death
3020	M	3/Mid dose (0.5 mg/m2/day)	US/ Day 82	NA	Skin ulcer/erosion
5513	F	5/Low dose (0.125 mg/m²/day)	FD/ Day 48	NA	Physical trauma due to accidental cage closure
7519	F	7/High dose (0.50 mg/m²/day)	FD/ Day 2	NA	Bleeding accident

Sex: M = Male; F = Female.
Fate: US = Unscheduled sacrifice; FD = Found dead.
NA = Not Applicable Tissues were retained but not examined.

Prior to euthanasia, mean body weight gain for high dose group males was reduced, from Week 3 to Week 7 (a gain of 27.8 g compared to a gain of 89.5 g in the control group, a decrease of 69%). The highest reduction in food consumption during was during Week 6 (decrease of 14% compared to a control value of 38 g/animal/day). During Week 3 to Week 7, the high dose females gained 20.3 g compared to 30.3 g in the control (a decrease of 33%).

Table 33: Changes in Hematologic parameters of preterminal rats (Day 50)

Parameter	Control*		0.5 mg/m^2	1 mg/m^2
	F	M	F	M
Neutrophils (10^9/L)	1.37	1.69	↑151%	↑270%
Monocytes (10^9/L)	0.21	0.25	↑41%	↑149%
Lymphocytes (10^9/L)	7.44	9.62	↓63%%	↓50%
RBC (10^{12}/L)	8.47	8.90	↓23%	↓25%
Hemoglobin (g/L)	159.3	165.6	↓19%	↓24%
Hematocrit (%)	47.2	50.2	↓25%	↓30%

Table 34: Changes in Clinical Chemistry parameters of preterminal rats (Day 50)

Parameter	Control*		0.5 mg/m^2	1 mg/m^2
	F	M	F	M
AST (U/L)	166.6	126.9	↑44%	↑100%
ALT (U/L)	47.4	47.6	↑50%	↑51%
Phosphorus (mmol/L)	2.28	2.53	↑21%	↑23%
Creatinine (µmol/L)	47.2	39.6	↓33%	↓29%
Total protein (g/L)	72.8	66.3	↓17%	↓11%
Albumin (g/L)	49.7	43.7	↓37%	↓48%
Potassium (mmol/L)	5.78	5.55	↓22%	↓12%

*Group mean values for hematology (Table 33) and clinical chemistry (Table 34) parameters on Day 50 were compared to vehicle control group means from Week 4 due to the lack of a concurrent control group on Day 50 (Week 8).

Hematology changes on Day 50 included increased absolute neutrophil count (up to 270%), absolute monocyte count (up to 150%) and decreased absolute lymphocyte count (by 63%), RBC count (by 25%), hemoglobin (by 24%) and hematocrit (by 30%). Clinical chemistry changes on Day 50, included increased AST (up to 100%), ALT (up to 70%) and phosphorus (up to 34%). There were decreases in creatinine (by 33%), total protein (by 17%), albumin (by 48%) and potassium (by 22%).

Histopathologic findings included focal or multifocal hepatocellular necrosis in the liver. All other microscopic findings for this group were similar to those in surviving dose groups (Table 40).

Clinical Signs

Table 35: Incidence of Clinical Observations

Sign	M		0.25 mg/m2	0.5 mg/m2	1.0 mg/m2
	F	0	0.125 mg/m2	0.25 mg/m2	0.5 mg/m2
Number of animals	M	18	12	18	18
	F	18	12	18	18
Skin scabs	M	9	12	18	18
	F	11	11	18	18
Skin Lesions	M	4	2	3	11
	F	--	--	2	10
Skin Red	M	--	2	7	11
	F	--	--	7	10
Hypersensitive	M	1	--	2	2
	F	--	--	1	5

-- - no remarkable observation compared to corresponding controls

Body Weights

Body weight was not significantly affected by administration of GSK1120212, except for the high dose (1.0 mg/m^2 in males and 0.5 mg/m^2 in females).

Feed Consumption

Like body weight, food consumption was not significantly affected by administration of GSK1120212, except for the high dose group males (1.0 mg/m^2).

Ophthalmoscopy

No remarkable observations

Hematology

Table 36: Percentage change in hematology parameters from control values

Parameter	M / F	0			0.25 mg/m^2 / 0.125 mg/m^2		0.5 mg/m^2 / 0.25 mg/m^2			1.0 mg/m^2 / 0.5 mg/m^2	
Terminal (wk 13)/*Recovery (wk 17)*											
		4 wks	13 wks	*17 wks*	4 wks	13 wks	4 wks	13 wks	*17 wks*	4 wks	13 wks
Monocytes 10^9/L	M	0.25	0.30	*0.32*	--	--	↑30%	↑32%	*↑19%*	↑131%	NA
	F	0.21	0.17	*0.14*	↑13%	↓10%	↓22%	↓17%	*↑9%*	↑59%	NA
Esonophils 10^9/L	M	0.12	0.16	*0.24*	↓36%	↓30%	↓49%	↓33%	*↑22%*	↓59%	NA
	F	0.12	0.15	*0.10*	↓22%	↓29%	↓34%	↓37%	*↑48%*	↓32%	NA
Neutrophils 10^9/L	M	1.69	1.84	*1.60*	↑8%	↑29%	↑42%	↑137%	*↑62%*	↑191%	NA
	F	1.37	1.03	*0.90*	↑13%	↑27%	--	↑72%	*↑42%*	↑140%	NA
Lymphocytes 10^9/L	M	9.62	8.14	*9.39*	↓27%	↓16%	↓24%	↓24%	*↓15%*	↓34%	NA
	F	7.44	5.29	*5.11*	↓5%	↓14%	↓31%	↓41%	*↓24%*	↓29%	NA
WBC	M	11.85	10.59	*11.67*	↓22%	--	↓14%	--	--	--	NA
	F	9.28	7.53	*6.30*	--	--	↓27%	↓25%	--	--	NA
Platelets 10^9/L	M	1138	1112	*1050*	--	--	↑6%	↑12%	--	15%	NA
	F	1212	1060	*909.0*	↓5%	--	↓7%	--	--	↑7%	NA
Basophils 10^9/L	M	0.03	0.03	*0.03*	↓43%	↓40%	↓20%	--	--	↓20%	NA
	F	0.02	0.02	*0.01*	↓32%	↓25%	↓50%	↓50%	--	↓14%	NA

↑ - increase, ↓ - decrease, -- - no remarkable difference from corresponding control value; NA - not applicable.

As shown in Table 36, treatment with trametinib led to increases in monocyte and neutrophil counts in Week 4 in male and female rats. There were also decreases in lymphocytes count, up to 41% at mid dose. These changes may be in response to skin and GI tract inflammation.

Clinical Chemistry

Table 37: Percentage change in clinical chemistry parameters from control values

Parameter	M / F	0			0.25 mg/m^2 / 0.125 mg/m^2		0.5 mg/m^2 / 0.25 mg/m^2			1.0 mg/m^2 / 0.5 mg/m^2	
Terminal (wk 13)/*Recovery (wk 17)*											
		4 wks	13 wks	*17 wks*	4 wks	13 wks	4 wks	13 wks	*17 wks*	4 wks	13 wks
AST U/L	M	126.9	175.3	*144.5*	↑10%	--	--	↑21%	--	↑44%	NA
	F	166.6	177.0	*108.7*	--	--	--	↑19%	*↑55%*	↑13%	NA
ALT U/L	M	47.6	51.2	*53.2*	↑16%	--	↑39%	↑24%	--	↑70%	NA
	F	47.4	60.5	*46.7*	↑10%	--	↑14%	↑22%	*↑37%*	↑59%	NA
Phosphorus mmol/L	M	2.53	2.28	*2.07*	↑3%	--	↑14%	↑9%	*↓5%*	↑23%	NA
	F	2.28	1.75	*1.45*	↑15%	↑9%	↑15%	↑16%	--	↑34%	NA
Albumin g/L	M	43.7	46.0	*45.7*	↓5%	↓5%	↓9%	↓17%	*↓6%*	↓19%	NA
	F	49.7	52.2	*52.5*	--	--	↓10%	↓8%	--	↓18%	NA

↑ - increase, ↓ - decrease, -- - no remarkable difference from corresponding control value; NA – not applicable.

Although increases in ALT were minimal, they were dose-related in Week 4 (Table 37) in male and female rats and may be due to the minimal to mild liver vacuolation. There were also dose-related increases in phosphorus in Week 4 (up to 34% in females at the high dose).

Urinalysis

No significant changes in urinalysis were observed.

Gross Pathology

Table 38: Incidence of gross pathology observations

Macroscopic Findings		0		0.25 mg/m^2	0.125 mg/m^2	0.5 mg/m^2	0.25 mg/m^2	1.0 mg/m^2	0.5 mg/m^2
		M	F	M	F	M	F	M	F
	Group Size:	**0**/12/*6*	**0**/12/*6*	**0/**12	**0**/12	**2**/11/*5*	**1**/11/*6*	**18**	**18**
	Grade	**Preterminal/**Terminal/*Recovery*							
Lymph node (LN) Enlargement	Present	--	--	1	--	5	3	**18**	**17**
LN Mandibular Enlargement	Present	--	1	2	1	7	5	**16**	**18**
Skin miscellaneous Scabs	Present	1	--	1	--	8	7	**17**	**17**
Stomach									
Area depressed	Present	--	1	3	--	3	1	**1**	**--**
Foci dark	Present	1	--	2	--	3	--	**5**	**3**
Thickening	Present	--	1	2	1	5	2	**2**	**3**

Organ Weights

Changes in organ weights were not determined for high dose males and females at early termination of dosing. In general, changes in organ weights were minimal and even when significant, they were non-dose-related. The decrease in ovarian weight (Table 39) is correlated with decrease in corpora lutea, while the increase in adrenal weights correlated with the microscopic observation of cortical hypertrophy/hyperplasia.

Table 39: Percentage change in absolute organ weights from control

	M		0.25 mg/m^2	0.5 mg/m^2	1.0 mg/m^2
Organ	F	0	0.125 mg/m^2	0.25 mg/m^2	0.5 mg/m^2
Preterminal/Terminal/*Recovery*					
Adrenals	M	0.0101	↓4%	↑3%	ND
	F	0.0213	↑8%	↑13%	ND
Ovary	M	N/A	N/A	N/A	N/A
	F	0.0306	↓7%	↓16%	ND

Organ weights relative to body weight were similar. ND – Not determined, N/A – Not applicable, ↓ - decrease, ↑ - increase

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Table 40: Incidence of histopathological observations

Microscopic Findings		0		0.25 mg/m^2	0.125 mg/m^2	0.5 mg/m^2	0.25 mg/m^2	1.0 mg/m^2	0.5 mg/m^2
		M	F	M	F	M	F	M	F
	Group Size:	12/*6*	12/*6*	12	12	**2**/11/*5*	**1**/11/*6*	**18**	**18**
	Grade	**Preterminal/**Terminal/*Recovery*							
Adrenals									
Hypertrophy/Hyperplasia: cortical	Minimal	--	--	--	--	1	--	**1**	**5**
	Mild	--	--	--	--	--	--	**4**	**3**
Bone Marrow									
Hypercelluarity: Myeloid	Min.	--	--	--	--	--	--	**1**	**2**
	Mild	--	--	--	--	2	4	**9**	**11**
	Moderate	--	--	--	--		1	**1**	**2**
Degeneration/ Necrosis	Minimal	--	--	--	--	3	--	**2**	--
	Mild	--	--	--	--	4	--	**7**	**1**
	Moderate	--	--	--	--	4	--	**7**	--
	Marked	--	--	--	--	1	--	--	--
Liver									
Vacuolation: Periportal	Minimal	6		5	1	8	2	**14**	**5**
	Mild	--	--	--	--	3	--	**1**	**1**
	Moderate	--	--	--	--	--	--	**1**	
Necrosis	Minimal	1	1	--	--	--	--	**1**	**3**
	Mild	--	--	--	--	--	--	**3**	
	Moderate	--	--	--	--	--	--	--	**1**
Lymph Nodes (LN)									
Hyperplasia/Lymphoplasmacytic	Minimal	--	--	1	--	1	1	**1**	**1**
	Mild	--	--	--	--	3	2	**4**	**9**
	Moderate	--	--	--	--	1	--	**5**	**6**
	Marked	--	--	--	--	--	--	**6**	**--**
LN Mandibular									
Hyperplasia/Lymphoplasmacytic	Minimal	--	--	--	--	2	1	**4**	**7**
	Mild	--	--	--	--	2		**3**	**6**
	Moderate	--	--	--	--	--	--	**5**	**2**
	Marked	--	--	--	--	--	--	**2**	**--**
Ovary									
Cyst	Minimal	--	--	--	--	--	2	--	**1**
	Mild		1	--	3	--	3	--	**4**
Corpora Lutea: Decreased	Mild		1	--	--	--	2	--	**4**
	Moderate	--	--	--	--	--	2	--	**4**
	Marked	--	--	--	2	--	3	--	**2**

Microscopic Findings		0		0.25 mg/m^2	0.125 mg/m^2	0.5 mg/m^2	0.25 mg/m^2	1.0 mg/m^2	0.5 mg/m^2
		M	F	M	F	M	F	M	F
	Group Size:	12/*6*	12/*6*	12	12	**2**/11/*5*	**1**/11/*6*	**18**	**18**
	Grade	**Preterminal/**Terminal/*Recovery*							
Skin									
Acanthosis	Minimal	--	--	--	--	2	--	**5**	**2**
	Mild	--	--	--	--	1	1	**10**	**5**
	Moderate	--	--	--	--	--	--	**--**	**2**
Ulceration/Erosion	Mininmal	--	--	--	--	1	--	**3**	**1**
	Mild	--	--	--	--	1	1	**8**	**5**
	Moderate	--	--	--	--	--	--	**1**	**1**
Exudate/Crust	Minimal	--	--	--	--	1	--	**5**	**2**
	Mild	--	--	--	--	1	1	**8**	**5**
	Moderate	--	--	--	--	--	--	**1**	**1**
Inflammation: Acute/subacute	Minimal	--	--	--	--	1	--	--	--
	Mild	--	--	--	--	2	--	**7**	**3**
	Moderate	--	--	--	--	--	--	**3**	**1**
Spleen									
Hematopoiesis/Extramedullary: increased	Minimal	--	--	--	--	1	2	**5**	**--**
	Mild	--	1	--	2	--	4	**1**	**3**
Stomach									
Mineralization: Glandular mucosa	Minimal	--	--	--	--	1	--	**--**	**5**
	Mild	--	--	--	--		--	**3**	**3**
	Moderate	--	--	--	--	1	--	**12**	--
	Marked	--	--	--	--	--	--	**1**	--
Erosion: Squamous mucosa	Minimal	--	--	--	--	--	--	**2**	--
	Mild	--	--	--	--	2	--	**1**	--
	Moderate			1		4	1	**2**	**2**
	Marked		1	1	--	--	1	**1**	**1**
Hyperplasia: Squamous mucosa	Minimal	--	--		--	--	--	**2**	**--**
	Mild	--	--	1	--	--	--	**6**	**1**
	Moderate			4	--	2	--	**3**	**2**
	Marked	--	1	--	--	--	2	**4**	**1**
Inflammation: Squamous mucosa	Minimal	--	--	--	--	--	1	**--**	**--**
	Mild	--	--	1	--	--	--	**2**	**1**
	Moderate	--	1	3	--	3	1	**2**	**2**
	Marked	--	--	--	--	2	1	**1**	**1**
Dilatation: Glandular	Min	--	--	--	--	1	--		**1**
	Mild	--	--	--	--	1	--	**7**	**1**
	Moderate	--	--	--	--	--	--	**2**	**--**

↑ - increase, ↓ - decrease, -- - no remarkable difference from corresponding control value.

Incidence of histopathologic findings are summarized in Table 40. Adrenal cortical hypertrophy/hyperplasia correlated with the slight increases in adrenal weights. In the bone marrow, myeloid hypercellularity may be secondary to inflammatory stimuli as many of these same rats had ulcer/erosions and inflammation at several skin sites. In addition, bone marrow degeneration/necrosis, present in male and female rats at doses ≥ 0.5 mg/m^2, was reportedly characterized by the loss of all cellular marrow elements in the metaphysis of the femur and/or tibia. In the liver,

vacuolation of periportal hepatocytes and focal or multifocal hepatocellular necrosis were increased in male and female rats. Lymphoplasmacytic hyperplasia of the lymph nodes correlated with the macroscopic observation of enlargement (Table 38). These changes were believed to be secondary to macroscopic and microscopic changes in the skin. Ovarian cysts were increased and the number of corpora lutea was decreased in female rats at all dose levels compared to control animals along with minimal decreases in ovarian weights. Histopathologic findings in the skin included: acanthosis, ulcer/erosion, exudate/crust, inflammation (acute or subacute), and/or hypotrichosis/alopecia.

Skin lesions were present in male rats and in female rats and correlated with the macroscopic observations of scabs (Table 38). Ulcerative/erosive skin lesions were considered the cause of death in most rats euthanized early due to poor and/or debilitating condition and were responsible for the early termination (Day 50) of high-dose male and female rats from the main and recovery study groups. Increased splenic extramedullary hematopoiesis was reportedly secondary to skin changes (ulcers/erosions and inflammation). Gastrointestinal toxicity was exemplified by mineralization of the glandular muocsa, hyperplasia and inflammation of the squamous mucosa, and erosions involving the squamous mucosa of the stomach in male and female rats. Erosions of the squamous mucosa had the macroscopic correlation of depressed areas in some rats.

Toxicokinetics

In males, mean AUC_{0-t} and C_{max} values were a little more than dose proportional from 0.5 mg/m^2 to 1 mg/m^2 on Day 1 but a little less than dose proportional in Week 4. Mean AUC_{0-t} and C_{max} values increased significantly (up to 5.7- and 5.5-fold, respectively) from Day 1 to Week 4 in male rats and continued to increase marginally (<2-fold) between Week 4 and Week 13 (Table 41), showing accumulation during repeated dosing

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Table 41: Mean toxicokinetic parameters for GSK1120212 in male rats

Parameter[a]	Period	Male		
		Dose of GSK1120212 (mg/m²/day)		
		0.25	0.5	1.0
AUC0-t (ng.h/mL)	Day 1	NC	33.1 [28.8 – 36.5]	76.5 [58.0 – 103]
	Week 4	72.7 [59.0 – 84.8]	188 [124 – 286]	285 [247 – 350]
	Week 13	95.4 [82.0 – 104]	277[b] [230, 323]	NA
Cmax (ng/mL)	Day 1	0.635 [0.561 – 0.763]	1.82 [1.37 – 2.14]	4.19 [3.17 – 5.55]
	Week 4	3.47 [2.82 – 4.03]	10.1 [6.37 – 16.6]	19.3 [15.6 – 26.7]
	Week 13	5.34 [4.57 – 6.45]	15.4[b] [13.1, 17.8]	NA
Median Tmax (h)	Day 1	4.00 [2.00 – 8.00]	2.00 [2.00 – 4.00]	8.00 [4.00 – 8.00]
	Week 4	1.00 [1.00 – 2.00]	4.00 [4.00 – 8.00]	8.00 [2.00 – 8.00]
	Week 13	4.00 [1.00 – 4.00]	4.00[b] [4.00, 4.00]	NA

NC = Not calculated due to insufficient plasma concentration data.
NA = Not applicable. Dosing for these animals was discontinued with the last dose given on Day 48.

a. Results are reported as mean unless stated otherwise and [range].
b. n = 2 One mid dose toxicokinetic male given 0.50 mg/m²/day was euthanized on Day 82 prior to Week 13 toxicokinetic sampling due to deteriorating clinical condition related to skin lesions/ulcerations.

In females, mean AUC_{0-t} and C_{max} values were a little more than dose proportional from 0.25 mg/m^2 to 0.5 mg/m^2 on Day 1 and Week 4. Mean AUC_{0-t} and C_{max} values increased significantly (up to 6.5-fold) from Day 1 to Week 4 in female rats and continued to increase marginally (<2-fold) between Week 4 and Week 13 (Table 42), showing accumulation during repeated dosing.

In terms of AUC_{0-t} and C_{max} values, female rats had exposures of about 1.5-fold compared to males at dose levels of 0.25 mg/m^2 and 0.5 mg/m^2.

Table 42: Mean toxicokinetic parameters for GSK1120212 in female rats

Parameter[a]	Period	Female		
		Dose of GSK1120212 (mg/m²/day)		
		0.125	0.25	0.5
AUC0-t (ng.h/mL)	Day 1	NC	18.7 [18.3 – 19.5]	49.1 [43.5 – 54.4]
	Week 4	80.1 [71.7 – 96.2]	122 [103 – 135]	287 [265 – 304]
	Week 13	102[b] [99.2, 105]	158 [132 – 192]	NA
Cmax (ng/mL)	Day 1	NC	1.03 [0.920 – 1.16]	2.82 [2.39 – 3.22]
	Week 4	3.92 [3.23 – 5.04]	6.63 [5.54 – 7.45]	16.1 [14.3 – 18.1]
	Week 13	5.30[b] [4.86, 5.75]	8.03 [6.79 – 9.91]	NA
Median Tmax (h)	Day 1	NC	4.00 [4.00 – 8.00]	4.00 [4.00 – 8.00]
	Week 4	8.00 [1.00 – 8.00]	8.00 [1.00 – 8.00]	2.00 [1.00 – 8.00]
	Week 13	2.50[b] [1.00, 4.00]	4.00 [1.00 – 4.00]	NA

NC = Not calculated due to insufficient plasma concentration data.
NA = Not applicable. Dosing for these animals was discontinued with the last dose given on Day 48.

a. Results are reported as mean unless stated otherwise and [range].
b. n = 2 One low dose toxicokinetic female given 0.125 mg/m²/day was found dead on Day 48 prior to Week 13 toxicokinetic sampling as a result of physical trauma related to a handling accident.

Study title: GSK1120212B: A 13-week Oral Gavage Toxicity Study in the Beagle Dog With a 4-week Recovery

Study no.: 803421
Study report location: 4.2.3.2.1
Conducting laboratory and location: (b) (4)
Date of study initiation: August 17, 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: GSK1120212B, Lot # 091216933, Purity 99.4%

Key Study Findings

- Major organs of toxicity included the skin, GI tract, lungs, and lymph nodes
- One male dog at the high dose level (0.6 mg/m^2) was euthanized early.

Methods

Doses:	0, 0.15, 0.3, 0.6/0.45 mg/m^2
Frequency of dosing:	Once Daily
Route of administration:	Oral (Gavage)
Dose volume:	40 mL/m^2
Formulation/Vehicle:	1.5% Hydroxypropylmethylcellulose (HPMC; Pharmacoat 603), 5% Mannitol P60, 0.2% Sodium Lauryl Sulphate (SLS)
Species/Strain:	Beagle Dogs
Number/Sex/Group:	4 dogs/sex/group for main study, and 2/sex/group for recovery, except the LD group
Age:	11-12 months
Weight:	Males 6.9 – 9.9 kg and Females 7.7 – 9.8 kg
Satellite groups:	None

Observations and Results

Mortality

One male dog (# 404) dosed at 0.6 mg/m^2 was euthanized on Day 14. Prior to euthanasia, this animal's clinical signs suggesting gastrointestinal toxicity included red/black feces, liquid feces, decreased fecal output, salivation, and red/yellow vomitus. In addition, this dog had a skin lesion in the prepucial area, red gums, was weak and dehydrated, had decreased activity, hematuria, decreased muscle tone, tremors, increased rectal temperature to 40°C, decreased food consumption (≤50 g/day from days 9-14), and body weight loss of 1.3 kg from Days 4 to 14. An unscheduled hematologic assessment conducted for Dog # 404 on Day 12 revealed increased WBC count (201% above baseline value of 6.76 x10^3/µL) increased neutrophils (320% above baseline value of 4.07 x10^3/µL) and monocytes (246% above baseline value of 0.41 x10^3/µL). Clinical chemistry changes included increased AST (50% above baseline value of 24 U/L), ALP (78% above baseline value of 96 U/L), triglycerides (55% above baseline value of 0.29 mmol/L), and cholesterol (65% above baseline value of 3.18 mmol/L) and decreased glucose (by 49% of the baseline value of 5.7 mmol/L).

Gross pathological examination revealed changes in the digestive tract including depressed and/or dark areas/foci in the tongue, pharynx, esophagus, stomach, duodenum, cecum, colon, and rectum. There was histopathologic evidence of dark areas/foci, as well as a dark depressed area in the rectum, correlated with minimal to mild multifocal hemorrhage in the lamina propria of the stomach, colon, and rectum, and minimal to moderate ulceration or erosion in the tongue, esophagus and stomach. In addition, minimal to mild neutrophilic inflammation or infiltrates were seen in several organs including the submucosa of the cecum and rectum, lymph nodes, the Gut Associated Lymphoid Tissue (GALT) of the ileum, and minimal sinusal neutrophilia in the spleen. In the bone marrow, there was slight myeloid hyperplasia and severe lymphoid atrophy in the thymus correlating to

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small thymus. The digestive tract lesions were considered to be the cause of morbidity of the animal.

Clinical Signs

Salivation, preputial and vaginal discharge were seen at mid and high doses. The dose of 0.6 mg/m^2/day resulted in severe skin lesions (scabs or lesions with associated skin redness (Table 43)) and gastrointestinal toxicity characterized by body weight loss, decreased food consumption and/or abnormal feces. The observations at 0.6 mg/m^2 were reported to resolve following a 10-day dosing holiday and resumption at 0.45 mg/m^2.

Table 43: Incidence of clinical observations

Sign	Sex	0	0.15 mg/m^2	0.3 mg/m^2	0.6/0.45 mg/m^2
	M	6	4	6	6
	F	6	4	6	6
Prepuce discharge, Liquid	M	--	--	--	2
Vagina discharge, Liquid	F	2	--	2	6
Salivation	M	--	--	1	2
	F	--	--	4	--
Skin Scab	M	--	--	--	4
	F	--	--	--	--
Skin Lesions	M	--	--	1	3
	F	--	--	--	--
Swollen Firm prepuce	M	--	--	--	2
Swollen soft vulva	F	--	--	--	5

-- - No remarkable observation

Body Weights

Except for the dog that was euthanized on Day 14 (Dog # 404), no remarkable treatment-related weight changes in surviving animals were reported during the study.

Feed Consumption

Except for the dog that was euthanized on Day 14 (Dog # 404), no remarkable treatment-related effects on food consumption in surviving animals were observed during the study.

Ophthalmoscopy

The ophthalmology report includes data for 1/4 male dogs at the dose level of 0.15 mg/m^2, 1/6 dogs at the dose level of 0.3 mg/m^2 and 0/5 dogs at the dose level of 0.6/0.45 mg/m^2. In the two male dogs studied, there were no remarkable findings.

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In female dogs, the report includes data for 0/4 dogs at the 0.15 mg/m^2 dose level, 1/6 dogs at the 0.3 mg/m^2 dose level, and 2/6 dogs at the 0.6/0.45 mg/m^2 dose level. The dog at the 0.3 mg/m^2 dose level had slight diffuse conjunctiva hyperemia and focal cornea opacity both on the right eye, while one of the 2 dogs at the 0.6/0.45 mg/m^2 dose level had moderate serous adnexa discharge on the left eye. The findings at both dose levels were made at the end of Week 13

ECG

No significant effects on ECG recordings were observed in Beagle dogs when GSK1120212 was administered by oral gavage at dose levels ≤0.6010.45 mg/m^2 for 13 weeks.

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Hematology

Table 44: Percentage change in hematologic parameters from control values

Parameter	Sex	0			0.15 mg/m^2			0.3 mg/m^2			0.6/0.45 mg/m^2		
		Pret	4 wk	13 wk	Pret	4 wk	13 wk	Pret	4 wk	13 wk	Pret	4 wk	13 wk
Monocytes 10^9/L	M	0.43	0.36	0.32	↑14%	↑73%	↑47%	↑31%	↑35%	--	↑37%	↑58%	↑52%
	F	0.32	0.33	0.31	--	↑9%	↑4%	--	↑4%	↑17%	↑5%	↓40%	↑16%
Eosinophils 10^9/L	M	0.17	0.14	0.20	↑24%	↑20%	↑12%	--	↓21%	↓36%	↑74%	↑104%	↑21%
	F	0.17	0.13	0.22	↓35%	--	↑47%	↑199%	--	↑28%	↑32%	↓31%	↑43%
Reticulocytes 10^9/L	M	24.9	23.7	39.2	↓34%	↓12%	↓30%	↓25%	↓15%	↓47%	↓8%	↑19%	↓41%
	F	25.1	31.8	40.4	--	--	↑13%	--	↓61%	↑42%	--	↓48%	↑67%
Platelets 10^9/L	M	257.3	286.7	301.7	--	↑3%	↑8%	--	--	↓8%	--	↑20%	↑20%
	F	253.5	318.7	322.8	↑24%	--	↑20%	↑26%	↑15%	↑19%	↑20%	↑6%	↑14%
WBC 10^9/L	M	7.86	8.29	8.17	--	↑37%	↑14%	--	--	↓5%	--	↑20%	↑22%
	F	8.36	8.56	8.59	--	--	↑13%	--	--	↑6%	--	--	↑15%
Neutrophils 10^9/L	M	4.68	4.90	4.75	--	↑53%	↑10%	--	↓9%	↓11%	--	↑15%	↑27%
	F	4.81	4.89	4.95	--	↑7%	↑15%	--	--	--	--	↓6%	↑19%
Lymphocytes 10^9/L	M	2.42	2.76	2.77	--	↑13%	↑18%	--	↑10%	↑5%	--	↑21%	↑13%
	F	2.92	3.09	3.02	--	--	↑12%	--	↑17%	↑17	--	--	↑10%
Basophils 10^9/L	M	0.08	0.06	0.10	--	↓19%	↓34%	--	↑45%	↓23%	--	↓6%	↓35%
	F	0.09	0.05	0.06	--	↑23%	↑87%	--	↑43%	↑85%	--	--	↑104%
LUC 10^9/L	M	0.08	0.08	0.05	--	↑21%	↓5%	--	↑50%	↓40%	--	↓4%	↓15%
	F	0.06	0.07	0.05	--	--	↑49%	--	--	↑77%	--	--	↑102%
RBC 10^{12}/L	M	6.73	6.44	7.48	--	--	↓16%	--	--	↓18%	--	--	↓22%
	F	6.40	6.22	6.77	--	--	--	--	--	--	--	--	↓14%
Hemoglobin g/L	M	154.3	148.7	170.5	--	--	↓16%	--	--	↓19%	--	--	↓21%
	F	148.5	143.0	156.3	--	--	--	--	--	--	--	--	↓13%
Hematocrit %	M	43.77	42.98	50.2	--	--	↓15%	--	--	↓19%	--	--	↓21%
	F	41.57	40.73	45.0	--	--	--	--	--	--	--	--	↓14%

Pret – Pretreatment; wk . weeks; ↑ - increase; ↓ - decrease; -- - no remarkable difference from corresponding control value.

Reticulocyte count increased in a dose-related manner in week 13. Other changes in hematologic parameters were minimal and not dose-related (Table 44) given the changes some in pretreatment values in the same groups of animals at

corresponding dose levels. Minimal decreases in RBC, hemoglobin and hematocrit in male dogs, and increases in LUC in female dogs at week 13 were dose-related. Following a 4-week recovery period, monocytes and platelets in female dogs treated with 0.3 mg/m^2 and 0.6/0.45 mg/m^2 significantly increased compared to their corresponding control values (Table 45).

Table 45: Percentage change in monocyte and platelet counts from control at weeks 13 and 17

		0		0.3 mg/m^2		0.6/0.45 mg/m^2	
		Wk 13	*Wk 17*	Wk 13	*Wk 17*	Wk 13	*Wk 17*
Monocytes 10^9/L	M	0.32	*0.31*	↑17%	*↑3%*	↑52%	*↑33%*
	F	0.31	*0.22*	↑5%	*↑30%*	↑16%	*↑72%*
Platelets 10^9/L	M	301.7	*312.0*	↓8%	*↓9%*	↑20%	*↑23%*
	F	322.8	*282.5*	↑19%	*↑25%*	↑14%	*↑68%*

↑ - increase, ↓ - decrease, -- - no remarkable difference from corresponding control value.

Clinical Chemistry

Table 46: Percentage change in blood chemistry parameters from control

Parameter	Sex	0			0.15 mg/m^2			0.3 mg/m^2			0.6/0.45 mg/m^2		
		Pret	4 wk	13 wk	Pret	4 wk	13 wk	Pret	4 wk	13 wk	Pret	4 wk	13 wk
Total Bilirubin µmol/L	M	2.0	2.0	2.8	↓25%	↓10%	↓18%	↓25%	↓15%	↓21%	↓35%	↓30%	↓29%
	F	1.5	1.3	2.2	--	--	↓9%	↓13%	--	↓18%	↓13%	--	↓32%
Triglycerides mmol/L	M	0.33	0.32	0.36	↓23%	↓20%	↓25%	↓5%	↓8%	↓32%	↓14%	↓4%	↓29%
	F	0.35	0.33	0.28	--	↑32%	↑35%	↓15%	--	↓12%	↓22%	--	--

Pret. – Pretreatment; wk – weeks; ↓ - decrease; -- - no remarkable difference from corresponding control value.

Changes in clinical chemistry parameters were minimal and not dose-related (Table 46), given the changes in pretreatment values in the same groups of animals at corresponding dose levels.

Urinalysis

No significant treatment-related changes were reported in urinalysis.

Gross Pathology

Table 47: Incidence of Macroscopic Findings

Macroscopic Findings		0		0.15 mg/m^2		0.3 mg/m^2		0.6/0.45 mg/m^2	
		M	F	M	F	M	F	M	F
	Group Size:	4/*2*	4/*2*	4	4	4/*2*	4/*2*	3/*2*	4/*2*
	Grade	Terminal/*Recovery*							
Adrenals Area dark	Present	--	--	--	--	--	--	**--**	*1*
Brain Dilatation	Present	--	--	--	1/*1*	--	--	--	--
Cecum									
Area dark	Present	--	--	--	--	1/*1*	--	**--**	--
Dialatation	Present	--	--	--	--		1/*1*	--	--
Area depressed	Present	--	--	--	--	--	--	*1*	--
Colon Area dark	Present	--	--	--	--	--	--	*1*	--
Duodenum Area dark	Present	--	--	--	--	--	--	*1*	--
Esophagus Area depressed	Present	--	--	--	--	--	--	*1*	--
Heart Area dark	Present	--	--	1/*1*	--	--	--	1	--
Lungs									
Area pale	Present	--	--	1/*1*	--	--	--	--	--
Area raised	Present	--	--	--	--	1/*1*	--	--	--
Area dark	Present	--	--	--	--	--	--	*1*	--
Lymph Node									
Mottled	Present	--	--	--	--	1/*1*	1/*1*	2/*2*	2/*2*
Foci dark	Present	--	--	--	--	--	1/*1*	--	--
Lymph Node Popliteal									
Enlargement	Present	--	--	1/*1*				1/*1*	1/*1*
Foci dark	Present	--	--	1/*1*		--	--	--	1/*1*
Mottled	Present	--	--	--	1/*1*	--	--	--	--
Pharynx Area depressed	Present	--	--	--	--	--	--	*1*	--
Pituitary Cyst	Present	--	--	--	--	*1*	--	--	--
Rectum Area depressed	Present	--	--	--	--	--	--	*1*	--
Thymus Foci dark	Present	--	--	*1*	--	--	1/*1*	--	1/*1*
Small	Present	--	--	--	--	--	*1*	**--**	--

Macroscopic Findings		0		0.15 mg/m^2		0.3 mg/m^2		0.6/0.45 mg/m^2	
		M	F	M	F	M	F	M	F
	Group Size:	4/*2*	4/*2*	4	4	4/*2*	4/*2*	3/*2*	4/*2*
	Grade	Terminal/*Recovery*							
Stomach									
Area depressed	Present	--	--	--	--	--	--	*1*	--
Foci dark	Present	--	--	*1*	--	--	--	*1*	--
Tongue Area depressed	Present	--	--	--	--	--	--	**--**	--

-- - no remarkable difference from corresponding control value

Organ Weights

There were no consistent significant treatment-related changes in organ weights.

Histopathology

For animals that survived until the scheduled time, histopathologic findings (Table 48) were minimal or mild except in the lymph nodes where sinusal erythrocytosis/hemorrhage was classified as moderate.

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

Table 48: Incidence of Histopathologic Findings

Microscopic Findings		0		0.15 mg/m^2		0.3 mg/m^2		0.6/0.45 mg/m^2	
		M	F	M	F	M	F	M	F
	Group Size:	4/*2*	4/*2*	4	4	4/*2*	4/*2*	3/*2*	4/*2*
	Grade	Terminal/*Recovery*							
Aorta Mineralization	Minimal	--	--	--	--	--	1		*1*
Brain Dilatation: ventricles	Mild	--	--	--	1	--	--	--	--
Heart Fibrosis: epicardium	Minimal	--	--	--	--	--	--	1	--
Kidney									
Lipidosis: glomerular	Minimal	--	--	--	--	--	--	--	1
Basophilia: tubular	Minimal	--	--	--	--	--	1	--	--
Fibrosis	Minimal	--	--	--	--	--	--	--	*1*
Liver									
Necrosis	Minimal	--	--	1	--	--	--	--	--
Macrophage aggregates	Minimal	--	--	--	--	1	--	--	--
Lungs									

Microscopic Findings		0		0.15 mg/m^2		0.3 mg/m^2		0.6/0.45 mg/m^2	
		M	F	M	F	M	F	M	F
	Group Size:	4/*2*	4/*2*	4	4	4/*2*	4/*2*	3/*2*	4/*2*
	Grade	Terminal/*Recovery*							
Hemorrhage	Minimal	--	--	--	1		1	--	1
Infiltration: mononuclear cells	Minimal	--	--	--	1	--	--	--	--
Fibrosis: pleura	Minimal	--	--	--	--	--	--	--	*1*
Macrophage accumulation	Mild	--	--	--	--	--	--	--	*1*
Lymph Node	Mild	--	--	--	--	--	1	--	--
Erythrocytosis/hemorrhage: sinusal	Moderate	1	1	--	--	1	--	2	4/*1*
Lymph Node Meseneric									
Erythrocytosis/hemorrhage	Mild	--	--	--	--	--	--	--	*1*
Lymph Node Popliteal									
Plasmacytosis	Mild	--	--	1	--	--	--	1	1
Erythrocytosis/hemorrhage	Minimal	--	--	--	--	--	--	--	1
	Mild	--	--	--	1	--	*1*	--	--
Pituitary	Minimal	--	--	2			1/*1*	1	*1*
Cyst	Mild	--	--	--	--	--		--	1/*1*
Skin Ulceration	Mild	--	--	--	--	--	1	--	--
Stomach									
Hemorrhage	Minimal	--	--	1	--	1	--	--	--
Testis									
Atrophy/Hypoplasia	Mild	--	--	2	--	--	--	--	--
Inflammation: vascular	Minimal	--	--	1	--	--	--	--	--
Thymus									
Hemorrhage	Minimal	--	--	--	--		1/*1*	--	1/*1*
Atrophy: lymphoid	Mild	--	--	--	--	--	--	--	1
Thyroid Inflammation: subacute	Minimal	--	--	--	--	1	--	--	--
Cyst	Minimal	--	--	--	--	--	*1*	--	--
Tongue									
Inflammation	Minimal	--	--	--	--	1	--	--	--

-- - no remarkable difference from corresponding control value.

Toxicokinetics

In males (Table 49), the mean AUC_{0-t} on Day 1 was more than dose proportional from 0.3 mg/m^2 to 0.6 mg/m^2 (exposure was 9.8-fold at the high dose), while the C_{max} for the same dose levels increased 3-fold at the high dose. There was a 31.9-fold increase in exposure from Day 1 to Week 4 at 0.3 mg/m^2 dose level, while the increase in C_{max} for the same period was 3.5-fold. There was no further increase in AUC_{0-t} or C_{max} from Week 4 to Week 13.

Table 49: Mean Toxicokinetic Parameters for GSK1120212 in Male Dogs Following Oral Administration

Parameter [a]	Period	Male			
		Dose of GSK1120212 (mg/m²/day)			
		0.15 (n = 4)	0.3 (n = 6)	0.6 (n = 6)	0.45 (n = 5)
AUC_{0-t} (ng.h/mL)	Day 1	NC	2.96 [1.99 – 3.78]	28.9 [26.1 – 31.6]	NA
	Week 4	46.0 [34.7 – 62.4]	94.5 [82.1 – 115]	NA	131 [112 – 150]
	Week 13	45.6 [32.8 – 59.4]	95.5 [67.1 – 146]	NA	128 [118 – 140]
C_{max} (ng/mL)	Day 1	0.838 [0.803 – 0.907]	1.57 [1.09 – 2.13]	4.63 [3.72 – 6.05]	NA
	Week 4	2.55 [2.09 – 3.21]	5.45 [4.73 – 6.44]	NA	8.91 [7.85 – 10.2]
	Week 13	2.32 [1.54 – 2.84]	5.15 [3.45 – 6.41]	NA	8.42 [6.73 – 9.76]
Median T_{max} (h)	Day 1	0.50 [0.50 – 0.50]	0.50 [0.50 – 1.00]	0.50 [0.50 – 1.00]	NA
	Week 4	0.50 [0.50 – 1.00]	2.00 [0.50 – 2.00]	NA	1.00 [0.50 – 2.00]
	Week 13	1.00 [0.50 – 2.00]	1.00 [0.50 – 4.00]	NA	1.00 [0.50 – 2.00]

NC = Not calculated. There were insufficient plasma concentration data to calculate AUC.
NA = Not applicable. Dosing of 0.6 mg/m²/day was stopped on Day 11 for the main study female animals (451-454) and on Day 12 for the main study (401-404) and recovery (405-406) male animals and recovery female animals (455-456). Dosing resumed at a lower dose of 0.45 mg/m²/day on Day 21 for the main study female animals (451-454) and on Day 22 for the main study (401-403) and recovery (405-406) male animals and recovery female animals (455-456)..
a. Results are reported as mean unless stated otherwise and [range].

In females (Table 50), the mean AUC_{0-t} on Day 1 was more than dose proportional from 0.3 mg/m^2 to 0.6 mg/m^2 (exposure was 4.8-fold at the high dose), while the C_{max} for the same dose levels increased dose proportionally. There was a 16.6-fold increase in exposure from Day 1 to Week 4 at 0.3 mg/m^2 dose level, while the increase in C_{max} for the same period was 3-fold. There was slight decrease in AUC_{0-t} and C_{max} from Week 4 to Week 13.

Table 50: Mean Toxicokinetic Parameters for GSK1120212 in Female Dogs Following Oral Administration

Parameter [a]	Period	Female			
		Dose of GSK1120212 (mg/m²/day)			
		0.15 (n = 4)	0.3 (n = 6)	0.6 (n = 6)	0.45 (n = 6)
AUC_{0-t} (ng.h/mL)	Day 1	NC	6.98 [2.22 – 17.3]	33.3 [24.2 – 49.6]	NA
	Week 4	60.7 [52.3 – 82.4]	116 [95.1 – 158]	NA	177 [115 – 245]
	Week 13	51.8 [41.8 – 69.2]	107 [89.7 – 130]	NA	150 [113 – 197]
C_{max} (ng/mL)	Day 1	0.870 [0.710 – 1.15]	2.44 [1.63 – 3.06]	5.42 [2.09 – 9.02]	NA
	Week 4	3.56 [2.65 – 4.80]	7.73 [6.27 – 9.10]	NA	12.0 [9.10 – 16.9]
	Week 13	2.71 [2.17 – 3.68]	7.24 [6.21 – 8.34]	NA	9.78 [8.64 – 11.7]
Median T_{max} (h)	Day 1	0.50 [0.50 – 1.00]	0.50 [0.50 – 0.50]	0.50 [0.50 – 2.00]	NA
	Week 4	0.50 [0.50 – 0.50]	0.50 [0.50 – 1.00]	NA	1.00 [0.50 – 2.00]
	Week 13	0.75 [0.50 – 1.00]	0.50 [0.50 – 1.00]	NA	0.50 [0.50 – 1.00]

NC = Not calculated. There were insufficient plasma concentration data to calculate AUC.
NA = Not applicable. Dosing of 0.6 mg/m²/day was stopped on Day 11 for the main study female animals (451-454) and on Day 12 for the main study (401-404) and recovery (405-406) male animals and recovery female animals (455-456). Dosing resumed at a lower dose of 0.45 mg/m²/day on Day 21 for the main study female animals (451-454) and on Day 22 for the main study (401-403) and recovery (405-406) male animals and recovery female animals (455-456).
a. Results are reported as mean unless stated otherwise and [range].

7 Genetic Toxicology

The full battery of genetic toxicology studies (including *in vitro* and *in vivo* assays) for the effect of GSK1120212 were previously submitted under IND 102,715 and reviewed by Dr. Robeena Aziz. Dr. Aziz concluded that GSK1120212 with or without S9 activation was not mutagenic in multiple strains of *Salmonella typhimurium* or *Escherichia coli*. GSK1120212 was not genotoxic in the mouse lymphoma L5178Y TK+/- cells or in rat bone marrow *in vivo*. Thus, GSK1120212 was negative for genotoxicity.

Impurities

(b) (4)

The Applicant states that the (b) (4) is a potentially genotoxic impurity (b) (4). All clinical phase batches synthesized to date (n=9), including batches run on production scale at the proposed commercial site, have been tested for (b) (4) using LC-MS. Levels of (b) (4) have been confirmed to be not greater than (b) (4) based on the highest expected daily dose of 2 mg/day).

According to the sponsor's submission, the genotoxic impurity, [redacted] itself has two additional potentially genotoxic impurities, [redacted], though [redacted] was found not to be mutagenic in the Ames assay. The specifications for [redacted] are listed as NMT [redacted] respectively. The Applicant reports that analysis of commercial scale batches of intermediate grade trametinib dimethyl sulfoxide that were representative of the manufacturing route and process, manufactured at the commercial site showed that the levels of all 3 potential genotoxic impurities were below the threshold of toxicological concern (TTC; [redacted]). The Applicant set the specification for each impurity at [redacted] following [redacted] for the drug substance and does not expect further formation of the impurities beyond this stage.

At the [redacted] specification proposed by the Applicant for each of the genotoxic impurites, the level of exposure in humans at the recommended daily dose of 2 mg of Mekinist is [redacted]. Thus, at the clinically recommended dose, the levels of either individual or combined impurities remain below the level of toxicological concern and the proposed specifications are acceptable from a pharmacologic/toxicologic safety standpoint.

[redacted]

The Applicant reports increased levels of two impurities, [redacted] in the 0.5 mg and 1 mg tablets of Mekinist during the [redacted] test (Table 51). Due to the high levels of these [redacted] observed in Mekinist (Table 52), and the possibility that they could be genotoxic, the impurities, [redacted] were screened for genotoxicity using an in silico prediction tool (Derek v.13). [redacted] was identified as a potential genotoxin and underwent screening in an Ames test. In this assay, [redacted] was negative for genotoxicity.

Table 51: Degradation of Trametinib in Trametinib Tablets, 1 mg

Compound/RRT	Unstressed (%)[4]	80°C[1] (%)[4]	80°C/75% RH[1] (%)[4]	Light Control[2,3] (%)[4]	Light[3] (%)[4]
(b) (4)	< 0.05	< 0.05	0.10	0.05	< 0.05
	0.11	0.11	0.12	0.11	0.12
	0.06	< 0.05	< 0.05	< 0.05	0.90
	ND	ND	0.09	ND	1.2
Trametinib (% of claim)	100.1	99.7	100.2	99.9	97.1
% Recovery[5]	100.3	99.8	100.5	100.1	99.3
% Total impurities[6]	(b) (4)				

Notes:
ND denotes not detected (less than detection limit for the method presented in P.5.2 Analytical Procedures_ Identification, Content, Uniformity and Drug-Related Impurities by HPLC).
1. Exposed for 14 days.
2. Light Control sample was wrapped in foil before exposure to light source.
3. Exposed to 2 x ICH Q1B, Option 1 conditions.
4. % of claim for trametinib, % area for all degradants/impurities.
5. The sum of each individual impurity (% area) and trametinib (% of claim).
6. Peaks arising from the system or excipients not included.

Table 52: (b) (4) Impurity levels

Impurity	Specification	Dose mg/day*	Impurity Level 0.5 mg	Impurity Level 1 mg	Dose mg/day‡
(b) (4)					

*Impurity dose based a specification (b) (4) and a daily dose of 2 mg of mekinist
‡ Impurity dose based on actual levels of impurities and a daily dose of 2 mg of mekinist using 0.5 mg or 1 mg tablets.

Study title: GSK2646258A: Bacterial Mutation Assay (Ames Test) With *Salmonella typhimurium* and *Escherichia coli* (screening study).

Study no.:	Ames-851
Study report location:	eCTD: 4.2.3.7.6.1.
Conducting laboratory and location:	Not given
Date of study initiation:	07/12/2010
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	GSK2646258, Lot and purity not given

Key Study Findings

- GSK2646258 was found not to be mutagenic in the bacterial mutation assay

Methods

Strains: *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA102) and *Escherichia coli* WP2 *uvrA* (pKM101).

Concentrations in definitive study: 50, 150, 500, 1500, 2500, 5000 µg/plate ±S9

The purpose of this study was reportedly to assess the potential of [redacted] (a [redacted] to induce gene mutations *in vitro* in bacterial strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA102) and *Escherichia coli* WP2 *uvrA* (pKM101). A single plate incorporation test was conducted for each tester strain both in the presence and absence of rat liver S9-mix, together with appropriate vehicle and positive controls.

Results

Summary data for the vehicle controls were reported to be within the laboratory historical vehicle control ranges. The unequivocal increases in numbers of revertant colonies induced by the positive controls suggest that the performance of the vehicle and positive controls were consistent with a valid assay.

The highest concentration of [redacted] tested was [redacted] per plate, in the presence and absence of S9-mix respectively. No treatment-related increases in the number of revertant colonies were observed at any of the concentrations tested in this study.

Thus, under the conditions of this experiment, [redacted] was found not to be mutagenic in the bacterial mutation assay, when tested in the presence or absence of S9-mix.

Given a daily dose of 2 mg of mekinist, and a specification of [redacted] (Table 52) the daily dose of [redacted] would be [redacted]. Although these [redacted] are not genotoxic, their actual levels in the 0.5 and 1 mg mekinist tablets exceeded the specifications. Since these impurities exceed their specifications when the 0.5 mg and 1 mg tablets of Mekinist are exposed to light, the Applicant has [redacted] The Applicant did not list [redacted] individually in the drug product specification, stating that these impurities are controlled as “any unspecified impurity” in accordance with ICH Q3B.

Reference ID: 3293647

Study title: [redacted] Bacterial Mutation Assay (Ames Test) With *Salmonella typhimurium* and *Escherichia coli* (screening study)

Study no.:	Ames-1121
Study report location:	eCTD: 4.2.3.7 6 1.
Conducting laboratory and location:	Not given
Date of study initiation:	06/23/2011
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	[redacted], Lot and purity not given

Key Study Findings

- [redacted] was found not to be mutagenic in the bacterial mutation assay

Methods

Strains:	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and *Escherichia coli* WP2 *uvrA* (pKM101)
Concentrations in definitive study:	[redacted]: 50, 150, 500, 1500, 2500, 5000 µg/plate ±S9

The purpose of this study was to assess the potential of [redacted] in GSK1120212) to induce gene mutations *in vitro* in bacterial strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA102) and *Escherichia coli* WP2 *uvrA* (pKM101). A single plate incorporation test was conducted for each tester strain both in the presence and absence of rat liver S9-mix (Table 53, Table 54; Test 1). A second test was conducted with strain TA100 in the presence of S9-mix only (Table 55; Test 2). Plates treated with appropriate vehicle and positive controls were included for all strains in the presence and absence of S9-mix.

Results

Summary data for the vehicle controls were reported to be within the laboratory historical vehicle control ranges. The unequivocal increases in numbers of revertant colonies induced by the positive controls suggest that the performance of the vehicle and positive controls were consistent with a valid assay.

The highest concentration of [redacted] tested was [redacted] per plate, in the presence and absence of S9-mix respectively. A small non-concentration related increase in the number of revertant colonies (approaching 2-fold for a positive response) was observed in strain TA100 only in the presence of S9-mix (Table 54). Therefore, [redacted] was tested again against this strain in the presence of S9-mix (Table 55). No treatment related increases in the number of revertant colonies were observed in the second test. Thus, no reproducible treatment-related increases in the numbers of revertant colonies were observed.

Table 53: Bacterial Mutation Assay (Ames Test) – Test 1

Table 1 Test 1 Without Metabolic Activation

Strain	Compound	Dose level per plate (µg)	Mean revertants per plate	Standard Deviation	Ratio treated / Vehicle	Individual revertant colony counts
TA100	(b) (4)	50	123.7	18.0	1.2	141, 125, 105
		150	116.0	11.0	1.1	116, 127, 105
		500	105.3	3.1	1.0	102, 108, 106
		1500	110.7	3.1	1.1	108, 114, 110
		2500	106.7	16.8	1.0	126, 98, 96
		5000	113.7	9.3	1.1	124, 106, 111
			103.5	8.7		94, 112, 94, 113, 108, 100
TA1535		50	22.0	2.6	1.1	24, 23, 19
		150	16.0	8.0	0.8	24, 8, 16
		500	18.0	6.9	0.9	14, 14, 26
		1500	24.0	5.0	1.2	19, 24, 29
		2500	21.7	4.9	1.1	25, 16, 24
		5000	23.0	6.0	1.1	17, 29, 23
			20.5	5.9		19, 25, 21, 13, 29, 16
TA1537		50	13.7	2.3	0.9	15, 15, 11
		150	12.7	2.3	0.8	10, 14, 14
		500	17.3	6.5	1.1	17, 11, 24
		1500	16.7	0.6	1.1	17, 17, 16
		2500	17.3	4.9	1.1	14, 15, 23
		5000	14.3	4.5	0.9	14, 19, 10
			15.5	4.4		22, 16, 17, 10, 11, 17
TA98		50	26.0	7.8	1.1	30, 31, 17
		150	29.7	6.8	1.2	32, 35, 22
		500	20.0	5.3	0.8	16, 26, 18
		1500	20.7	5.7	0.8	27, 16, 19
		2500	20.3	4.7	0.8	24, 15, 22
		5000	26.0	4.4	1.1	23, 24, 31
			24.3	6.2		33, 19, 31, 21, 23, 19
WP2 uvrA (pKM101)		50	96.3	10.7	1.0	103, 84, 102
		150	106.3	16.1	1.1	113, 88, 118
		500	99.7	10.6	1.0	90, 111, 98
		1500	103.3	6.4	1.0	96, 108, 106
		2500	94.3	14.6	1.0	79, 108, 96
		5000	95.7	7.1	1.0	88, 102, 97
			98.7	10.3		80, 100, 97, 103, 111, 101

Strain	Compound	Dose level per plate (µg)	Mean revertants per plate	Standard Deviation	Ratio treated / Vehicle	Individual revertant colony counts
TA100	(b) (4)	2	1038.5	51.6	10.0	1075, 1002
TA1535		2	1154.0	9.9	56.3	1147, 1161
TA1537		50	1112.0	193.7	71.7	1249, 975
TA98		1	494.0	18.4	20.3	481, 507
WP2 uvrA (pKM101)		2	4003.0	432.7	40.6	3697, 4309

Key to Positive Controls — Key to Plate Postfix Codes

NaAz	Sodium Azide
9-AAC	9-Amino Acridine
2NF	2-Nitrofluorene
4NQO	4-Nitroquinoline-1-oxide

(Excerpted from the sponsor's submission)

Table 54: (b) (4) Bacterial Mutation Assay (Ames Test) – Test 1

Table 2 Test 1 With Metabolic Activation

Strain	Compound	Dose level per plate (µg)	Mean revertants per plate	Standard Deviation	Ratio treated / Vehicle	Individual revertant colony counts
TA100	(b) (4)	50	83.3	5.7	0.9	88, 77, 85
		150	100.7	16.1	1.1	119, 89, 94
		500	119.3	41.6	1.3	90, 101, 167
		1500	173.0	9.6	1.8	166, 169, 184
		2500	138.7	62.6	1.5	211, 103, 102
		5000	109.3	7.0	1.2	116, 110, 102
			94.3	5.5		92, 97, 94, 101, 85, 97
TA1535		50	17.7	6.4	0.8	14, 14, 25
		150	23.3	3.8	1.1	19, 26, 25
		500	22.3	11.6	1.1	24, 33, 10
		1500	23.0	1.7	1.1	22, 22, 25
		2500	17.7	0.6	0.8	18, 17, 18
		5000	11.7	1.2	0.6	13, 11, 11
			21.2	5.2		14, 19, 22, 27, 27, 18
TA1537		50	24.0	7.0	0.7	24, 31, 17
		150	28.3	2.1	0.9	29, 26, 30
		500	34.0	2.6	1.0	37, 33, 32
		1500	24.3	7.5	0.7	32 T4, 17 T4, 24 T4
		2500	21.7	3.5	0.7	25 T3, 22 T3, 18 T3
		5000	16.3	2.1	0.5	14 T3, 18 T3, 17 T3
			32.5	4.7		32, 26, 35, 40, 30, 32
TA98		50	45.7	7.1	1.0	38, 47, 52
		150	44.0	5.6	1.0	45, 38, 49
		500	41.0	4.4	0.9	46, 39, 38
		1500	49.7	3.1	1.1	49, 47, 53
		2500	49.7	4.7	1.1	48, 55, 46
		5000	56.3	2.5	1.2	54, 56, 59
			45.8	6.1		48, 57, 41, 45, 41, 43
WP2 uvrA (pKM101)		50	135.0	7.8	0.9	140, 139, 126
		150	147.0	21.4	1.0	130, 140, 171
		500	125.7	4.5	0.9	121, 130, 126
		1500	151.0	1.7	1.0	150, 150, 153
		2500	163.3	16.9	1.1	144, 175, 171
		5000	178.0	16.4	1.2	192, 182, 160
			147.0	10.7		139, 141, 159, 157, 133, 153

Strain	Dose level per plate (µg)	Mean revertants per plate	Standard Deviation	Ratio treated / Vehicle	Individual revertant colony counts
TA100	5	4017.0	251.7	42.6	4195, 3839
TA1535	5	542.5	82.7	25.6	601, 484
TA1537	5	238.5	41.7	7.3	268, 209
TA98	10	439.0	18.4	9.6	452, 426
WP2 uvrA (pKM101)	10	1078.0	7.1	7.3	1073, 1083

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)

(Excerpted from the sponsor's submission)

Table 55: Repeat Assay for *S. typhimurium* TA100

Table 3 Test 2 With Metabolic Activation

Strain	Compound	Dose level per plate (µg)	Mean revertants per plate	Standard Deviation	Ratio treated / Vehicle	Individual revertant colony counts
TA100	(b) (4)	150	59.0	11.5	0.7	47, 60, 70
		500	66.0	4.6	0.8	71, 62, 65
		1000	65.3	7.4	0.8	71, 68, 57
		1500	73.0	3.6	0.9	74, 76, 69
		2000	77.0	6.1	0.9	84, 74, 73
		2500	71.0	1.0	0.9	71, 70, 72
		5000	83.7	11.6	1.0	76, 97, 78
TA100		5	3068.0	247.5	36.9	2893, 3243

Key to Positive Controls Key to Plate Postfix Codes

2-AAN 2-Aminoanthracene

(Excerpted from the sponsor's submission)

Under the conditions of these experiments, (b) (4) was not mutagenic in the bacterial mutation assay, when tested in the presence or absence of S9-mix.

8 Carcinogenicity

No carcinogenicity studies with trametinib were submitted. For justification, the Applicant cited ICH S1A: The Need for Long-term Rodent Carcinogenicity Studies of Pharmaceuticals (1996), and ICH S9: Nonclinical Evaluation for Anticancer Pharmaceuticals (2009), which state that carcinogenicity studies are not considered necessary to support the use of therapeutics (trametinib) intended to treat patients with advanced cancer (metastatic melanoma), where the life expectancy of the patients is short (i.e. less than 2 to 3 years).

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: GSK1120212B: Oral Embryo-Fetal Development Study in Rats

Study no.:	G10218
Study report location:	4.2.3.5.2.1
Conducting laboratory and location:	GlaxoSmithKline (GSK) Safety Assessment 709 Swedeland Rd King of Prussia, PA
Date of study initiation:	01/07/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK1120212B (b) (4) dimethyl sulfoxide (DMSO) solvate), Lot # 091450 and purity 99.8%

Key Study Findings

- 3 of 4 rats dosed at 2.86/1.0 mg/m^2 had increased incidence of scabs on the face, ears, neck, shoulders, and abdomen
- Decrease in maternal body weight reported at all dose levels on Days 6-9. Decrease in body weight at 2.86/1.0 mg/m^2 correlated with decrease in food consumption
- Increased post-implantation loss at 2.86/1.0 mg/m^2
- Decrease in fetal body weight (4-fold in males and 5-fold in females) at 2.86/1/0 mg/m^2 dose level compared to 1.0/0.5 mg/m^2 dose level
- Increase in AUC 4.6-fold and C_{max} 5-fold at 2.86/1.0 mg/m^2 compared to 1.0/0.5 mg/m^2.

Methods

*Doses: 0, 0.5/0.125, 0.75/0.25, 1.0/0.5, 2.86/1.0 mg/m^2/day.
Frequency of dosing: once daily on Days 6 through 17 postcoitum (pc)
Dose volume: 40 mL/m^2 (30 mL/m^2 for the 1 mg/m^2 dose on Days 26-28)
Route of administration: Oral gavage
Formulation/Vehicle: 1.5% hydroxypropylmethylcellulose (Pharmacoat 603), 5% mannitol 60 and 0.2% sodium lauryl sulfate solution
Species/Strain: Crl:CD Sprague Dawley
Study design:

Group Number	Treatment[a]	Loading Dose (mg/m^2/day) Day 6 pc	Main Dose (mg/m^2/day) Days 7 to 17 pc	Number of Females
Pregnant				
1	Vehicle	0	0	22
2	GSK1120212	0.5	0.125	22
3	GSK1120212	0.75	0.25	22
4	GSK1120212	1.0	0.5	22
Non-pregnant TK Arm[b]				
5	GSK1120212	0.5	0.125	3
6	GSK1120212	0.75	0.25	3
7	GSK1120212	1.0	0.5	3
Pregnant				
8	GSK1120212	0	0	4
9	GSK1120212	2.86	1.0	4
Non-pregnant TK[b]				
10	GSK1120212	2.86	1.0	3

a. Doses are expressed as the parent compound, GSK1120212. Individual dose volumes will be determined in mL based on body surface area (BSA in m^2) calculated from the most recently collected body weight; BSA = 0.105 x (body weight in kg)$^{2/3}$ [Harkness, 1989]. Doses to be determined by body surface area (mg/m^2) calculated from the most recent body weight.
b. Non-pregnant females will be dosed from Day 1 through Day 6 [Day 1 loading dose, Days 2-6 with main dose].

Deviation from study protocol:
*A single loading dose was given on Day 6 pc followed by repeat maintenance doses on Days 7 through 17 pc (loading dose/maintenance dose).

Mated (F0) female rats were assigned to 4 treatment groups balanced by body weight. Groups of 22 (Groups 1-4) or 4 (Groups 8 and 9) mated females were given vehicle or test article formulations by oral gavage once daily on Days 6 to 17 post coitum (pc) at the dose levels shown in the study design above. Each group of rats was given a loading dose on Day 6 (see study design table above) and maintenance dose on Days 7-17.

Reference ID: 3293647

Observations and Results

Mortality

No unscheduled deaths or deliveries were reported on this study

Clinical Signs

GSK1120212-related skin lesions observed at high doses in animals used in embryo-fetal development studies were consistent with the skin lesions reported in repeat-dose toxicity studies.

Body Weight

Decrease in body weight gain compared to control animals was reported on days 6-9 pc at all dose levels: 0.5/0.125 mg/m^2 (31%), 0.75/0.25 mg/m^2 (37%), 1.0/0.5 mg/m^2 (39%), and 2.86/1.0 mg/m^2 (41%) compared to mean control body weight gain of 22.5 g.

Feed Consumption

Administration of ≤1.0/0.5 mg/m^2 GSK1120212 to pregnant rats had no remarkable effect on food consumption. There was a 21% decrease in food consumption on days 6-9 in pregnant rats administered 2.86/1.0 mg/m^2 of GSK1120212 compared to mean control (70 g) which correlated with the decrease in body weight gain compared to controls observed during the same period.

Toxicokinetics

Non-pregnant female rats were given a loading dose of GSK1120212 orally by gavage on Day 1 and were subsequently given daily doses of GSK1120212 from Day 2 through Day 6, as shown in Table 56.

Blood samples were collected into tubes containing EDTA (as anti-coagulant) on Day 1 at the following nominal times: 0.5, 1, 2, 4, 8 and 24 hours after dosing. On Day 4, a single sample was collected at predose (0) and on Day 7 a single sample was collected prior to sacrificing the animals.

Reference ID: 3293647

Table 56: Dose levels of GSK1120212 for Toxicokinetics

Group Number	Treatment	Loading Dose (mg/m^2/day)	Main dose given daily (mg/m^2/day)
5	GSK1120212	0.5	0.125
6	GSK1120212	0.75	0.25
7	GSK1120212	1	0.5
10	GSK1120212	2	1

(Excerpted from the sponsor's submission)

**Group 10 was given GSK1120212 2.86 mg/m^2 and not 2.0 mg/m^2.

The mean AUC_{0-t} and C_{max} values increased approximately dose proportionally from 0.5 to 1.0 mg/m^2 but greater than dose proportionately from 1.0 to 2.0 mg/m^2. Plasma concentrations on Days 4 and 7 were similar for each dose level (Table 57). Given that the toxicokinetic evaluation was conducted in non-pregnant rats, increases in toxicity noted in the pregnant rats between the 1.0 and 2.86 mg/m^2 dose levels support the presumption that exposures in pregnant rats follow a similar pattern and can be used for comparisons to clinical exposures.

Table 57: Mean TK Parameters from non-pregnant Female Rats Following Oral Administration of GSK1120212 (Embryo-Fetal Study)

Parameter [a]	Period	Female			
		Loading Dose & [Main Dose] of GSK1120212 (mg/m^2/day)			
		0.5 [0.125]	0.75 [0.25]	1 [0.5]	2 [1]
AUC_{0-t} (ng.h/mL)	Day 1	52.3 [47.3 – 57.3]	110 [108 – 113]	149 [130 – 160]	684 [604 – 772]
C_{max} (ng/mL)	Day 1	2.79 [2.57 – 2.99]	6.12 [5.87 – 6.32]	8.60 [7.41 – 9.36]	43.6 [39.4 – 51.4]
Plasma Concentrations [b] (ng/mL)	Day 4	1.90 [1.73 – 2.20]	3.44 [3.18 – 3.60]	5.11 [4.48 – 5.54]	8.90 [8.25 – 10.2]
Plasma Concentrations [c] (ng/mL)	Day 7	1.98 [1.80 – 2.28]	3.30 [2.75 – 3.80]	4.97 [3.39 – 5.87]	10.5 [8.65 – 11.4]
Median T_{max} (h)	Day 1	2.00 [2.00 – 4.00]	8.00 [4.00 – 8.00]	8.00 [8.00 – 8.00]	8.00 [4.00 – 8.00]

a. Results are reported as mean unless stated otherwise and [range].
b. Sample collected prior to dosing
c. Sample collected prior to sacrificing the animal

Necropsy

Fertility Parameters

Administration of GSK1120212 to pregnant female rats from Days 6-17 pc had no effect on numbers of corpora lutea, implantations, resorptions, live and dead fetuses per litter, or placental morphology. The percentage of post-implantation loss was mildly increased compared to the untreated controls at all dose levels (≥0.5/0.25 mg/m^2 Table 58). Although post-implantation loss in the group treated at the dose level 2.86/1.0 mg/m^2 appeared high, it was 2.1 times that of its own control group (12.5%). Thus compared to control values, post-implantation loss did not seem to be dose-dependent Table 58.

Table 58: Summary of Pre- and Post-implant observations

Dose	0	0.5/0.125 mg/m^2	0.75/0.25 mg/m^2	1.0/0.5 mg/m^2	2.86/1.0 mg/m^2
Number of Pregnant Females	21	22	21	22	4
Corpora Lutea	281	279	287	284	56
Pre-implant Loss	9	21	15	13	3
% Pre-implant Loss	3.2	7.5	5.2	4.6	5.4
Implants	272	258	272	271	53
Early Resorption	11	11	14	21	13
Late Resorption	0	6*	1	1	1
Dead Fetuses	0	1*	2	1	0
All Post-implant Loss	11	18	17	23	14
% Post-implant Loss	4.0	7.0	6.3	8.5	26.4
Live Fetuses	261	240	255	248	39

*6 late resorptions and a dead fetus were from the same female rat.

Sex ratio was unaffected by trametinib exposure in utero. At the high dose there was a decrease in gravid uterine weight. There were decreases in male and female fetal body weights from pregnant rats administered GSK1120212 compared to the control animals at dose levels ≥ 0.75/0.25 mg/m^2. At the 0.75/0.25 and 1.0/0.5 mg/m^2 dose levels decreases were mild (<5%); however at the high dose level of 2.86/1.0 mg/m^2 there was a decrease in fetal body weight of approximately 21% compared to the control group (Table 59). The decrease in fetal body weight at 2.86/1.0 mg/m^2 compared to 1.0/0.5 mg/m^2 was 4-fold in males and 5-fold in females. Although the toxicokinetic study (Table 57) was conducted in non-pregnant rats, the results show 4.6-fold exposure at 2.86/1/0 mg/m^2 compared to 1.0/0.5 mg/m^2. Thus, the increased fetal toxicity appears consistent with increased maternal exposure to GSK1120212 and increased maternal toxicity.

Table 59: Percentage decrease in Fetal body weight and gravid uterus

	0	0.5/0.125 mg/m^2	0.75/0.25 mg/m^2	1.0/0.5 mg/m^2	2.0/1.0 mg/m^2
M	5.85 g	0	↓4.7%	↓4.7%	↓21.0%
F	5.50 g	0	↓3.1%	↓3.8%	↓21.3%
Gravid uterus	94.8 g	↓10%	↓6%	↓13%	↓23%

Fetal abnormalities in pregnant rats administered GSK1120212 were noted at the high dose level of 2.86/1.0 mg/m^2: about 17% of 20 fetuses from dams treated at this dose level were reported to have incompletely ossified sternebrae and 10% had no evidence of the innominate great vessel. No abnormalities were noted at frequencies significantly exceeding those seen in control animals at lower dose levels

9.2 Embryonic Fetal Development

Study title: GSK1120212B: Oral Embryo-Fetal Development Study in Rabbits

Study no.:	G11166
Study report location:	eCTD: 4.2.3.5.2.1
Conducting laboratory and location:	GlaxoSmithKline (GSK) Safety Assessment 709 Swedeland Rd King of Prussia, PA
Date of study initiation:	09/06/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	GSK1120212B, Lot # 091450 and Purity 99.8%

Key Study Findings

- One of 4 rabbits at HD aborted on day 18 pc
- Decrease in maternal body weight gain at MD and HD post dosing period. Decrease in body weight gain not correlated with food consumption.
- Increased post-implantation loss at HD
- Dose-related decrease in fetal body weight
- Increased incidence of incomplete ossification of bones

Methods

Doses:	0, 1.0/0.5, 2.0/1.0, 4.0/2.0 mg/m^2 [single loading dose/maintenance dose]
Frequency of dosing:	Loading dose on Day 7, then Daily from Day 8 to 19 post coitum (pc)
Dose volume:	66 mL/m^2 based on daily body weight
Route of administration:	Oral gavage
Formulation/Vehicle:	1.5% hydroxypropylmethylcellulose (Pharmacoat 603), 5% mannitol and 0.2% sodium lauryl sulfate
Species/Strain:	Dutch Belted rabbit [Haz:(DB)SPF]
Number/Sex/Group:	22/group
Satellite groups:	3/group for TK

Reference ID: 3293647

Study design:

Group	Treatment[a]	Loading Dose (mg/m^2)	Maintenance Dose (mg/m^2/day)	Number of Mated Females
1	vehicle	0	0	22
2	GSK1120212	1	0.5	22
3	GSK1120212	2	1	22
4	GSK1120212	4	2	22

a. Dose is expressed as the parent compound, GSK1120212.

Observations and Results

Mortality

One rabbit administered 2/1 mg/m^2 was reported to have died on Day 13 pc approximately 10 minutes after dosing. The sponsor attributed the death to a dosing error because a small amount of foam came out of the mouth and nose of the carcass prior to necropsy.

Clinical Signs

One rabbit dosed at 4/2 mg/m^2 of GSK1120212 was reported to have aborted on Day 18 pc. The abortion was considered test-article related in the absence of correlative effect on food consumption.

This reviewer notes that in a dose range finding study, 1 of 4 pregnant rabbits dosed at 4/2 mg/m^2 (same dose as in this pivotal study) aborted on day 22. Also of 4 rabbits dosed at 8/4 mg/m^2, one aborted, one had 100% fetal resorption, and one was euthanized.

Body Weight

Pregnant rabbits administered GSK1120212 at the 1.0/0.5 and 4.0/2.0 mg/m^2 dose levels had increases in mean body weight gains of about 20% from 7-20 days pc compared to baseline weights. Pregnant rabbits administered GSK1120212 had dose-dependent decreases in mean body weight gain of 11%, 22%, and 33% at 1/0.5, 2/1, and 4/2 mg/m^2, respectively, compared to controls during the post-dosing phase of the study (Days 20 to 29 pc). Changes in body weight were not correlated with effects on food consumption.

A slight decrease in mean food consumption (5% of control Mean, 65 g) was reported at 4/2 mg/m^2 between Days 12 and 13 pc. The sponsor states that the amount of food consumed during this interval was within normal variation for Dutch Belted rabbits and so the decrease in consumption is not considered test article-related.

Toxicokinetics

Mated female rabbits were administered GSK1120212 daily on Days 7 through 19 post coitum (pc). On Day 7 pc (Day 1 of dosing), the rabbits were given a loading dose of 1,

Reference ID: 3293647

2, or 4 mg/m^2, and on Days 8 through 19 pc, the rabbits were given a main dose of 0.5, 1, or 2 mg/m^2, respectively. All mated female rabbits (3/dose level) were pregnant. Blood samples were collected on Day 11 pc into tubes containing EDTA at the following nominal times 0.5, 1, 2, 4, 8 and 24 hours after dosing and stored at approximately -20 °C or below until analyzed. For composite sampling, at each of the 0.5, 1, and 2 mg/m^2 doses, the mean plasma concentrations of GSK1120212 at each time point within each dose group were calculated (Table 60).

Table 60: Composite TK Parameters from Female Rabbits Following Oral Administration on Day 11 pc

Parameter n=3/timepoint	Dose of GSK1120212 (mg/m²/day) (loading/main dose)		
	1/0.5	2/1	4/2
AUC_{0-t} (ng.h/mL)	31.9	56.4	127
C_{max} (ng/mL)	2.10	3.55	8.93
T_{max} (h)	2.00	1.00	1.00

The AUC_{0-t} and C_{max} values increased approximately dose proportionally from 1-2 mg/m^2 (~2-fold).

Necropsy

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

As shown in Table 61 whereas the percentage post-implantation loss in pregnant rabbits dosed at ≤ 2/1 mg/m^2 was similar to control animals (~5%), there was a mild increase in post-implantation loss at the high dose level 4/2 mg/m^2 (8.7%), primarily due to an increase in late resorptions.

Table 61: Summary of Pre- and Post-implantation observations

Dose	**0**	**1.0/0.5 mg/m^2**	**2.0/1.0 mg/m^2**	**4.0/2.0 mg/m^2**
Number of Pregnant Females	22	22	21	20
Corpora Lutea	176	185	170	168
Pre-implant Loss	20	20	15	18
% Pre-implant Loss	11.4	10.8	8.8	10.7
Implants	156	165	155	150
Early Resorption	6	6	7	3
Late Resorption	2	3	1	9
Dead Fetuses	0	0	0	0
All Post-implant Loss	8	9	8	13
% Post-implant Loss	5.1	5.5	5.2	8.7
Live Fetuses	148	156	147	137

Reported mean fetal body weights were dose-dependently decreased compared to controls, as shown in Table 62. The reduced fetal weight were consistent with reduced gravid uterus weights

Table 62: Percentage Change in Fetal body weight and gravid uterus

	0	1.0/0.5 mg/m^2	2.0/1.0 mg/m^2	4.0/2.0 mg/m^2
M	33.2 g	↓8%	↓12%	↓18%
F	32.82 g	↓11%	↓14%	↓21%
Gravid uterus	318.5 g	↓5%	↓12%	↓16%

Offspring (Malformations, Variations, etc.)

A dose dependent increase in the incidence of skeletal defects was observed at all doses, primarily related to incomplete ossification of the bones, including the extremities, skull, vertebrae, and sternebrae. Whereas the Applicant reported most of the skeletal defects as variations, the marked enlargement of the anterior fonticulus of the skull (involving 3.6% of fetuses from females dosed at 4/2 mg/m^2), the curved scapula (involving 2.9% of fetuses from females dosed at 4/2 mg/m^2), and the failure of ossification of pubis (involving 1.4% of fetuses from females dosed at ≥2/1 mg/m^2) were reported as malformations (Table 63). Cleft palate was also reported in 2.2% of the fetuses from females dosed at 4/2 mg/m^2.

Table 63: Percentage Incidence of Fetal and Litter Observations

	0	1/0.5 mg/m^2	2/1 mg/m^2	4/2 mg/m^2
Number of Fetuses Examined	**148**	**156**	**147**	**137**
Number of litters	**22**	**22**	**21**	**20**
Mouth/Jaw				
Cleft Palate-(Malformation) (Fetuses)	0	0	0	3(2.2%)
Litters	0	0	0	1(5%)
Heart				
Papillary Muscle Small-(Variation w Review) (Fetuses)	0	0	0	2(1.5%)
Litters	0	0	0	2(10%)
Lung				
Lobe Small-(Variation w Review)	0	0	0	2(1.5%)
Litters	0	0	0	1(5%)
Thorax				
Excessive Fluid-(Variation w Review)	0	1(0.64%)	0	2(1.5%)
Litters	0	1(4.5%)	0	1(5%)
Thymus				
Split-(Variation w_Review)	1(0.68%)	1(0.64%)	1(0.68%)	2(1.5%)
Litters	1(4.5%)	1(4.5%)	1(4.8%)	2(10%)
Caudal Vertebrae				
Arch and/or Centrum Incompletely Ossified-(Variation)	0	2(1.3%)	1(0.68%)	6(4.4%)
Litters	0	2(9.1%)	1(4.8%)	3(15%)
Cervical Vertebrae				
Centrum Incompletely Ossified-(Variation)	4(2.7%)	11(7.1%)	4(2.7%)	10(7.3%)

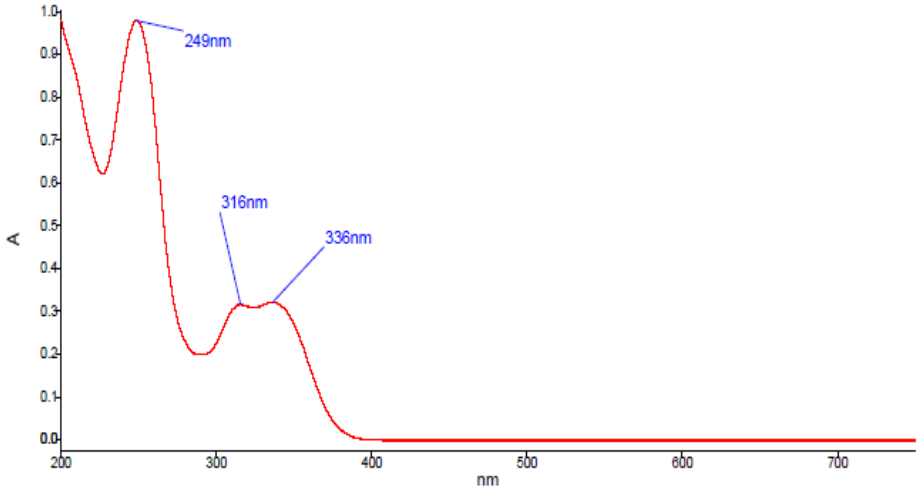
	0	1/0.5 mg/m^2	2/1 mg/m^2	4/2 mg/m^2
Number of Fetuses Examined	**148**	**156**	**147**	**137**
Number of litters	**22**	**22**	**21**	**20**
Litters	4(18.2%)	6(27.3%)	3(14.3%)	6(30%)
Forepaw				
Metacarpal- Less Than the Expected Number are Ossified-(Variation)	5(3.4%)	16(10.3%)	14(9.5%)	31(22.6%)
Litters	2(9.1%)	4(18.2%)	5(23.8%)	11(55%)
Phalanges - Less Than the Expected Number are Ossified-(Variation)	0	0	0	2(1.5%)
Litters	0	0	0	1(5%)
Hindpaw				
Talus Not Ossified-(Variation)	1(0.68%)	1(0.64%)	2(1.4%)	4(2.9%)
Litters	1(4.5%)	1(4.5%)	1(4.8%)	1(5%)
Pubis				
Incompletely Ossified-(Variation)	1(0.68%)	6(3.8%)	3(2.0%)	5(3.6%)
Litters	1(4.5%)	2(9.1%)	2(9.5%)	2(10%)
Not Ossified-(Malformation)	1(0.68%)	0	2(1.4%)	2(1.5%)
Litters	1(4.5)	0	2(9.5%)	1(5%)
Ribs				
T13 Supernumerary-(Variation)	10(6.8%)	22(14.1%)	15(10.2%)	17(12.7%)
Litters	5(22.7%)	10(45.5%)	7(33.3%)	8(40%)
Scapula				
Curved-(Malformation)	0	0	0	4(2.9%)
Litters	0	0	0	1(5%)
Skull				
Incompletely Ossified Frontal-(Variation)	0	0	3(2.0%)	2(1.5%)
Litters	0	0	3(14.3%)	2(10%)
Incompletely Ossified Interparietal-(Variation)	0	0	0	2(1.5%)
Litters	0	0	0	1(5%)
Incompletely Ossified Hyoid-(Variation)	2(1.4%)	4(2.6%)	6(4.1%)	8(5.8%)
Litters	2(9.1)	4(18.2%)	3(14.3%)	4(20%)
Incompletely Ossified Parietal-(Variation)	20(13.5%)	24(15.4%)	30(20.4%)	43(31.4%)
Litters	10(45.5%)	10(45.5%)	13(61.9%)	12(60%)
Anterior Fonticulus Slightly Enlarged-(Variation)	2(1.4%)	1(0.64%)	3(2.0%)	12(8.8%)
Litters	2(9.1%)	1(4.5%)	3(14.3%)	6(30%)
Anterior Fonticulus Markedly Enlarged-(Malformation)	0	0	0	5(3.6%)
Litters	0	0	0	1(5%)
Nasal Variation in Shape-(Variation)	0	0	0	5(3.6%)
Litters	0	0	0	1(5%)
Sternebrae				
Incompletely Ossified-(Variation)	1(0.68%)	3(1.9%)	3(2.0%)	6(4.4%)
Litters	1(4.5%)	3(13.6%)	2(9.5%)	3(15%)

Phototoxicity

Although the Applicant agrees that the photoabsorption spectrum for trametinib shows peaks in the region of concern for photosafety, at 314 and 337 nm (Figure 19), and that trametinib demonstrates a wide tissue distribution of drug-related material, no dedicated phototoxicity studies were conducted. The Applicants justification is that in the uveal tract and pigmented skin, radioactivity was below the limit of quantification by 72 hours post dose, suggesting no selective association of trametinib with melanin containing tissues. The Applicant concludes that while trametinib absorbs light in the region of concern for photosafety and has secondary effects on the skin, there's minimal risk for photosafety to patients receiving oral treatment because of the very low systemic exposure at the recommended clinical dose. Furthermore, to date, there has not been a signal for trametinib-related adverse events due to photosensitivity in clinical studies conducted to date.

Although the argument on the basis of the nonclinical observations appears weak, the justification on the basis of clinical experience appears acceptable.

Figure 19: Photoabsorption Spectrum for Trametinib

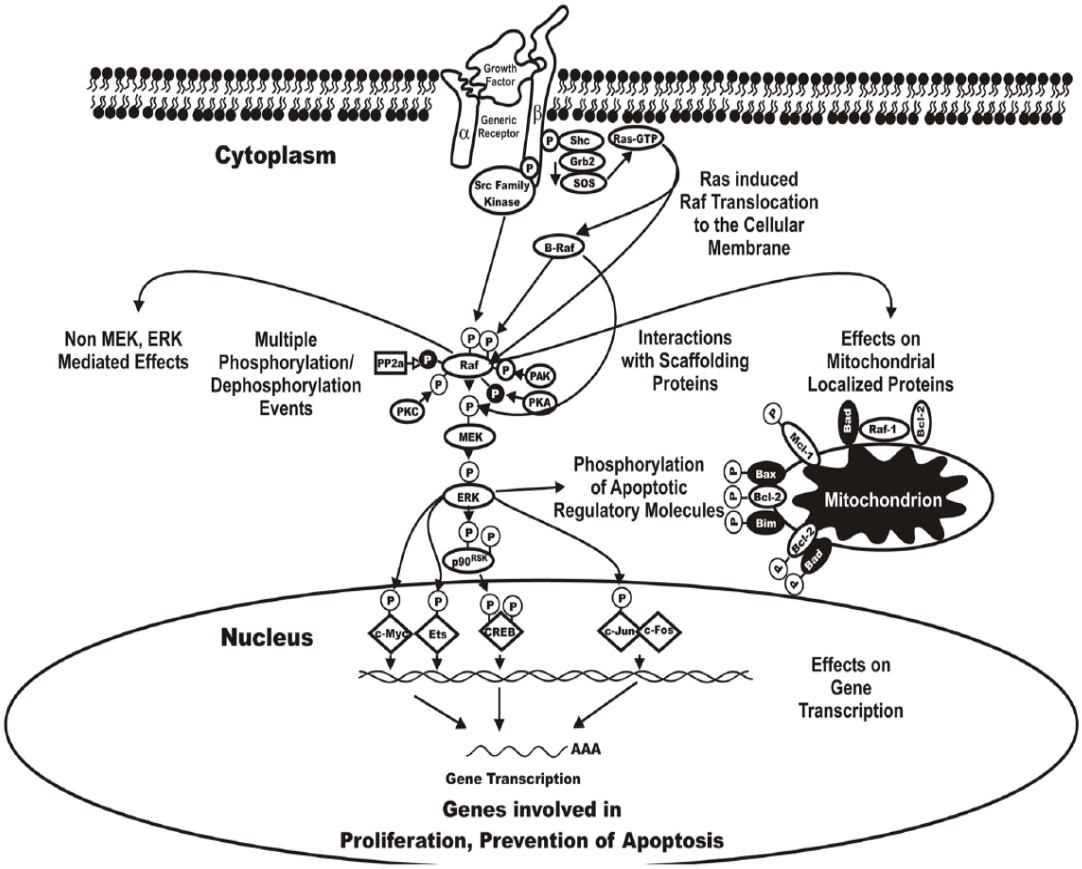


11 Integrated Summary and Safety Evaluation

MEK1 and MEK2 are ubiquitously expressed proteins that participate in the MAPK/ERK signal transduction cascade. MEK proteins propagate signals between the small GTPase Ras, its downstream immediate effector Raf and the extracellular signal-regulated kinases (ERK1/2), which are ultimately responsible for transducing growth-

promoting extracellular signals to the nucleus, via their interactions with c-jun and other transcription factors (Figure 20; McCubrey, 2007[1]).

Figure 20: RAS/MEK/ERK Signaling

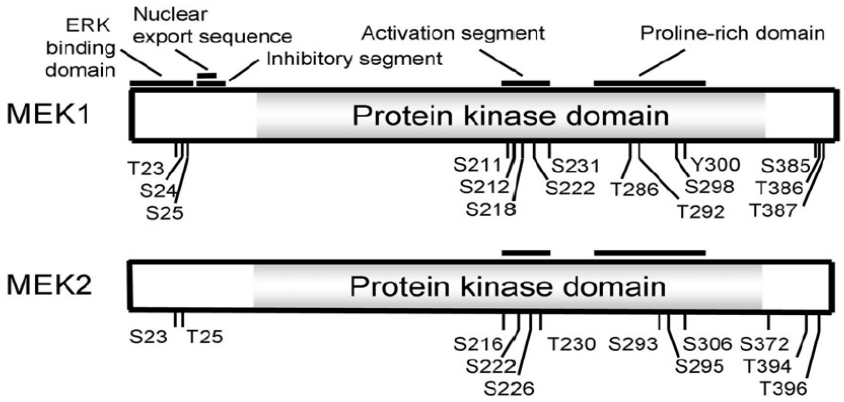


The processes of MEK regulation are highly intricate. MEK1 is activated by phosphorylation of two serine residues (S218 and S222) located in its activation segment. This process is mediated by RAF kinases in the context of a protein scaffold complex called kinase suppressor of Ras (KSR). Conversely, phosphorylation of MEK1 on S212 is inhibitory, and ERK phosphorylation of MEK1 at the T292 residue downregulates the pathway (Figure 21– Roskoski, 2012[2]). Thus, there exist multiple opportunities for dysregulation of MEK signaling including enhanced activity of enzymes that activate MEK, mutations that render the activation segments constitutively active, inhibition of inhibitory enzymes, or mutations that ablate the inhibitory site.

[1] McCubrey, et al., 2007. Biochimica et Biophysica Acta. 1773(8):1263-1284.

[2] Roskoski R Jr. ERK1/2 MAP kinases: structure, function, and regulation. *Pharmacol Res.* 2012 Aug;66(2):105-43.

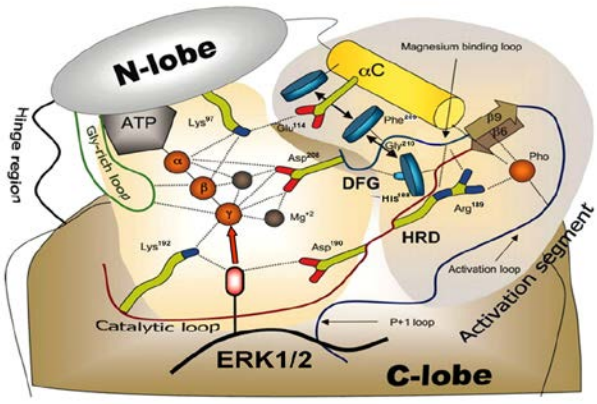
Figure 21: Location of Regulatory Phosphorylation Sites on MEK1 and MEK2 Proteins



GSK2110212B inhibits both the activation of MEK by Raf kinases, and inhibits the activity of phosphorylated MEK (pMEK; activated MEK); however its potency for blocking MEK activation is approximately 10-20-fold greater than its potency for inhibition of pMEK, once the protein has become activated.

BRAF, a serine-threonine kinase, is a proto-oncogene in the ras/raf intracellular signaling pathway that is frequently found to be altered in a number of tumor types, including melanoma. BRAF mutations such as the common V600E mutation, in which a glutamic acid is substituted for valine resulting in a constitutively active protein, can activate growth-promoting pathways. Constitutive activation of these pathways is thought to confer a wide range of neoplastic properties, including enhanced cell growth, proliferation and migration. A number of mutations in the ras/raf pathway render cells susceptible to GSK2110212B-mediated growth inhibition. The enhanced susceptibility observed in these cells to inhibition of MEK activation is hypothesized to be due to dependence on pERK signaling.

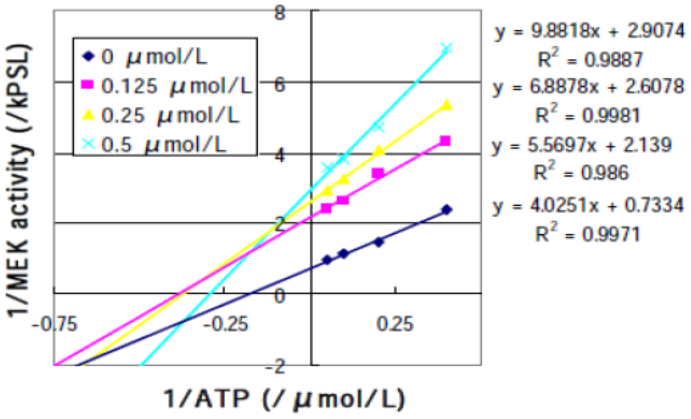
Figure 22: MEK Protein Structure



Structurally, MEK consists of an N-terminal sequence, a kinase domain and a C-terminal sequence. The N-terminal sequence contains an inhibitory and nuclear export sequence as well as a docking sequence for its target (ERK). The catalytic site lies in

the cleft between the small and large lobes (Figure 22– Roskoski, 2012). The surrounding lobes each contain a regulatory site. The Applicant has claimed that GSK1120212B is an (b) (4) inhibitor, (b) (4) however, the disparate location of the intercepts in the Lineweaver-Burk plot (Sponsor-Figure 23) between inhibited and uninhibited enzyme suggest a mixed mode of inhibition. The Applicant has not provided more definitive data on the location of GSK1120212 binding to the MEK protein.

Figure 23: Lineweaver-Burk Plot of Inhibition MEK by GSK1120212



$$Ki = 380 \pm 120 \text{ nmol/L}$$
$$Ki' = 290 \pm 80 \text{ nmol/L}$$

(Excerpted from the Applicant's submission)

The Applicant submitted studies that evaluated *in vitro* activity of trametinib using recombinant kinase domains of various kinases to determine the potency and selectivity of the compound. Trametinib was shown to inhibit the phosphorylation of ERK (IC_{50} values 3.5 to 11 nM), to block BRAF catalyzed MEK1/MEK2 activation by binding to the unphosphorylated MEK1/MEK2 (IC_{50} =0.7 and 0.9 nM, respectively), and to inhibit the kinase activity of phosphorylated MEK1/MEK2 (IC_{50}=13.2 and 10.7 nM, respectively. Trametinib was shown to be an inhibitor of growth in BRAF mutant melanoma cell lines *in vitro* and in BRAF mutant tumor xenografts in mice. Inhibition of tumor growth was coupled with a reduction in phosphorylation of ERK.

Safety Pharmacology

The safety pharmacology assessment for trametinib included *in vitro* assessment of hERG channel activity and cardiac electrophysiology, as well as *in vivo* assessments of neurobehavior, respiratory function, and cardiovascular parameters following drug administration. Trametinib inhibited hERG channel activity in a concentration dependent manner in assays conducted on CHO-K1 and HEK293 cells with IC_{50} values of 3.7 μM and 1.54 μM, respectively, suggesting low potential for causing QTc prolongation at

physiological levels. Trametinib had no effect on QT prolongation when tested in the rabbit left ventricular wedge preparation though there was a decrease in contractility in wedges at high concentrations of the drug, and no effect on cardiovascular parameters when studied in either anesthesized or conscious dogs following single dose administration.

In an attempt to further characterize the cardiac toxicity noted during the clinical development of trametinib, the Applicant conducted a study using male mice administered GSK1120212B at dose levels of 0.25 and 0.5 mg/kg/day by oral gavage once daily for 21 days. Trametinib dependent decreases in mean heart rate and lower mean absolute and relative heart weights were observed, regardless of dose level. In addition, trametinib-treated mice had lower left ventricular functional parameters, but preserved response to dobutamine induced contractility. The decrease in left ventricular function was similar to that reported in humans though mice tolerated higher trametinib exposures than either humans or other animal models and the cardiac findings in this study occurred at exposures 3- to 7-fold higher than the clinical exposure at the recommended dose. .

Neurotoxic findings observed with trametinib in the exploratory neurobehavioral study were considered to be a result of the extreme dose level. Neurotoxicity was not observed at tolerated doses of trametinib. There were no changes in respiratory parameters following administration of up to 1 mg/m^2 trametinib.

ADME

The predominant circulating species of trametinib in both the rat and the dog was unchanged parent, which in rats was detectable for >24 hours post-dose in males and females. In the rat, and to a lesser extent, in the dog, GSK1120212 underwent extensive biotransformation; however, the circulating concentrations of all metabolites were low (<10%). GSK1120212 was metabolized primarily by deacylation, demethylation, ketone formation, mono-oxygenation and glucuronidation. At least one metabolite, designated M5, or GSK1790627, was demonstrated to exhibit pharmacological activity against ERK phosphorylation in $BRAF^{V600E}$ mutant cell lines. GSK1790627 is produced by hydrolytic cleavage of GSK1120212 at the amide group, and is formed in all species tested in vitro; however, given the relatively low systemic exposure to M5, its contribution to the clinical activity of tremetinib remains unclear.

GSK1120212 is considered a concentration-dependent inhibitor of CYPs 2C9 (IC_{50} = 2.2-5 µM), and 3A4 (IC_{50} = 3.2 µM). CYP1A2 activity was increased (activation) in the presence of GSK1120212. Concentrations at which GSK1120212 was demonstrated to have an effect on CYP isoenzyme activity were well above those achieved clinically. Though GSK1120212 was a substrate for multiple CYP enzymes, the overall contribution of all of them to trametinib metabolism was small.

Following administration of GSK1120212, tissue concentrations were highest in the liver, intestines, kidney, adrenals, harderian glands, preputial glands, stomach mucosa,

pituitary glands, pancreas, salivary glands, and thyroid. Penetration into the brain was low, however, the drug did cross the blood brain barrier and was detected in the brain following even a single dose of trametinib at concentrations of approximately 10% of those found in plasma. Compared with blood levels, the compound exhibited modest accumulation in pigmented skin and the uveal tract, indicating that the compound may have a potential for phototoxicity; however, the affinity was evidently low, as radioactivity was not detected by 72 hours in these tissues. *In vitro* plasma protein binding was high in all species tested: 95% in the mouse, 96% in the rat, 97% in the dog, 98% in the monkey, and 97% in humans.

Excretion of trametinib was examined in rats and dogs. The predominant route of elimination in both species was via the feces. Biliary elimination was examined in an experiment using bile duct cannulated male rats; in this experiment bile accounted for approximately 51% of the fecal elimation of trametinib. Total levels of unchanged trametinib (parent) in the feces were between 50-85% in this study. Of that, unabsorbed parent presumably accounted for the bulk of fecal elimination, as biliary excretion of parent was low (<10%). Urinary excretion was <1% of the presumed administered dose.

General Toxicology

The Applicant submitted two definitive GLP-compliant chronic repeat-dose toxicology studies in Sprague-Dawley rats and Beagle dogs. Male rats were administered trametinib (GSK1120212) at doses of 0, 0.25, 0.5 and 1.0 mg/m^2 while female rats were administered trametinib at doses of 0, 0.125, 0.25, 0.5 mg/m^2, daily for 13 weeks. Male and female dogs were administered trametinib at planned doses of 0, 0.15, 0.3, 0.6 mg/m^2 daily for 13 weeks; at the 0.6 mg/m^2 dose level, dosing was stopped on Day 11 for females and Day 12 for males due to the deteriorating clinical condition of the animals and dosing was resumed at the dose level of 0.45 mg/m^2 on Day 21 for females and Day 22 for males.

In rats, because of toxicities resulting in 10 deaths (5M/5F) in the main study groups, dosing at the 1 mg/m^2 level in males and 0.5 mg/m^2 level in females was stopped on Day 48 and all remaining animals in these dose groups were necropsied on Day 50. Also, one male rat at the 0.5 mg/m^2 level died due to skin ulceration/erosion coupled with stomach inflammation and erosion.

Dose levels chosen for female rats were lower than those of male rats due to differences in exposure and the resulting toxicity between the sexes demonstrated in a previous study. At the 0.25 mg/m^2 dose level female rats demonstrated exposures (measured by both AUC and C_{max}) that were approximately 1.5 times higher than those reported in males at the dose level of 0.5 mg/m^2. There was evidence of accumulation of trametinib in both male and female rats following repeated dosing. The exposure measured by AUC in females rats at the 0.5 mg/m^2 dose level during Week 4 was approximately 287 ng·h/mL and was similar to the exposure in male rats at the 1 mg/m^2

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dose level of 285 ng·h/mL; exposure at the high dose level in each sex was approximately 0.77 times the human exposure at the recommended daily dose of 2 mg, (370 ng·h/mL).

Generally, toxicities in high dose groups of rats that were found dead or sacrificed early were similar to the low and mid dose groups of rats that were sacrificed at the end of the experiment, except that toxicities at the high dose appeared to be more severe/intense. Hematology changes included increased absolute neutrophil count, absolute monocyte count, and decreased absolute lymphocyte count, RBC count, hemoglobin and hematocrit. Clinical chemistry changes included increased AST, ALT, and phosphorus. There were decreases in creatinine, total protein, albumin and potassium.

Overall findings in the 4-week rat study submitted at the time of original IND submission were similar to those seen in the 13-week study with the skin being the major target organ for toxicity; however, in the 4-week study the kidney was identified as a potential target organ for trametinib with dose dependent increases in calcium in the serum and increases (>100% compared to controls) in urinary protein levels at the high dose level of 1 mg/m^2.

Histopathologic findings in the rat included skin toxicities (acanthosis, ulcer/erosion, exudate/crust, inflammation (acute or subacute), and/or hypotrichosis/alopecia), gastrointestinal toxicities (mineralization of the glandular muocsa, hyperplasia and inflammation of the squamous mucosa, and erosions involving the squamous mucosa of the stomach), adrenal cortical hypertrophy/hyperplasia, myeloid hypercellularity and degeneration/necrosis in the bone marrow, vacuolation of periportal hepatocytes and focal or multifocal hepatocellular necrosis in the liver, lymphoplasmacytic hyperplasia of the lymph nodes, ovarian cysts and decreases in the number of corpora lutea. Marked decreases in corpora lutea were noted even at the lowest dose in female rats. Hypertrophy/hyperplasia of the cortical adrenal gland occurred at the high dose level. Similar toxicities were reported in the acute toxicology studies in the rat submitted to IND 102175.

In the 13-week dog study, one male dog dosed at 0.6 mg/m^2 was euthanized on Day 14. The dose was reduced to 0.45 mg/m^2 for the rest of the high dose animals and there were no additional unscheduled deaths. Findings in the early sacrifice dog included increased neutrophils, monocytes, AST, and triglycerides. Histopathologic evidence of dark areas/foci, and a dark depressed area in the rectum correlated with minimal to mild multifocal hemorrhage in the lamina propria of the stomach, colon, and rectum, and minimal to moderate ulceration or erosion in stomach were observed. In addition, slight myeloid hyperplasia in the bone marrow and severe lymphoid atrophy in the thymus correlated with the gross observation of a small thymus in this animal. The digestive tract lesions were considered to be the cause of morbidity of the animal. In animals that survived until the end of the study, toxicological findings were generally mild. The skin was a target of toxicity with increases in scabs and lesions noted in the clinical observations. The lungs were also identified as a target in this study: gross findings of pale, raised, or dark areas corresponded with histopathological findings of minimal

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hemorrhage, mononuclear infiltration, pleural fibrosis, and macrophage accumulation. All findings in the lung were classified as minimal to mild; however, clinically treatment with trametinib has been associated with cases of interstitial lung disease and pneumonitis. In dogs the 21-day study submitted to support initiation of clinical dosing, findings in the GI tract and lymphoid tissues were similar to those reported in the longer term study although skin toxicities were not noted in the shorter study (see Appendix). There was an increase in the incidence of delayed capillary refill time in high dose males in the 21-day study. While delays in capillary refill time may suggest cardiovascular insufficiency, there were no histopathological findings in the heart and the general poor condition of high dose animals in this study confounds a clear association. In the 13-week study in dogs there were no significant effects of trametinib treatment on ECG parameters. Minimal epicardial fibrosis was detected in the histopathology for one male dog at the high dose level.

In the 13-week dog study, other than a 2-fold greater AUC_{0-t} at the 0.3 mg/m^2 dose level on Day 1 in females, there were no marked differences in systemic exposure (mean AUC_{0-t} or C_{max} values) between males and females at any dose level. Exposures at the maximum dose level in dogs were all less than half the human exposure at a daily clinical dose of 2 mg measured by AUC (370 ng.h/mL). In male dogs exposures at the 0.45 mg/m^2 dose level were approximately 0.35 times the human exposure at either Week 4 (131 ng·h/mL) or Week 13 (128 ng·h/mL); in females exposures were 0.45 and 0.41 times the human exposure at Week 4 (177 ng·h/mL) and Week 13 (150 ng·h/mL), respectively. The lower toxicity seen in dogs compared to rats may be explained by the lower exposure of dogs to trametinib, about half the exposure compared to that observed in rats in terms of AUC.

Trametinib was negative in *in vitro* and *in vivo* genetic toxicology tests, however, three trametinib impurities were identified as potentially genotoxic: the genotoxic (b) (4) (b) (4) and 2 impurities of (b) (4) though (b) (4) was found to be non-genotoxic in the Ames assay. The specifications for (b) (4) in the (b) (4) are listed as NMT (b) (4) respectively. The Applicant reports that analysis of commercial scale batches of intermediate grade trametinib dimethyl sulfoxide that were representative of the manufacturing route and process, manufactured at the commercial site showed that the levels of all 3 potential genotoxic impurities were below the threshold of toxicological concern (TTC; (b) (4) The Applicant set the specification for each impurity at (b) (4) following (b) (4) for the drug substance and does not expect further formation of the impurities beyond this stage.

At the (b) (4) specification proposed by the Applicant for each of the genotoxic impurities, the level of exposure in humans at the recommended daily dose of 2 mg of Mekinist is (b) (4). At this dose, the level of either the individual or the combined impurities does not exceed the threshold of toxicological concern and the proposed specifications are acceptable from a pharmacologic/toxicologic safety perspective.

The potential for phototoxicity was addressed by assessment of the photoabsorption spectrum for trametinib. In this assessment trametinib showed peaks in the range of 314 to 337 nm, which is a range suggestive of the potential for photosafety concern, particularly given the wide tissue distribution of the drug demonstrated in animal distribution studies. The Applicant states that there has not been a signal for trametinib-related adverse events due to photosensitivity in clinical studies conducted to date.

In summary, animal toxicities were predictive of clinical toxicities except for ocular toxicity. Although the decrease in isometric contractile force of rabbit ventricular wedge preparation and the decreased ventricular performance in mice occurred at concentrations/doses higher than would be expected in humans at the recommended dose of 2 mg, they appear to correlate with decreased LVEF reported clinically.

The doses of potential genotoxic impurities are low and are not likely to pose a health risk in the intended patient population.

Reproductive and Developmental Toxicology

The Applicant conducted GLP-compliant embryo-fetal developmental toxicology studies for GSK1120212 in pregnant Sprague-Dawley rats and pregnant Dutch Belted rabbits. Rats were administered GSK1120212 at dose levels of 0, 0.5/0.125, 0.75/0.25, 1.0/0.5 and 2.86/1.0 mg/m^2 (loading dose/maintenance dose). The loading dose was administered on Day 6 post coitum (pc) and the maintenance dose was administered daily from Days 7-17. In rats, increased maternal toxicity was evident in the increased incidence of scabs on the lips or snout in 2/22 rats at the 1.0/0.5 mg/m^2 and on the face, ears, neck, shoulders, and abdomen in 3/4 rats at the 2.86/1.0 mg/m^2 dose level. At the high dose level these scabs were considered dose limiting and so a full cohort of pregnant animals was not included in the experiment. Decreased maternal body weight gain in animals treated at the 2.86/1.0 mg/m^2 dose level on Days 6-9 correlated with a decrease in food consumption. Fetal toxicity evidenced by increased post-implantation loss, decreased fetal body weight (approximately 20%) compared both to control and mid dose group animals, and increased observations of delayed ossifications and great vessel malformations (missing inominate) were also evident at the 2.86/1.0 mg/m^2 dose level. No 100% litter losses were reported at any dose level. In the main group animals at the 0.75/0.25, and 1.0/0.5 dose levels decreased fetal weight was also observed in the absence of effects on maternal food consumption. There were no clear increases in fetal variations or malformations at dose levels <2.86/1.0 mg/m^2. A mild increase in post-implantation loss in treated dams compared to untreated controls was evident at all dose levels; however, this increase was not clearly dose-related at doses between 0.5/0.125 and 1/0.5 mg/m^2.

Although the toxicokinetic sampling included in this study was conducted in non-pregnant rats, the results show a 4.6-fold increase in exposure measured by AUC at the 2.8.6/1.0 mg/m^2 dose level (684 ng·h/mL, approximately 1.9 times the human exposure at the recommended dose) compared to exposure at the1.0/0.5 mg/m^2 dose level (149

ng·h/mL, approximately 0.4 times the human exposure at the recommended dose). Thus, the increased maternal and fetal toxicities appear consistent with increased maternal exposure to GSK1120212.

Dutch Belted rabbits were administered GSK1120212 at dose levels of 0, 1.0/0.5, 2.0/1.0, and 4.0/2.0 mg/m^2 (loading dose/maintenance dose). The loading dose was administered on Day 7 pc and the maintenance dose was administered daily from Days 8-19. At the high dose of 4/2 mg/m^2 the maternal exposure measure by AUC was 127 ng·h/mL, approximately 0.3 times the exposure in humans at the recommended dose of 2 mg. In rabbits, administration of the high dose of GSK1120212 in the pivotal embryo-fetal development study (4.0/2.0 mg/m^2) resulted in 100% loss of fetuses (abortion) in one of 4 rabbits on day 18 pc. A decrease in maternal body weight gain at dose levels ≥1.0/0.5 mg/m^2 occurred only after the end of dosing and was not correlated with decreased food consumption; however there was a trend in reduced fetal body weight at all dose levels of GSK1120212 compared to controls. At the 4.0/2.0 mg/m^2 dose level fetal toxicity was further evidenced by increased post-implantation loss, increased incidence of incomplete ossification of bones, and increased incidence of cleft palate and markedly enlarged anterior fonticulus. In addition, in a dose range finding study, 1 of 4 pregnant rabbits dosed at the 4/2 mg/m^2 dose level aborted on Day 22 and of the 4 rabbits dosed at the 8/4 mg/m^2 dose level, one aborted, one had 100% fetal resorption, and one was euthanized. Given the maternal and fetal toxicities observed in these studies, Pregnancy Category D is recommended.

Based on the review of the supporting nonclinical studies, there are no outstanding nonclinical issues that must be addressed to support the use of this drug in patients with advanced cancer, thus, the pharmacology/toxicology review team recommends the approval of this marketing application.

12 Appendix/Attachments

Reviews of 3-week repeat dose toxicology and genetic toxicology studies are excerpted from IND 102,175, reviewed by Dr. Robeena Aziz, 2008.

2.6.6.3 Repeat-dose toxicity

Study title: GSK1120212B: 3-Week Oral Toxicity Study in Sprague-Dawley Rats Followed by a 2-Week Recovery Period.

Key study findings:

- Drug-related mortalities: 1 F at HD, 1.00 mg/m2/day, on Study Day 20.
- Skin was prominent target organ of toxicity leading to mortality in females only.
- Other target organ of toxicity: glandular stomach and liver. Secondary effects in lymph nodes of males and females and bone marrow of females only.
- STD_{10} is 1.00 mg/m2/day, the high-dose tested.

Study no.:	CD2007/00984/00/G07042
Volume #, and page #:	Volume 8; Pages 1-784
Conducting laboratory and location:	GlaxoSmithKline King of Prussia, PA, USA
Date of study initiation:	October 3, 2007
GLP compliance:	yes
QA report:	yes (X) no ()
Drug, lot #, and % purity:	GSK1120212B Lot No.: eE 169079 Purity: 99.4%

Dose justification: In a previous 14-day oral toxicity study in rats (Study No. CD2006/01117/00), GSK1120212B was administered at 0, 0.1, 0.3 or 1 mg/kg/day (approximately 0, 0.7, 2 and 7 mg/m2/day, respectively). The HD of 7 mg/m2/day resulted in ulcerations of the skin in females, transient body weight loss in males and gastric fudic gland mineralization with hypertrophy in both sexes. Mean total white cell counts were decreased for males $\geq$2 mg/m2/day but were slightly increased for females at 0.7 mg/m2/day. Body weight loss and/or reduced body weight gain were seen for females at 0.7 mg/m2/day, associated with reduced food consumption. In a follow-up oral toxicity study (Study No. CD2006/01957/00J), GSKl120212B was administered at 0, 1, 2 and 3 mg/kg/day (approximately 0, 7, 14, and 21 mg/m2/day, respectively). Mortality occurred at the MD and HD between Days 6-12. Mortality was attributed to decreased body weight and microscopic changes in skin, liver, kidney, heart, aorta, bone, stomach, lung, ovaries, mammary gland, and small and large intestines including rectum. Test article-related findings in moribund sacrificed rats were similar to findings noted for the remainder of rats at $\geq$7 mg/m2/day. Day 6 predose plasma concentrations indicated significant accumulation at all doses. A single oral dose rat pharmacokinetic study (Study No. CD2007 /00787 /00) at doses of 0.9 and 5 mg/m2 resulted in AUCs of 140 and 1700 ng.hr/mL,

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respectively. Based on systemic exposure comparison between the single dose pharmacokinetic study and previous 14-day studies, a high dose of 1 mg/m2/day was selected for this study. Doses of 0.125, 0.25, 0.5 mg/m2/day were selected to explore dose-response relationships.

Methods:

Doses:	0, 0.125, 0.250, and 0.500, 1.00 mg/m2/day
Species/strain:	Sprague-Dawley Rats Crl:CD IGS
Number/sex/group or time point (main study):	16/sex/dose in Cont., MD2 and HD 10/sex/dose in LD and MD1
Satellite groups used for toxicokinetics:	3/sex/dose in Cont.-HD
Formulation/vehicle**:**	1.5% HPMC/5% Mannitol 60/0.2% SLS
Route/dose volume:	oral gavage, 40 mL/m2/day
Age:	10-weeks old
Weight:	M: 318−440 g/F: 212−285 g
Unique study design or methodology (if any):	none
Schedule:	once daily x 21

Observations and times:

Mortality:	Once daily during treatment, and recovery
Clinical signs:	Daily during pretest and 1x/daily during treatment and recovery
Body weights:	At least once pretest (Day -8) and on Days 1, 4, 8, 11, 15, 18, 21, 25, 28, and 31, and 35
Food consumption:	At least once pretest and 1x/week during treatment and recovery
EKG	Not done
Ophthalmoscopy:	Once pretest (Day -13) and on Day 18
Hematology:	Once pretest and on Days 3 and 21, 30, and 36
Clinical chemistry:	Once pretest and on Days 3 and 21, 30, and 36
Coagulation	Once pretest and on Days 3 and 21, 30, and 36
Urinalysis:	Once pretest and on Days 3 and 21, 30, and 36
Gross pathology:	Conducted on all animals including those found dead, euthanized *in extremis*, and those euthanized on Days 22 (terminal) and 36 (recovery).
Organ weights:	Days 22 (terminal) and 36 (recovery).
Histopathology:	All groups (terminal and recovery) Adequate Battery: yes (X), no ( )—explain Peer review: yes (X), no ( )
Toxicokinetics:	0, 0.50, 1.0, 2.0, 4, 8, and 24 hrs. after dosing Days 1 and 21 .

Results:

Mortality:

Index	No. of animals affected
Gender	Female
Dose (mg/m2/day)	1.00
No. of animals	16
No. of deaths[a]	1
Day of study	20

[a]= animal was sacrificed in moribund condition

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Clinical signs:

- At ≥0.500 mg/m2/day: Raised scabs located around the snout, lower lip, periocular area and/or dorsal neck with an earlier onset and greater severity in females than males.
- At 1.00 mg/m2/day: Raised scabs to exudative lesions were more widespread and progressed in most male and female rats.
- Skin lesions were still evident at the end of the recovery period for male and female rats at 1.00 mg/m2/day only.

Body weight**:** unremarkable.

Food consumption**:** unremarkable.

Ophthalmoscopy: unremarkable

EKG: not done

Hematology:

Index	% Control			
Gender	Males		Females	
Dose (mg/m2/day)	0.500	1.0	0.500	1.0
No. of animals	16	16	16	16
WCC				
Week 4			+57**	+84**
Week 5				+56*
Week 6				-36*
NEUT				
Week 4		+60*	+145**	+616**
Week 5				+406**
Week 6				+42*
MONO				
Week 4			+80**	+316**
Week 5		+174*		+233**
ESO				
Week 4	-48**	-65**		
BASO				
Week 4				+100*

– **=p≤0.001; *=p≤0.05

Clinical Chemistry:

Index	% Control			
Gender	Males		Females	
Dose (mg/m2/day)	0.500	1.0	0.500	1.0
No. of animals	16	16	16	16
PHOS				

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Day 3	+6*	+32**	+14**	+38**
Week 4		+33**	+27**	+36**
CA				
Day 3	+9**	+24**	+15**	+40**
Week 4		+36*	+27*	+29*
ALT				
Week 4			+74**	+106**

- Note: Recovery animals: unremarkable
- **=p≤0.001; *=p≤0.05

Coagulation: unremarkable

Urinalysis:

Index	No. of animals affected
Gender	Females
Dose (mg/m2/day)	1.00
No. of animals	16
Total protein excretion	
Week 4	+162**
Protein: Creatinine ratio	
Week 4	+153**

- Note: Recovery animals: unremarkable
- **=p≤0.001

Gross Pathology-**Terminal Sacrifice:**

- At ≥0.500 mg/m2/day: Skin crusts and/or skin discolorations involving the nares/rostrum, chin/ventral neck, lateral/ventral and dorsal thoracic and scapular regions, abdomen, back and axilary areas.

Gross Pathology-**Recovery:**

- At 1.00 mg/m2/day: Raised skin crusts and/or skin discoloration involving the ventral chin/dorsal back and thorax in 3/6 female rats.

Organ weights: unremarkable

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Histopathology – **Terminal Sacrifice:**

Index	No. of animals affected							
Gender	Males				Females			
Dose (mg/kg/m2)	0.125	0.250	0.500	1.00	0.125	0.250	0.500	1.00
Skin								
Acanthosis; localized								
– *minimal*				1/10			2/10	3/9
– *mild*			1/10	2/10				6/9
Ulcer; focal								
– *minimal*							1/10	1/9
Ulcer; multifocal								
– *minimal*								1/9
– *mild*								2/9
– *moderate*								6/9
Stomach								
Mineralization; glandular region								
– *minimal*		1/10		1/10	2/10	2/10	5/10	5/9
– *mild*				1/10				2/9
– *moderate*								1/9
Liver								
Vacuolation; periportal; multifocal								
– *minimal*	2/10	2/10	6/10	6/10	1/10	3/10	4/10	8/9
– *mild*								1/9
Bone marrow								
Hyperplasia; myeloid								
– *minimal*								1/9
– *mild*								5/9
– *moderate*								1/9
Lymph node-mandibular/cervical								
Cellularity increased; lymphoplasmacytic								
– *mild*				1/10			1/10	2/9
– *moderate*								6/9
Lymph node-axillary								
Cellularity increased; lymphoplasmacytic								
– *mild*								1/9
– *moderate*								1/9

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Histopathology – **Recovery:**

Index	No. of animals affected			
Gender	Males		Females	
Dose (mg/kg/m2)	0.500	1.00	0.500	1.00
Skin				
Acanthosis; localized				
– *minimal*	1/6			1/6
– *mild*				1/6
Fibrosis; dermis; localized				
– *minimal*				1/6
Ulcer; focal				
– *mild*				1/6
Ulcer; multifocal				
– *mild*				1/6
Stomach				
Mineralization; glandular region				
– *minimal*	1/6			2/6
– *moderate*		1/6		
Liver				
Vacuolation; periportal; multifocal				
– *minimal*				1/6

Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Toxicokinetics:

- 3/sex/dose-in Cont.- HD
- Cmax and AUC increased in a dose-proportional manner.
- Maximum plasma concentrations were observed between 0.5 and 8 hours after dosing with T_{max} values were large ranging from 2 to 8 hours.
- Drug accumulation apparent between Days 1 and 21.
- Mean AUC values were higher in females compared to males on Day 1 and 21.
- $T_{1/2}$ were not provided by the Sponsor.
- Details are listed in the Sponsor's table below:

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Parameter	Period (Day)	Male (n = 3) Dose of GSK1120212 (mg/m²/day)			
		0.125	0.250	0.500	1.00
AUC_{0-t} [a] (ng.h/mL)	1	NC	3.65[c] [2.46, 4.84]	33.3 [32.3 – 34.5]	64.2 [37.6 – 83.2]
	21	35.0 [31.5 – 39.7]	64.2 [54.7 – 78.2]	129 [102 – 160]	218 [195– 255]
C_{max} [a] (ng/mL)	1	NC	0.780 [0.694 - 0.844]	2.16 [1.81 – 2.36]	5.48 [4.95 – 6.28]
	21	1.78 [1.56 – 2.16]	3.50 [3.09 – 4.23]	7.78 [6.48 – 9.68]	13.3 [12.2 – 14.8]
T_{max} [b] (h)	1	NC	2.00 [2.00 - 2.00]	8.00 [2.00 – 8.00]	2.00 [1.00 – 2.00]
	21	4.00 [2.00 – 4.00]	4.00 [4.00 – 8.00]	4.00 [4.00 – 4.00]	4.00 [2.00 – 4.00]
Parameter	Period (Day)	Female (n = 3) Dose of GSK1120212 (mg/m²/day)			
		0.125	0.250	0.500	1.00
AUC_{0-t} [a] (ng.h/mL)	1	NC	18.1 [16.0 – 20.0]	52.2 [46.0 – 62.1]	193 [175 – 228]
	21	60.2 [45.6 – 75.6]	126 [99.8 – 144]	211 [154 – 291]	460 [442 – 473]
C_{max} [a] (ng/mL)	1	0.596[c] [0.529, 0.663]	1.19 [1.04 – 1.29]	3.53 [2.50 – 4.88]	11.2 [9.82 – 12.2]
	21	3.33 [2.58 – 3.72]	6.28 [5.08 – 7.11]	13.0 [10.6 – 16.5]	29.4 [28.0 – 31.3]
T_{max} [b] (h)	1	4.50[c] [1.00, 8.00]	2.00 [1.00 – 2.00]	2.00 [2.00 – 2.00]	2.00 [1.00 – 2.00]
	21	2.00 [0.50 – 4.00]	4.00 [1.00 – 8.00]	1.00 [1.00 – 4.00]	4.00 [2.00 – 4.00]

a. Results are reported as Mean and [Range].
b. Results are reported as Median and [Range].
c. n = 2. No or insufficient quantifiable plasma concentration data in the third animal.
NC = Not Calculated due to no or limited quantifiable plasma concentration data.

[Table excerpted from Sponsor]

Other: none

Study Title: GSK1120212B: 3-Week oral toxicity study in dogs followed by a 2-week recovery period

Key study findings:

- Drug-related mortalities in males only at 0.75 mg/m2/day (1 death) and 1.5 mg/m2/day (3 deaths).
- Due to mortality and clinical signs, remaining male dogs (N=2) at the 1.5 mg/m2/day dose group were not dosed after Study Day 8.
- Target organ of toxicity: GI, bone marrow, spleen, liver and mandibular/cervical lymph nodes (Males only).
- HNSTD is 0.5 mg/m2/day, the low-dose tested in males and high-dose tested in females.

Study no.: CD2007/00966/00/G07043
Volume #, and page #: Volume 11; Pages 1-572
Conducting laboratory and location: GlaxoSmithKline
King of Prussia, PA, USA

Reference ID: 3293647

Date of study initiation: August 31, 2007
GLP compliance: yes
QA report: yes (X) no ()
Drug, lot #, and % purity: GSK1120212B
Lot No.: eE 169079
Purity: 99.4%

Dose justification: Dose selection was based on dose-ranging study in dogs (Study No CD2006/01539/00). GSK1120212B was administered via oral gavage at 0, 2.5, 5, and 10 mg/m2/day for 10 days. Dogs at dose levels of ≥5 mg/m2/day were sacrificed in moribund condition on Days 4 and 7. Early deaths were attributed to body weight loss and gastrointestinal injury. Clinical signs included abnormal fecal discoloration/consistency, reduced food intake and emesis. Microscopic observations included gastrointestinal toxicity (mucosal necrosis, hemorrhage, inflammation, mucosal and vilus atrophy), with associated clinical pathology changes (including decreased red blood cell parameters, increased total white cell counts, decreased platelet count and decreased serum protein and albumin concentrations), and lymphoid necrosis and atrophy. Dogs at the 2.5 mg/m2/day dose level survived until the end of the study, but had reduced food intake, absent/scant feces and pallor of the pinna. Body weight loss, emesis, abnormal fecal discoloration/consistency and coldness-to-touch were the only clinical findings observed in females only. Microscopic observations at this dose included duodenal crypt necrosis in the female, minimally increased granulocytic and mildly decreased erythroid progenitor cells in the bone marrow with associated decreased reticulocyte counts. In a single-dose toxicokinetic study (Study No. RD2007/00991/00), apparent gender differences were noted with lower AUC and Cmax values in males compared to females (Male doses: 0, 0.5,1 and 2 mg/m2; AUC(0-t) values 6, 64 and 153 ng.h/mL, respectively, and Cmax values 2, 11 and 15 ng/mL, respectively. Females doses: 0, 0.25,0.325,0.425 and 0.5 mg/m2; AUC(0-t) values of 2, 21,33 and 96 ng.h/mL, respectively, and Cmax values of 1, 2, 4 and 16 ng/mL, respectively). Therefore, based on duodenal crypt necrosis and bone marrow changes at 2.5 mg/m2/day as well as gender differences from the toxicokinetic study, a high dose of 1.5 and 0.5 mg/m2/day were selected for males and females, respectively as the MTD. Male doses of 0.75 and 0.5 mg/m2/day and female doses of 0.4 and 0.3 mg/m2/day were selected to evaluate potential dose-response relationships.

Methods:

Doses:	M: 0, 0.5, 0.75, and 1.5 mg/m2/day F: 0, 0.3, 0.4, and 0.5 mg/m2/day
Species/strain:	beagle dogs
Number/sex/group or time point (main study):	5/sex/dose in Cont., MD and HD 3/sex/dose in LD
Satellite groups used for toxicokinetics:	3/sex/dose in Cont.-HD
Formulation/vehicle**:**	1.5% HPMC/5% Mannitol 60/0.2% SLS
Route/dose volume:	oral gavage, 40 mL/m2/day
Age:	12-21 months old
Weight:	M: 8-11 kg/F: 6-9 kg
Unique study design or methodology (if any):	

Reference ID: 3293647

1. Due to severe clinical signs and BW loss, 3/5 male dogs at 1.5 mg/m2/day and 1/5 male dogs at the 0.75 mg/m2/day were sacrificed in moribund condition on Study Day 8 and 11, respectively.
2. Remaining recovery dogs (N=2) at the 1.5 mg/m2/day were not dosed after Study Day 8.
3. No male dogs at the 1.5 mg/m2/day (HD) were available for recovery.

Schedule: once daily x 21

Observations and times:

Mortality:	Twice daily during treatment, and recovery
Clinical signs:	Daily during pretest and 1x/daily during treatment and recovery
Body weights:	At least once pretest and on Days 4, 8, 11, 14, 17, 22, 25, 29, and 32
Food consumption:	At least once pretest and on Days 3, 4, 10, 11, 12 (Group 3 only), 16, 17, 24, 25, 31 and 32
EKG	Once pretest (Day -15) and Day 16
Ophthalmoscopy:	Once pretest (Day -13) and on Day 18
Hematology:	Once pretest (Day -13) and on Days 18 and 35
Clinical chemistry:	Once pretest (Day -13) and on Days 18 and 35
Coagulation	Once pretest (Day -13) and on Days 18 and 35
Urinalysis:	Once pretest (Day -13) and on Days 18 and 35
Gross pathology:	Conducted on all animals including those found dead, euthanized *in extremis*, and those euthanized on Days 21 or 22 (terminal) and 36 (recovery).
Organ weights:	Days 21 or 22 (terminal) and 36 (recovery).
Histopathology:	All groups (terminal and recovery) Adequate Battery: yes (X), no ( )—explain Peer review: yes (X), no ( )
Toxicokinetics:	0, 0.50, 1.0, 2.0, 4, 8, and 24 hrs. after dosing Days 1 and 21 .

Results:

Mortality:

Index	No. of animals affected	
Gender	Male	
Dose (mg/m2/day)	0.75	1.5
No. of animals	5	5
No. of deaths	1*	3*
Day of study	8	11

*All animals were sacrificed in moribund condition.

Reference ID: 3293647

Clinical signs:

Index	No. of animals affected	
Gender	Males	
Dose (mg/m2/day)	0.75	1.5
No. of animals	5	5
Study Days 1-5		
– Inappetence	1	4
– Soft, mucoid feces	1	4
– Loose, watery feces	1	4
– Unkempt coat	1	4
– Red discharge	1	4
– Delayed capillary time	1	4
Study Days 6-10		
– Inappetence	1	2
– Subdued behavior	1	2
– Absent/mucoid feces	1	2
– Loose, watery feces	1	2
– Brown/red discharge	1	2
– Red/brown urine	1	
– Salivation	2	2

– Note: Female animals: unremarkable
– Note: Recovery animals: unremarkable

Body weight**:**

- M at ≥0.75 mg/m2/day: Non-significant decrease (≤20%) during Days 4-8 and 4-11.
- Female animals: unremarkable.
- Recovery animals: unremarkable.

Food consumption**:**

- M at ≥0.75 mg/m2/day: Non-significant decrease (≤20%) during Days 4-8 and 4-11.
- Female animals: unremarkable.
- Recovery animals: unremarkable.

Ophthalmoscopy: unremarkable

ECG: unremarkable

Hematology:

Index	% Control			
Gender	Males		Females	
Dose (mg/m2/day)	0.75	1.5	0.75	1.5
No. of animals	2	1	3	3
Dosing Day 18				
HGB	-8	-22		

Reference ID: 3293647

HCT	-8	-17		
RETIC	-72	-86	-62	-51
MONO	+38	+29		
Dosing Day 35 (Recovery)				
RETIC	+49		+300	+84

– All values were significant; $p \leq 0.05$.

Clinical chemistry:

Index	% Control	
Gender	Males	
Dose (mg/m2/day)	0.75	1.5
No. of animals	2	1
Dosing Day 18		
ALP	+74	+152
GLDH	+47	+179
TRIGLY		+52
ALB	-14	-28

– Note: Female animals: unremarkable
– Note: Recovery animals: unremarkable
– All values were significant; $p \leq 0.05$.

Coagulation: unremarkable

Urinalysis: unremarkable

Gross pathology - **Early death**:

Index	No. of animals affected	
Gender	Males	
Dose (mg/m2/day)	0.75	1.5
No. of animals	1	3
Colon Contents, discolored, mucosa red	1	
Duodenum Discolored, mucosa, red, several		2
Esophagus Discolored, mucosa, red, multifocal	1	
Rectum Discolored, mucosa, red, multifocal	1	
Stomach Discolored, mucosa, red, mild fundic region Discolored, grandular region, red, mild Discolored, mucosa, red, mild cardia and pyloric regions	1	 1 1

Reference ID: 3293647

Gross pathology – **Terminal Sacrifice and Recovery**: unremarkable

Organ weights– **Terminal Sacrifice and Recovery**: unremarkable

Histopathology **– Early death:**

Index	No. of animals affected	
Gender	Males	
Dose (mg/m2/day)	0.75	1.5
No. of animals	1	3
Adrenals		
Hypertrophy; zona fasciculata		
– *moderate*	1	
Bone marrow		
Hyperplasia, myeloid		
– *minimal*		3
Cecum		
Inflammation, neutrophilic and erosive; mucosa		
– *minimal*		1
Lymphoid depletion; GALT		
– *moderate*		1

Index	No. of animals affected	
Gender	Males	
Dose (mg/m2/day)	0.75	1.5
No. of animals	1	3
Colon		
Inflammation, neutrophilic and erosive; mucosa		
– *minimal*		1
– *moderate*		1
Inflammation, neutrophilic and erosive; mucosa/submucosa		
– *moderate*	1	
Lymphoid depletion; GALT		
– *minimal*		1
– *moderate*		1
Duodenum		
Inflammation, neutrophilic; mucosa		
– *mild*		3
Esophagus		
Inflammation, neutrophilic; and erosive; mucosa/submucosa		
– *mild*		1

Reference ID: 3293647

Index	No. of animals affected	
Gender	Males	
Dose (mg/m2/day)	0.75	1.5
– *moderate*	1	
Ileum		
Inflammation, neutrophilic; and erosive; transmural		
– *moderate*		1
Jejunum		
Inflammation, neutrophilic; mucosa		
– *mild*		1
Liver		
Sinus neutrophilia, centrilobular		
– *minimal*	1	1
– *mild*		1
Kuppfer cell activation		
– *mild*	1	1
– *moderate*		1
Hyperplasia; bile duct		
– *mild*		1
Pigment; hepatocyte		
– *minimal*		1
Lymph node-Mandibular/cervical		
Sinus neutrophilia		
– *minimal*	1	1
Rectum		
Inflammation; neutrophilic and erosive; mucosa		
– *moderate*		1
Inflammation; neutrophilic and erosive; mucosa/submucosa		
– *moderate*	1	1
Lymphoid depletion; GALT		
– *minimal*		1
– *moderate*		1
Spleen		
Sinus neutrophilia; red pulp		
– *minimal*	1	1
– *moderate*		2
Stomach		
Inflammation; neutrophilic and erosive, mucosa; fundic region		

Reference ID: 3293647

Index	No. of animals affected	
Gender	Males	
Dose (mg/m2/day)	0.75	1.5
– *moderate* Inflammation; neutrophilic and erosive, mucosa; pyloric region	1	
– *minimal*		1
– *moderate*		1
Thymus Lymphoid depletion		
– *minimal*		1
– *moderate*		1
Tongue Inflammation; neutrophilic; perivascular;mucosa/submucosa		
– *mild*		1

Histopathology – **Terminal Sacrifice:**

Index	No. of animals affected	
Gender	Males	
Dose (mg/m2/day)	0.75	1.5
No. of animals	2	0
Bone marrow Hyperplasia, myeloid		
– *minimal*	1	
Spleen Sinus neutrophilia; red pulp		
– *minimal*	2	

– Note: Female animals: unremarkable

Histopathology – **Recovery:**

Index	No. of animals affected	
Gender	Males	
Dose (mg/m2/day)	0.75	1.5
No. of animals	2	2
Thymus Lymphoid depletion		
– *minimal*		2

– Note: Female animals: unremarkable

Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no (X)

Reference ID: 3293647

Toxicokinetics:

- 3/sex/dose-in Cont.- HD
- Maximum plasma concentrations were observed between 0.5 to 4 hours after dosing with T_{max} values were small ranging from 0.5 to 4 hours.
- Accumulation apparent between Day 1 and 21.
- Cmax and AUC increased in a dose-proportional manner.
- $T_{1/2}$ were not provided by the Sponsor.
- Details are listed in the Sponsor's table below:

Parameter	Period	Males		
		Dose of GSK1120212 (mg/m²/day)		
		0.5[a]	0.75[b]	1.5[b]
AUC_{0-t}[c] (ng.h/mL)	Day 1	12.2 [3.28 – 17.6]	27.6 [20.2 – 37.5]	73.2 [49.5 – 111]
	Day 21	159 [140 – 192]	282[e] [245 – 326]	ND
C_{max}[c] (ng/mL)	Day 1	1.70 [1.31 – 1.98]	3.25 [2.29 – 4.00]	7.31 [5.18 – 11.8]
	Day 21	9.37 [8.50 – 9.93]	19.0[e] [15.1 – 22.4]	ND
T_{max}[d] (h)	Day 1	1.00 [0.50 – 1.00]	0.50 [0.50 – 0.50]	0.50 [0.50 – 2.00]
	Day 21	1.00 [1.00 – 8.00]	1.00[c] [0.50 – 2.00]	ND
Parameter	Period	Females		
		Dose of GSK1120212 (mg/m²/day)		
		0.3[a]	0.4[b]	0.5[b]
AUC_{0-t}[c] (ng.h/mL)	Day 1	3.59 [2.11 – 6.19]	17.2 [3.04 – 26.0]	23.8 [15.6 – 34.7]
	Day 21	120 [112 – 132]	211 [142 – 261]	205 [129 – 277]
C_{max}[c] (ng/mL)	Day 1	1.42 [0.778 – 1.90]	2.14 [0.966 – 3.22]	2.48 [1.14 – 3.48]
	Day 21	7.19 [5.83 – 8.66]	11.6 [7.43 – 16.4]	12.3 [7.20 – 17.7]
T_{max}[d] (h)	Day 1	0.50 [0.50 – 2.00]	0.50 [0.50 – 1.00]	0.50 [0.50 – 1.00]
	Day 21	1.00 [1.00 – 2.00]	4.00 [1.00 – 4.00]	2.00 [1.00 – 4.00]

a. n=3
b. n=5
c. Results are reported as Mean and [Range].
d. Results are reported as Median and [Range].
e. n = 4. Dosing for Animal D07M-2596 was terminated on Day 9 due to adverse clinical observations.
ND = No data. The last day of dosing for these animals was Day 7.

[Tables excerpted from Sponsor]

Reference ID: 3293647

Histopathology inventory (optional)

Study	CD2007/00984/00	CD2007/00966/00
Species	Rat	Dog
Adrenals	X*	X*
Aorta	X	X
Bone Marrow smear	X	X
Bone (femur)	X	X
Brain	X*	X*
Cecum	X	X
Cervix	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Eye	X	X
Fallopian tube		
Galt (Peyer's patch)		X
Gall bladder		X
Gross lesions	X	
Harderian gland		
Heart	X*	X*
Ileum	X	X
Injection site	X	X
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland		
Larynx		X
Liver	X*	X*
Lungs	X	X
Lymph nodes, axillary		
Lymph nodes mandibular	X	
Lymph nodes, mesenteric	X	X
Lymph node (inguino-femoral)	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves		
Ovaries	X*	X*

Reference ID: 3293647

Study	CD2007/00984/00	CD2007/00966/00
Oviduct		
Pancreas	X	X
Parathyroid	X	X
Peripheral nerve		
Pharynx		
Pituitary	X	X
Prostate	X*	X*
Rectum		X
Salivary gland	X	X
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin and adnexa	X	X
Spinal cord	X	
Spleen	X*	X
Sternum	X	X
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X*	X
Tongue	X	X
Trachea		
Ureter		
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		

X* = organ weights taken

2.6.6.4 Genetic toxicology:

Study title: GSK1120212B: Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* – and *E. Coli*

Key findings:

- The test article was not mutagenic either in the presence or absence of microsomal enzymes when evaluated at up to and including 1500 and 2500 µg/plate (the maximum concentrations tested were limited by precipitation).

Reference ID: 3293647

Study no.: WD2007/00304/00
Volume #, and page #: Vol. 13, P.1-51
Conducting laboratory and location: (b) (4)

Date of study initiation: March 2, 2007
GLP compliance: Letter included and signed
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Drug: GSK1120212B (dimethyl sulphoxide solvate)
Lot #: 071131978
Purity: 99.2%

Methods

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

Concentrations used in definitive study:

First main plate incorporation test with S-9 activation:	1.5, 5, 15, 50, 150, 500 and 1500 µg/plate
First main plate incorporation test without S-9 activation:	5, 15,50, 150,500, 1500 and 2500 µg/plate
Second main plate incorporation test with and without S-9 activation:	1.5, 5, 15, 50, 150, 500 and 1500 µg/plate

Basis of concentration selection:
Concentration selection was based on previous precipitation of the test compound (assessed by eye at the end of incubation) observed on all plates treated with 500 µg/plate and above in the presence of S-9 mix and with 1500 µg/plate and above in the absence of S-9 mix following 3 days incubation at 37°C. There was clear evidence of bactericidal activity observed as a diminution of the background bacterial lawn in the absence of S9-mix at 4500 µg/plate in strain TA98. On the basis of this result, the maximum concentration tested in the main Ames tests was 2500 µg/plate for treatments in the absence of S-9 mix in Mutation Test 1 and 1500 µg/plate for all other treatments.

Negative controls:
DMSO

Positive controls:

Strain	**Without S9**	**With S9**
TA98	Benzo(a)pyrene	2-nitrofluorene
TA100	2- aminoanthrace	Sodium azide
TA1535	2- aminoanthrace	Sodium azide
TA1537	2- aminoanthrace	9-aminoacridine (AAC)
WP2*uvrA*pKM101	2- aminoanthrace	4-nitroquinoline N-oxide (NQO)

Reference ID: 3293647

Incubation and sampling times:
Incubated for 72 hours

Results

Study validity:

- Three replicate plates used in the confirmatory study
- Assay validation criteria:
    - The assay must be considered valid in accordance with the criteria for assay acceptance.
    - If the data for any treatment level shows a response ≥2 times the concurrent vehicle control value (TA98, TA100 and WP2 uvrA (pKM101)), or ≥3 times the concurrent vehicle control value (TA1535 and TAI537), in conjunction with a dose-related response, the result is considered positive.
    - Where the data for any strain shows a dose-related response, but does not exceed the 2 or 3-fold threshold as detailed above, the result is considered equivocal and further testing may be required for clarification as follows:
        a) Where an equivocal result is obtained in the presence of S9-mix, a second assay, Yahagi test, is conducted with pre-incubation (Yahagi, 1975). Alternatively a different concentration of S9-mix may be used.
        b) Where an equivocal result is obtained in the absence of S9-mix, a repeat assay is conducted to assess/confirm reproducibility.

The evaluation criteria (1-3 above) are applied to the results for either of the circumstances, "a" or "b" above.

- The negative and positive control values were within the historical control data ranges
- Study design is valid.

Study outcome:

- No positive increases in the mean number of revertants per plate was recorded in the TA98, TA100, TA1535, TA1537, and WP2uvrA strains in the presence or absence of S9 mix at concentrations of 1500 and 2500 µg/plate (See Sponsor's Tables below).

Genotoxicity: In Vitro **Report Title:** GSK1120212B: Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli*. **Test Compound:** GSK1120212B (dimethyl sulphoxide solvate) **Batch Number:** 071131978

Metabolic Activation	Test Compound	Concentration (µg/plate)[1]	Main Plate Incorporation (Ames) Test 1 Mean Number of Revertant Colonies per Plate				
			TA 98	TA 100	TA 1535	TA 1537	WP2 *uvrA* pKM101
Without Activation	DMSO	100 µL/plate	23	123	17	15	152
	GSK1120212	5	25	150	15	17	155
	GSK1120212	15	25	133	24	15	171
	GSK1120212	50	17	127	22	16	219
	GSK1120212	150	23	152	16	15	204
	GSK1120212	500	25[3]	134[3]	22[3]	16[3]	162[3]
	GSK1120212	1500	24[3]	148[3]	22[3]	17[3]	181[3]
	GSK1120212	2500	22[3]	156[3]	18[3]	11[3]	142[3]
	2-Nitrofluorene	5	1217[2]	NT	NT	NT	NT
	Sodium azide	2	NT	1207[2]	807[2]	NT	NT
	9-Aminoacridine	50	NT	NT	NT	304[2]	NT
	4-Nitroquinoline-1-oxide	2	NT	NT	NT	NT	1905[2]

1. All concentrations are 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 at 2500 on the test plates at the end of incubation

Genotoxicity: In Vitro **Report Title:** GSK1120212B: Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli*. **Test Compound:** GSK1120212B (dimethyl sulphoxide solvate) **Batch Number:** 071131978

Metabolic Activation	Test Compound	Concentration (µg/plate)[1]	Main Plate Incorporation (Ames) Test 1 Mean Number of Revertant Colonies per Plate				
			TA 98	TA 100	TA 1535	TA 1537	WP2 *uvr*A pKM101
With Activation	DMSO	100 µL/plate	39	137	18	23	219
	GSK1120212	1.5	45	146	20	24	223
	GSK1120212	5	37	168	26	23	237
	GSK1120212	15	46	158	25	20	232
	GSK1120212	50	44	187	20	21	228
	GSK1120212	150	49	152	19	25	224
	GSK1120212	500	36[3]	136[3]	25[3]	15[3]	206[3]
	GSK1120212	1500	32[3]	158[3]	14[3]	13[3]	206[3]
	Benzo[a]pyrene	10	533[2]	NT	NT	NT	NT
	2-Aminoanthracene	5	NT	2110[2]	330[2]	146[2]	NT
	2-Aminoanthracene	10	NT	NT	NT	NT	1352[2]

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

Genotoxicity: In Vitro **Report Title:** GSK1120212B: Bacterial Mutation Assay (Ames Test) with *Salmonella typhimurium* and *Escherichia coli*. **Test Compound:** GSK1120212B (dimethyl sulphoxide solvate) **Batch Number:** 071131978

Metabolic Activation	Test Compound	Concentration (µg/plate)[1]	Main Plate Incorporation (Ames) Test 2 Mean Number of Revertant Colonies per Plate				
			TA 98	TA 100	TA 1535	TA 1537	WP2 *uvr*A pKM101
Without Activation	DMSO	100 µL/plate	23	135	14	19	160
	GSK1120212	1.5	25	109	16	19	165
	GSK1120212	5	22	116	13	16	149
	GSK1120212	15	24	116	14	21	159
	GSK1120212	50	30	114	13	16	179
	GSK1120212	150	25[3]	110[3]	16[3]	16[3]	174[3]
	GSK1120212	500	30[3]	117[3]	19[3]	22[3]	166[3]
	GSK1120212	1500	30[3]	122[3]	13[3]	19[3]	173[3]
	2-Nitrofluorene	5	1465[2]	NT	NT	NT	NT
	Sodium Azide	2	NT	1282[2]	872[2]	NT	NT
	9-Aminoacridine	50	NT	NT	NT	426[2]	NT
	4-Nitroquinoline-1-Oxide	2	NT	NT	NT	NT	2147[2]

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

[Tables excerpted from Sponsor]

Metabolic Activation	Test Compound	Concentration (µg/plate)[1]	Main Plate Incorporation (Ames) Test 2 Mean Number of Revertant Colonies per Plate				
			TA 98	TA 100	TA 1535	TA 1537	WP2 *uvrA* pKM101
With Activation	DMSO	100 µL/plate	36	127	19	20	220
	GSK1120212	1.5	40	127	23	23	215
	GSK1120212	5	38	114	18	23	231
	GSK1120212	15	40	116	14	21	223
	GSK1120212	50	34	119	17	23	206
	GSK1120212	150	36[3]	123[3]	19[3]	22[3]	225[3]
	GSK1120212	500	39[3]	130[3]	13[3]	20[3]	210[3]
	GSK1120212	1500	35[3]	129[3]	14[3]	10[3]	197[3]
	Benzo[a]pyrene	10	487[2]	NT	NT	NT	NT
	2-Aminoanthracene	5	NT	2099[2]	426[2]	133[2]	NT
	2-Aminoanthracene	10	NT	NT	NT	NT	1145[2]

1. All concentrations are 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

[Tables excerpted from Sponsor]

Study title: GSK1l20212B: In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus

Key findings:

- The test article was negative for inducing non-lethal gene mutations and chromosome damage in L5178Y (TK+/-) mouse lymphoma cells with and without metabolic activation when evaluated at concentrations ranging from 45 to 150 µg/mL (The maximum concentrations tested were limited by precipitation observed by eye at the end of treatment).

Study no.: WD2007/00303/00
Volume #, and page #: Volume 11, 1-40
Conducting laboratory and location: (b) (4)
Date of study initiation: March 2, 2007
GLP compliance: Letter included and signed
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Drug: GSK1120212B (dimethyl sulphoxide solvate)
Lot #: 071131978
Purity: 99.2%

Methods:
Strains/species/cell line:
L5178Y (TK+/-) cells obtained from (b) (4)

Concentrations used in definitive study:

3 hours without S9 activation	5, 10,20, 30, 35, 40, 45, 50, 55, 60, 70 and 80 µg/mL
24 hours without S9 activation	5, 10,20, 30, 35, 40, 45, 50, 55, 60, 70 and 80 µg/mL
3 hours with S9 activation	20, 40, 60, 90, 120, 150, 180, 210, 240, and 280 µg/mL

Basis of concentration selection:
Concentration selection was based on preliminary cytotoxicity test. Precipitation (assessed by eye at the end of treatment) was observed at a concentration of 240 µg/mL in the presence and absence of S9-mix. Cytotoxicity (reduction in RTG) was observed with GSK1120212 at concentrations of 120 µg/mL and 30 µg/mL in the presence and absence of S9-mix, respectively, for the 3 hour treatment period, and at 60 µg/mL in the absence of S9-mix for the 24 hour treatment period. There was a clear increase in cytotoxicity with increasing concentration of GSK1120212. Based on these results, the maximum concentrations tested in the mutation assays were 280 and 80 µg/mL in the presence and absence of S9-mix respectively, for the 3 hour treatment period, and 80 µg/mL in the absence of S9-mix for the 24 hour treatment period.

Negative controls:
DMSO

Positive controls:
Methyl methanesulphonate
Benzo(a)pyrene

Incubation and sampling times:
3 and 24 hour treatment

Results
Study validity:
- Two replicate plates used in the confirmatory study
- Assay evaluation criteria:

1. The maximum concentration tested is one that allows the maximum exposure up to 5000 µg/mL or 10 mM for freely soluble compounds, or the limit of solubility or toxicity, whichever is the lower. If compound solubility in the test system is a limiting factor, the maximum concentration chosen will be the lowest level at which compound precipitate is observed by eye in treatment cultures following incubation. Where toxicity is a limiting factor, the maximum concentration will normally be the lowest concentration at which the relative total growth is reduced to approximately 10-20% of the concurrent vehicle control. In cases were a relative total growth of 10-20% is not obtained, the principles as discussed by (Moore 2002) will be followed.
2. Acceptance criteria for vehicle controls (Moore, 2006):
    a. The mean value for vehicle control mutant frequency (MF) must be between 50 -170 x 10-6.
    b. The mean cloning efficacy must be between 65 - 120%.
    c. The mean suspension growth must be between 8 - 32 on Day 2 following 3 hour treatments. Obvious outliers should be excluded. However, there must be at least 2 acceptable vehicle control cultures remaining.
3. Acceptance criteria for positive controls (Moore, 2006):
    a. Positive controls should show:
        - Either an absolute increase in mean total MF of at least 300x10-6. At least 40% of this value should be due to small colony MF. Or

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an increase in small colony MF of at least 150 x 10-6 above the concurrent vehicle control.

b. The mean RTG value for the positive controls should be greater than 10%.

4. There should be an absence of confounding technical problems such as contamination, excessive numbers of outliers and excessive toxicity.
5. There should not be excessive heterogeneity between replicate cultures.

Tests that do not fulfill the required acceptance criteria are rejected and these results are not reported.

– The negative and positive control values were within the historical control data ranges
– Study design is valid.

Study outcome:

3-hour treatment without metabolic activation:

- The concentrations analysed for mutant frequency were 5, 35, 40, 45, 50 and 60 µg/mL. The mean relative total growth (RTG) value was reduced to 16% at 60 µg/mL. The mutant frequencies of the concentrations plated were all less than the sum of the mean control mutant frequency plus the global evaluation factor (GEF), indicating a negative result (See Sponsor's Table below).

24-hour treatment without metabolic activation:

- The concentrations plated for mutant frequency were 5, 10, 20, 30, 35, 40 and 45 µg/mL. The mean relative total growth (RTG) value was reduced to 13% at 45 µg/mL. The mutant frequencies of the concentrations plated were all less than the sum of the mean control mutant frequency plus the global evaluation factor (GEF), indicating a negative result (See Sponsor's Table below).

3-hour treatment with metabolic activation:

- The concentrations plated for mutant frequency were 20, 40, 60, 90, 120 and 150 µg/mL. The mean relative total growth (RTG) value was 59% at 150 µg/mL where precipitation (assessed by eye at the end of treatment) was observed. The mutant frequencies of the concentrations plated were all less than the sum of the mean control mutant frequency plus the global evaluation factor (GEF), indicating a negative result (See Sponsor's Table below).

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TABULATED SUMMARY

Genotoxicity: In Vitro

Test for Induction of: Forward mutation at the TK$^{+/-}$ locus

Cell Type: L5178Y Mouse Lymphoma Cells

Report Title: GSK1120212B: In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus

No. of Independent Tests: 3

No. of Replicate Cultures: 4 (vehicle); 2 (treatment and positive controls)

Test Compound: GSK1120212B (dimethyl sulphoxide solvate salt)
Batch Number: 071131978
Study No: (b) (4) 2990/118
GSK Ref No: V27491
GSK Document Number: WD2007/00303/00
Location in CTD:

Metabolising System: Aroclor-induced rat liver S9-mix. Final concentration of S9-fraction in cultures = 2% v/v
Vehicles: Test Article: Dimethyl sulphoxide (DMSO) **Positive Controls:** DMSO
Treatment: 3 hr treatment with and without S9-mix; treatment for 24 hr without S9-mix

GLP Compliance: Yes
Date of Treatment(s): March - April 2007

Cytotoxic Effects: Relative total growth (RTG) was reduced to 16% at 60 µg/mL for 3 hour treatment in the absence of S9-mix, and 13% at 45 µg/mL for 24 hour treatment in the absence of S9-mix. No marked cytotoxicity occurred for 3 hour treatment in the presence of S9-mix
Genotoxic Effects: None

Test Article	Dose Level[1] µg/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})
DMSO	0	100	73.27	100	69.17	100	55.80
GSK1120212	5	102	83.11	NT	NT	106	47.91
GSK1120212	10	NE	NE	NT	NT	91	78.22
GSK1120212	20	NE	NE	95	81.78	82	57.59
GSK1120212	30	NE	NE	NT	NT	44	48.79
GSK1120212	35	85	67.56	NT	NT	40	51.86
GSK1120212	40	67	69.27	113	62.71	26	56.06
GSK1120212	45	25	93.09	NT	NT	13	62.71
GSK1120212	50	22	57.40	NT	NT	NE	NE
GSK1120212	55	30	(72.14)	NT	NT	NE	NE
GSK1120212	60	16	100.33	67	68.55	NE	NE
GSK1120212	90	NT	NT	51	72.72	NT	NT
GSK1120212	120	NT	NT	48	79.58	NT	NT
GSK1120212	150	NT	NT	59[2]	60.21[2]	NT	NT
Methyl methane sulphonate	15	51	634.85	NT	NT	NT	NT
Methyl methane sulphonate	5	NT	NT	NT	NT	42	679.06
Benzo[a]pyrene	2	NT	NT	67	463.19	NT	NT

Footnotes to table detailed overleaf

[Table excerpted from Sponsor]

Study title: GSK1120212B: Oral Micronucleus Assay in Rats

Key findings:

- The test article was not genotoxic at doses up to 2 mg/kg/day, the highest dose tested.

Study no.: WD2007/01240/00
Volume #, and page #: Volume 13, 1-74 (b) (4)

Date of study initiation: August 13, 2007
GLP compliance: Letter included and signed
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Drug: GSK1120212B (dimethyl sulphoxide solvate)
Lot #: 071131978
Purity: 99.2%

Methods:
Strains/species/cell line:
Male and Female Crl:CD®(SD)IGS BR

Reference ID: 3293647

Doses used in definitive study:

- 0, 1, and 2 mg/kg/day at a dose volume of 5 mL/kg/hour.
- Doses were administered orally once on two consecutive days to 3 (control) and 7 (low and high dose) male rats.

Basis of dose selection:

Dose selection was based on oral dose range finding study in rats. Rats (3/sex/dose) were dosed at 0, 0.5, 1.5, and 3 mg/kg/day on two consecutive days. Rats were observed for clinical toxicity and/or mortality. Clinical signs included soft and liquid feces at doses of 1.5 and 3 mg/kg/day in males and females. One male and 1 female were found dead on Day 3 following 2 daily doses of 3 mg/kg/day. As there were no apparent differences in toxicity observed between males and females, a lower dose of 2.5 mg/kg/day was administered to males only, which also resulted in mortality on Day 3. There was a dose-related group mean bodyweight loss, with the surviving males losing up to approximately 12% bodyweight and females losing up to 14%, following doses of 3 mg/kg/day. Based on the clinical tolerability of GSK1120212, the MTD was considered to be less than 2.5 mg/kg/day. A reduction in group mean %PCE (19% reduction when compared to vehicle control) was observed in females only, following 2 daily doses of 1.5 mg/kg/day, which may be indicative of bone marrow toxicity. No notable increase in the number of MPCE was observed in males and females up to doses of 1.5 mg/kg/day. Based on these results, doses of 1 and 2 mg/kg/day in male rats were chosen for the micronucleus assay.

Negative controls:

20% (w/v) Captisol® /0.08N (v/v) HCI in Reverse Osmosis water

Positive controls:

Cyclophosphamide (CP)

Incubation and sampling times:

Bone marrow aspirated from rats 24-hrs after last drug administration.

Results:

Study validity:

- Slides were scored for micronuclei by eye.
- The assay was evaluated according to the following:
- If any treatment group shows a response (group mean frequency of MPCE) which is >4 times the concurrent group mean vehicle control value and exceeds the upper 98% tolerance limit of historical controls, the result is considered positive.
- If all treatment groups show responses (group mean frequency of MPCE) which are >4 times the concurrent group mean vehicle control value, and within the group mean historical control range (98% tolerance limits) the result is considered negative.
- If any treatment group shows a response (group mean frequency of MPCE) which is >4 times the concurrent group mean vehicle control value but exceeds the group mean historical control range (98% tolerance limits), an additional 2000 PCE will be analyzed from each animal (vehicle and all treated groups).
- If after additional analysis, all treatment groups show responses (group mean frequency of MPCE) which are >4 times the concurrent group mean vehicle control value, and within

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the group mean historical control range (98% tolerance limits) the result is considered negative.

- If after additional analysis, all treatment groups show responses (group mean frequency of MPCE) which are >4 times the concurrent group mean vehicle control value, and within the group mean historical control range (98% tolerance limits) the result is considered negative.
- If after additional analysis, one or more treatment groups show a group mean response >4 times the concurrent group mean vehicle control and this exceeds the group mean historical control range (98% tolerance limits), the data will be subjected to the following statistical analysis:
- Initially, evidence of heterogeneity between animals within dose groups will be assessed using a chi-squared test for heterogeneity (Lovell, 1989) or another appropriate method. If the test for heterogeneity is not significant at the 5% level then the following statistical analysis will be applied:
    - The numbers of MPCE in each treated group (males and females separately where appropriate) will be compared with the vehicle control group using Fisher's exact test (Lovell, 1989; Annitage, 1971). Probability values of P>0.05 will be accepted as significant. When three or more test compound treated groups are present, a Cochran-Aritage trend test (Annitage, 1955) will also be used to evaluate a possible dose-response relationship. However, significance in the trend test (P>0.05) will only be reported if none of the pair-wise (Fisher's) tests are significant.
    - When three or more test compound treated groups are present, a Cochran-Aritage trend test (Annitage, 1955) will also be used to evaluate a possible dose-response relationship. However, significance in the trend test (P>0.05) will only be reported if none of the pair-wise (Fisher's) tests are significant.
- If the test for heterogeneity is significant then methods that do not combine data from animals within a group and which assume the animal to be the unit of variance will be applied instead.
- An exact Wilcoxon's rank sum test (Lehmann, 1975) will be used to compare each treated group with the vehicle control. Probability values of P<0.05 will be accepted as significant.
- When three or more test compound treated groups are present, a Jonckheere-Terpstra Test (Lehmann, 1975) will also be used to evaluate a possible dose-response relationship. However, significance in the trend test (P<0.05) will only be reported if none of the pair-wise (Wilcoxon) tests are significant.
- Study design and findings are valid.

Study outcome:

No mortality occurred in the study. Drug related clinical signs were observed in both dose groups. These included staining of the fur (red and brown), piloerection, decreased activity, and suspected slight dehydration. There was a dose-related group mean bodyweight loss of approximately 7 and 10% in animals treated at 1 and 2 mg/kg/day, respectively.

There was a decrease in group mean %PCE at 2 mg/kg/day (approximately 14% reduction) when compared with the concurent vehicle control. This may be due to bone marrow toxicity, however, these values were within the ranges determined from laboratory historical data. Group mean values for MPCE/2000 PCE were similar to the concurrent control data.

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Therefore, the drug was considered to have given a negative result for micronucleus induction. Results are presented in Sponsor's Table below.

Test Compound	Dose[1] (mg/kg/day)	Dose[2] (mg/m2/day)	No. of Animals Analysed[3]	Group Mean %PCE	Group Mean MPCE[4]
Vehicle	0	0	7M	44	1.9
GSK1120212	1	11.1	7M	45	2.1
GSK1120212	2	22.2	7M	38	2.1
Cyclophosphamide	20	222.2	3M	37	41.3

1. All doses/concentrations are expressed in terms of parent compound.
2. Body surface area (m^2) = $(K \times (BW\,g)^{2/3})/10^4$, where K = 9 for rats [Freireich et al. 1966]
3. M = Male
4. Group mean number of micronucleated polychromatic erythrocytes (MPCE) per 2000 PCE analyzed.

[Table excerpted from Sponsor]

2.6.6.5 Carcinogenicity: No studies conducted
2.6.6.6 Reproductive and developmental toxicology: No studies conducted

TOXICOLOGY TABULATED SUMMARY

Title	**21-Day Oral Toxicity Study with 2-Week Recovery**	
Species	Rat	Dog
Route	Oral gavage	Oral gavage
Dose (mg/kg/day)	0, 0.02, 0.04, and 0.0.08, and 0.17	M: 0, 0.025, 0.037, and 0.075 F: 0, 0.01, 00.02, and 0.025
Dose (mg/m2/day)	0, 0.125, 0.250, and 0.500, and 1.00	M: 0, 0.5, 0.75, and 1.5 F: 0, 0.3, 0.4, and 0.5
TK (AUC, ng.h/mL, at the end of main study)	M: 35, 64, 129, and 218 (LD, MD1, MD2, HD) F: 60, 126, 211, and 460 (LD, MD1, MD2, HD)	M: 159, 282, and ND (LD, MD, HD) F: 120, 211, and 205 (LD, MD, HD)
Mortality	**Females only**: 1 at 1.00 mg/m2/day.	**Males only.** 1 M at 0.75 mg/m2/day and 3 M at 1.5 mg/m2/day.
Clinical signs	**M and F at 0.500 mg/m2/day:** Raised scabs located around the snout, lower lip, periocular area and/or dorsal neck with an earlier onset and greater severity in females than males. Recoverable. **M and F at 1.00 mg/m2/day:** Raised scabs to exudative lesions were more widespread and progressed in most male and female rats. Not recoverable.	**M at ≥0.75 mg/m2/day:** inappetence; soft, mucoid feces; loose, watery feces; unkempt coat; red discharge; delayed capillary time; subdued behavior; red/brown discharge; red/brown urine; and salivation. Recoverable.
Body weight	Unremarkable.	**M at ≥0.75 mg/m2/day:** Non-significant (≤20%) during Days 4-8 and 4-11, respectively.
Food consumption	Unremarkable.	**M at ≥0.75 mg/m2/day:** Non-significant (≤20%) during Days 4-8 and 4-11, respectively.
Hematology	**M at ≥1.00 mg/m2/day:** ↑ in NEUT, MONO and ↓ in ESO (also at 0.500 mg/m2/day) during Weeks 4 and 5. Recoverable. **F at ≥0.500 mg/m2/day:** ↑ in WCC, NEUT, MONO, and BASO during Weeks 4 and 5. Recoverable except WCC and NEUT.	**M at ≥0.75 mg/m2/day:** ↓ in HGB, HCT, RETIC (F also) and ↑ in MONO. Recoverable except RETIC.
Clinical chemistry	**M and F at ≥0.500 mg/m2/day:** ↑ in PHOS and CA during Day 3 and Week 4. Recoverable. **F at 1.00 mg/m2/day:** ↑ in ALT during Week 4. Recoverable.	**M at ≥0.75 mg/m2/day:** ↑ in ALP, ALT, GLDH, TRIGLY, and ↓ in ALB. Recoverable.
Ophthalmoscopy	Unremarkable.	Unremarkable.
Urinalysis	**F at 1.00 mg/m2/day:** ↑ in total protein excretion and protein: creatinine ratio during Week 4. Recoverable.	Unremarkable

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Organ weight	Unremarkable.	Unremarkable.
Gross pathology	**Terminal sacrifice- M and F at ≥0.500 mg/m2/day:** Skin crusts and/or skin discolorations involving the nares/rostrum, chin/ventral neck, lateral/ventral and dorsal thoracic and scapular regions, abdomen, back and axilary areas. Greater degree in females. Recoverable in males only. **F at 1.00 mg/m2:** Raised skin crusts and/or skin discoloration involving the ventral chin/dorsal back and thorax in 3/6 rats during recovery.	**Early deaths:** discolored, mucosa, red and multifocal in colon, duodenum, rectum, and stomach. **M and F at ≥0.0.5 mg/m2/day:** Unremarkable.
Histopathology	**M and F at ≥0.250 mg/m2/day:** Minimal to mild acanthosis and minimal to moderate multifocal ulcer (F only) of skin. Not recoverable in females. Minimal to moderate glandular mineralization of stomach (F>M). Not recoverable. Minimal to mild periportal vacuolation of liver. Not recoverable in females. **F at 1.00 mg/m2/day:** Minimal to moderate myeloid hyperplasia in bone marrow. Recoverable. Mild to moderate increased cellularity lymphoplasmacytic in lymph node mandibular/cervical and lymph node axillary. Recoverable.	**Early deaths:** Minimal myeloid hyperplasia in bone marrow. Mild inflammation of neutrophilic mucosa in duodenum. Minimal to mild sinus neutrophilia and Kuppfer cell activation in liver. Minimal to moderate sinus neutrophilia, red pulp in spleen. **M at 0.75 mg/m2/day:** Minimal myeloid hyperplasia in bone marrow. Minimal to moderate sinus neutrophilia, red pulp in spleen. Recoverable. **M at 1.5 mg/m2/day:** Minimal lymphoid depletion in thymus. Not recoverable.

Reference ID: 3293647

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/s/

GABRIEL S KHASAR
04/16/2013

SHAWNA L WEIS
04/16/2013

MARGARET E BROWER
04/16/2013

WHITNEY S HELMS
04/16/2013

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:	204114
Supporting document/s:	2
Applicant's letter date:	08/02/2012
CDER stamp date:	08/03/2012
Product:	Trametinib (GSK1120212)
Indication:	Patients with unresectable or metastatic melanoma with BRAF V600 mutations
Applicant:	Glaxosmithkline, LLC 1250 South Collegeville Rd Collegeville, PA 19426
Review Divisions:	Division of Hematology Oncology Toxicology (DHOT) Division of Drug Oncology Products 2 (DOP 2)
Reviewer:	G. Sachia Khasar, PhD.
Supervisor/Team Leader:	Whitney Helms, PhD.
Division Directors:	John Leighton, PhD., DABT (DHOT) Patricia Keegan, MD. (DOP 2)
Project Manager:	Norma S. Griffin

Reference ID: 3255230

MEMORANDUM

This memo is in response to a general consult from ONDQA seeking to confirm that the levels of three genotoxic impurities, [redacted] were within the threshold of toxicological concern (TTC).

According to the sponsor's submission, the genotoxic impurity, [redacted] itself has two additional potentially genotoxic impurities, [redacted], though [redacted] was found not to be mutagenic in the Ames assay. The specifications for [redacted] are listed as NMT [redacted], respectively. The Applicant reports that analysis of commercial scale batches of intermediate grade trametinib dimethyl sulfoxide that were representative of the manufacturing route and process, manufactured at the commercial site showed that the levels of all 3 potential genotoxic impurities were below the threshold of toxicological concern (TTC; [redacted] The Applicant set the specification for each impurity at [redacted] following [redacted] for the drug substance and does not expect further formation of the impurities beyond this stage.

At the [redacted] specification proposed by the Applicant for each of the genotoxic impurites, the level of exposure in humans at the recommended daily dose of 2 mg of Mekinist is [redacted]. At this dose the level of either individual or the combined impurities does not exceed the level of toxicological concern and the proposed specifications are acceptable from a pharmacologic/toxicologic safety standpoint.

Table 13 Batch Analysis Data for (b) (4) Content by HPLC in Intermediate Grade Trametinib Dimethyl Sulfoxide

Batch	Use	(b) (4)
0212A4B001 (IG)	Phase 3 clinical studies	
0212A4B002 (IG)	Phase 3 clinical studies	
0212A4B003 (IG)	Phase 3 clinical studies	
0212A4B004 (IG)	Phase 3 clinical studies	
0212A4B005 (IG)	Phase 3 clinical studies	
0212A4B006 (IG)	Phase 3 clinical studies	
0212A4B007 (IG)	Phase 3 clinical studies	
K281169 (IG)	Commercial supply	
K281214 (IG)	Commercial supply	
K281220 (IG)	Commercial supply	
K281221 (IG)	Commercial supply	
K281289 (IG)	Commercial supply	
K281379 (IG)	Commercial supply	
K281380 (IG)	Commercial supply	

Notes:
IG = intermediate grade trametinib dimethyl sulfoxide
NGT = Not greater than
1. For these batches, this test was performed on the corresponding non-micronized trametinib dimethyl sulfoxide batch (0212A5B001, 0212A5B002 and 0212A5B003)

(Excerpted from the sponsor's submission)

[The info in this in this table was submitted to CMC (and quality module) in response to IR and was not in the original submission. I will include this table in my review when I revise the review after your edits]

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/s/

GABRIEL S KHASAR
02/04/2013

WHITNEY S HELMS
02/05/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 204,114

NDA Number: 204,114 **Applicant:** GSK, LLC **Stamp Date:** 08/03/2012

Drug Name: Trametinib (GSK1120212) **NDA Type:** 505(b)(1)

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	Yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	Yes		
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)?	Yes		
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).			There is no change in formulation
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?	Yes		Nonclinical studies were conducted by oral gavage and the clinical formulation is for oral administration.
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?	Yes		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			N/A. No special studies/data were requested

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA 204,114

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 204,114

	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?	Yes		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			Impurity issues (if any) will be addressed during the review of the NDA
11	Has the applicant addressed any abuse potential issues in the submission?	N/A		Not applicable
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?	N/A		This NDA is not to support Rx to OTC.

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

If the NDA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

None

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

None

Sachia Khasar, PhD. And Margaret Brower, PhD	08/30/2012
Reviewing Pharmacologists	Date
Whitney S. Helms, PhD.	08/30/2012
Team Leader/Supervisor	Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA 204,114

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/s/

GABRIEL S KHASAR
09/05/2012

WHITNEY S HELMS
09/05/2012