

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

204790Orig1s000

PHARMACOLOGY REVIEW(S)

Division of Antiviral Products
Center for Drug Evaluation and Research

Date: May 10, 2013
Reviewer: Hanan Ghantous, PhD, DABT
Supervisory Interdisciplinary Scientist
NDA #/SS#/date: 204-790/0000/12/17/2012
Sponsor: ViiV Healthcare
Drug Product: Dolutegravir (TIVICAY)
Indication: Treatment of HIV infection
Recommended Action: Nonclinical data support approval

Dolutegravir (DTG) is an HIV integrase inhibitor (INI) that acts by binding to the integrase active site and blocking the strand transfer step of retroviral deoxyribonucleic acid integration. The applicant is seeking marketing approval for DTG as a treatment for HIV infection in combination with other antiretroviral agents in adults and children aged 12 to 18 years and weighing ≥ 40 kg.

The proposed dosage for DTG is 50 mg once daily (QD) in treatment naïve and treatment experienced INI- naïve, HIV-infected adults and INI- naïve children 12 to 18 years old, weighing ≥ 40 kg. In INI-resistant subjects the DTG dose is 50 mg twice daily (BID).

The applicant has submitted a complete nonclinical package of studies in mice, rats, rabbits, and nonhuman primates. In nonclinical toxicology studies, gastrointestinal effects including inflammation and hemorrhage were the principle finding in short term, subchronic, and chronic repeat dose studies, across species. Dolutegravir was not genotoxic, carcinogenic, or teratogenic and did not cause impairment of fertility in nonclinical studies.

Conclusion: I concur with the primary nonclinical reviewer, Dr. Mark Seaton that the nonclinical data support an approval action for Dolutegravir as a treatment for HIV infection in combination with other antiretroviral agents in adults and children aged 12 to 18 years and weighing ≥ 40 kg.

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/s/

HANAN N GHANTOUS
05/08/2013

Draft comments on N204-790 Dolutegravir

From: A. Jacobs

Date: 5/9/13

1. I concur that there are no nonclinical approval issues and that the pregnancy category is appropriate.
2. I made some editorial suggestions and comments on the text and on the description of the carcinogenicity studies, and the reviewer will address them as appropriate.

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

/s/

ABIGAIL C JACOBS
05/09/2013

**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:	204-790
Supporting document/s:	0000
Applicant's letter date:	12/16/2012
CDER stamp date:	12/17/2012
Product:	Dolutegravir (TIVICAY)
Indication:	Treatment of HIV infection in adults and children (aged 12 to 18 years and weighing 40 kilograms or more) in combination with other antiretroviral agents
Applicant:	ViiV Healthcare
Review Division:	Division of Antiviral Products
Reviewer:	Mark Seaton, Ph.D., DABT
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra Birnkrant, M.D.
Project Manager:	Sohail Mosaddegh, Pharm.D.

Disclaimer

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TABLE OF CONTENTS

1.1	INTRODUCTION.....	6
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS.....	6
1.3	RECOMMENDATIONS.....	7
2	DRUG INFORMATION	8
2.1	DRUG.....	8
2.2	RELEVANT INDS, NDAS, BLAS AND DMFS	9
2.3	DRUG FORMULATION.....	9
2.4	COMMENTS ON NOVEL EXCIPIENTS.....	9
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	9
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	10
2.7	REGULATORY BACKGROUND.....	10
3	STUDIES SUBMITTED	10
3.1	STUDIES REVIEWED	10
3.2	STUDIES NOT REVIEWED.....	15
3.3	PREVIOUS REVIEWS REFERENCED	16
4	PHARMACOLOGY.....	16
4.1	PRIMARY PHARMACOLOGY	16
4.2	SECONDARY PHARMACOLOGY	16
4.3	SAFETY PHARMACOLOGY	16
5	PHARMACOKINETICS/ADME/TOXICOKINETICS.....	16
5.1	PK/ADME.....	16
6	GENERAL TOXICOLOGY.....	20

6.1	SINGLE-DOSE TOXICITY	20
6.2	REPEAT-DOSE TOXICITY	20
7	GENETIC TOXICOLOGY	43
8	CARCINOGENICITY	44
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY.....	52
	PRENATAL AND POSTNATAL DEVELOPMENT.....	52
10	SPECIAL TOXICOLOGY STUDIES	62
11	INTEGRATED SUMMARY AND SAFETY EVALUATION.....	70
12	APPENDIX/ATTACHMENTS	77

Table of Tables

Table 1: Result of Genotoxic Impurity Purging Study.....	9
Table 2: Proposed Clinical Population and Dosing Regimen.....	10
Table 3: Toxicokinetics from 6-month Oral Toxicity Study of S-349572 sodium in Rats	26
Table 4: Plasma concentrations and toxicokinetic parameters in Male Monkeys on Day 14 of dosing (excerpted from sponsor)	31
Table 5: Plasma concentrations and toxicokinetic parameters in Female Monkeys on Day 14 of dosing (excerpted from sponsor)	31
Table 6: Toxicokinetic Parameters of ERC-349572 in Male Monkeys	35
Table 7: Toxicokinetic Parameters of ERC-349572 in Female Monkeys.....	36
Table 8: Systemic Exposure Following Nine-Month of Oral Administration of S-349572 sodium in Monkeys.....	40
Table 9: Clinical Chemistry Changes in Mice	42
Table 10: Test Article-related Microscopic Findings.....	42
Table 11: Toxicokinetic Parameters of Dolutegravir following Oral Administration to Mice.....	43
Table 12: Percentage of Animals Surviving at Terminal Sacrifice.	45
Table 13: Major Causes of death in found dead or unscheduled sacrifice mice dosed orally with S-349572-sodium for up to 24 months	46
Table 14: Plasma Toxicokinetic Parameters of S-349572 in Male and Female Mice on Days 26 and 182 following Oral (Gavage) Administration.....	47
Table 15: Percentage of animals surviving at terminal sacrifice.	50
Table 16: Primary causes of death.	50
Table 17: Values of Plasma Toxicokinetic Parameters of S-349572 in Male and Female Rats on Days 28 and 182 following Oral (Gavage) Administration of S-349572	51
Table 18: Toxicokinetic Parameters for Dolutegravir following Oral administration to Rabbits	55

Table 19: Toxicokinetic Parameters for Dolutegravir following Oral administration to Pregnant Rats58

Table 20: Group Designations (table excerpted from sponsor)64

Table 21: Observations (table excerpted from sponsor)65

Table 22: Toxicokinetic Parameters for Dolutegravir following Oral Administration in Juvenile Rats (table excerpted from sponsor).....68

Table 23: Safety Margins from Nonclinical Toxicology Studies.....72

1 EXECUTIVE SUMMARY

1.1 INTRODUCTION

Dolutegravir (DTG) is an HIV integrase inhibitor (INI) that acts by binding to the integrase active site and blocking the strand transfer step of retroviral deoxyribonucleic acid integration. The applicant is seeking marketing approval for DTG as a treatment for HIV infection in combination with other antiretroviral agents in adults and children aged 12 to 18 years and weighing ≥ 40 kg. The proposed dosage for DTG is 50 mg once daily (QD) in treatment naïve and treatment experienced INI- naïve, HIV-infected adults and INI- naïve children 12 to 18 years old, weighing ≥ 40 kg. In INI-resistant subjects the DTG dose is 50 mg twice daily (BID). The applicant has submitted a complete nonclinical package of studies in mice, rats, rabbits, and nonhuman primates.

1.2 BRIEF DISCUSSION OF NONCLINICAL FINDINGS

In nonclinical toxicology studies, gastrointestinal effects including inflammation and hemorrhage were the principle finding in short term, subchronic, and chronic repeat dose studies, across species. Gastrointestinal intolerance led to early deaths of three monkeys in two week (one female) or nine month (two males) studies. Gastric hemorrhage was noted microscopically in rats at exposure margins approximately 24X the anticipated human exposures and in monkeys at exposures approximately 3X the anticipated human exposures. Gastric-related adverse events were observed in clinical trials.

As gastrointestinal effects were considered to be due to local intolerance in the gastrointestinal tract, and not related to systemic exposure to DTG, safety margins (aka exposure margins, see above paragraph) calculated using systemic exposure comparisons can be considered conservative. Less conservative safety margins for DTG may be calculated using dose (mg/kg) comparisons (refer to Table 25). Using the nonhuman primate example, the NOAEL for the nine month monkey study (15 mg/kg/day) is 15X and 8X the human mg/kg equivalent QD and BID dose, respectively (based on a 50 kg human).

In addition to GI findings, hepatic toxicity was noted in a short term (two week) study in monkeys. Findings included hepatocellular single cell necrosis and diffuse hepatocellular hypertrophy and/or vacuolation in male monkeys given the high dose of 1000 mg/kg/day. Corresponding systemic exposures in high dose animals were approximately 7X and 5X the expected human exposures for a 50 mg QD or BID dose, respectively. There were no toxicologically-significant hepatotoxicity or other systemic effects of DTG administration in subchronic or chronic (i.e., one month or longer) nonclinical toxicology studies. In some hepatic cases observed in clinical trials, DTG related liver injury could not be ruled out.

Dolutegravir was neither genotoxic nor carcinogenic in nonclinical studies. Likewise, oral administration of DTG to pregnant rats and rabbits did not result in teratogenicity at systemic exposures up to 27X the human exposure for a 50 mg BID dose. Dolutegravir had no effect on maternal reproductive function (duration of gestation, gestation index, delivery and nursing behavior) but caused decreased food consumption and body weights during lactation in rats administered 1000 mg/kg DTG from Day 6 of gestation to Day 20 of lactation. Dose administration corresponded to the period from implantation to weaning of rats. In the offspring, decreased body weights were noted in the 1000 mg/kg group from pre-weaning until adolescence. The NOAEL was defined as 50 mg/kg for development of that generation, corresponding to systemic exposures greater than 14X the expected human exposures for a 50 mg BID dose.

In a juvenile rat study, the deaths of two male pups dosed with the high dose (75 mg/kg/day) were considered to be test article-related. Due to the deaths at 75 mg/kg, the NOAEL in juvenile rats was considered to be 2 mg/kg/day, corresponding to systemic exposures on Day 13 postpartum that were approximately 6X exposures at the recommended clinical dose of 50 mg QD and 4X exposures at the at 50 mg BID clinical dose. Systemic exposures at 75 mg/kg/day were approximately 10-fold higher than systemic exposures at the NOAEL.

The submitted studies represent a complete nonclinical toxicology package. It is recommended that DTG be approved as a treatment for HIV infection in combination with other antiretroviral agents in adults and children aged 12 to 18 years and weighing ≥ 40 kg.

1.3 RECOMMENDATIONS

1.3.1 Approvability

It is recommended that dolutegravir be approved.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies are recommended.

1.3.3 Labeling

8.1 Pregnancy

Pregnancy Category B. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, and dolutegravir was shown to cross the placenta in animal studies, this drug should be used during pregnancy only if clearly needed.

Antiretroviral Pregnancy Registry: To monitor maternal-fetal outcomes of pregnant women with HIV exposed to TIVICAY and other antiretroviral agents, an Antiretroviral Pregnancy Registry has been established. Physicians are encouraged to register patients by calling 1-800-258-4263.

Animal Data

Reproduction studies have been performed in rats and rabbits at doses up to 27 times the human dose of 50 mg BID and have revealed no evidence of impaired fertility or harm to the fetus due to TIVICAY.

Oral administration of Dolutegravir to pregnant rats at doses up to 1,000 mg/kg daily, approximately 27 times the recommended 50 mg twice daily human clinical exposure based on AUC, from days 6 to 17 of gestation did not elicit maternal toxicity, developmental toxicity, or teratogenicity.

Oral administration of Dolutegravir to pregnant rabbits at doses up to 1,000 mg/kg daily, approximately 0.4 times the recommended 50 mg twice daily human clinical exposure based on AUC, from days 6 to 18 of gestation did not elicit developmental toxicity or teratogenicity. In rabbits, maternal toxicity (decreased food consumption, scant/no feces/urine, suppressed body weight gain) was observed at 1,000 mg/kg.

8.3 Nursing Mothers

The Centers for Disease Control and Prevention recommend that HIV-1–infected mothers in the United States not breastfeed their infants to avoid risking postnatal transmission of HIV-1 infection.

Studies in lactating rats and their offspring indicate that Dolutegravir was present in rat milk. It is not known whether dolutegravir is secreted into human milk. Because of both the potential for HIV transmission and the

potential for adverse reactions in nursing infants, **mothers should be instructed not to breastfeed if they are receiving TIVICAY.**

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Two-year carcinogenicity studies in mice and rats were conducted with Dolutegravir. Mice were administered doses of up to 500 mg/kg, and rats were administered doses of up to 50 mg/kg. In mice, no significant increases in the incidence of drug-related neoplasms were observed at the highest doses tested resulting in Dolutegravir AUC exposures (b) (4)-fold higher than those in humans at the recommended dose of 50 mg twice daily. In rats, no increases in the incidence of drug-related neoplasms were observed at the highest dose tested resulting in Dolutegravir AUC exposures 10-fold and 15-fold higher in males and females, respectively, than those in human at the recommended dose of 50 mg twice daily.

Mutagenesis

Dolutegravir was not genotoxic in the bacterial reverse mutation assay, mouse lymphoma assay, or in the in vivo rodent micronucleus assay.

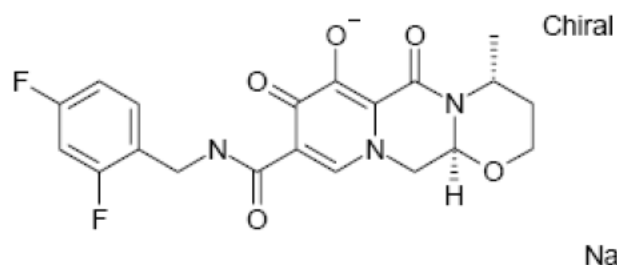
Impairment of Fertility

In a study conducted in rats, there were no effects on mating or fertility with Dolutegravir up to 1000 mg/kg/day. This dose is associated with an exposure that is approximately (b) (4) times higher than the exposure in humans at the recommended dose of 50 mg twice daily.

2 DRUG INFORMATION

2.1 DRUG

- I. CAS Registry Number
 - 1051375-19-9 (Na salt)
 - 1051375-16-6 (free acid)
- II. Generic Name: Dolutegravir
- III. Code Name: GSK1349572
- IV. Chemical Name: Sodium (4R,9aS)-5-Hydroxy-4-methyl-6,10-dioxo-3,4,6,9,9a,10-hexahydro-2H-1-oxa-4a,8adiazanthracene-7-carboxylic acid 2,4-difluorobenzylamide
- V. Molecular Formula/Molecular Weight: C₂₀H₁₈F₂NaO₅; MW=441.36
- VI. Structure or Biochemical Description



VII. Pharmacologic Class: Antiviral- HIV Integrase Inhibitor

2.2 RELEVANT INDS, NDAS, BLAS AND DMFS

IND 75,382

2.3 DRUG FORMULATION

Dolutegravir sodium is a white to light yellow powder and is slightly soluble in water.

The drug product is an immediate release tablet for oral administration. Each film-coated tablet of Dolutegravir for oral administration contains 52.6 mg of dolutegravir sodium, which is equivalent to 50 mg dolutegravir free acid, and the following inactive ingredients: D-mannitol, microcrystalline cellulose, povidone K29/32, sodium starch glycolate, and sodium stearyl fumarate. The tablet film-coating contains the inactive ingredients iron oxide yellow, macrogol/PEG, polyvinyl alcohol-part hydrolyzed, talc, and titanium dioxide.

2.4 COMMENTS ON NOVEL EXCIPIENTS

None.

2.5 COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN

Historical batch data along with spiking and purging studies ensure that no potential genotoxic impurities are present in Dolutegravir sodium drug substance (see table below, excerpted from sponsor).

Table 1: Result of Genotoxic Impurity Purging Study

(b) (4)

Genotoxic impurities will be controlled by specifications of the intermediates or by control of the manufacturing process.

During stability testing, no significant degradation of dolutegravir was observed. Only one process impurity, (b) (4) has been observed. The level of this impurity is controlled in the dolutegravir sodium drug substance specification.

2.6 PROPOSED CLINICAL POPULATION AND DOSING REGIMEN

Refer to Table 2 below (*excerpted from sponsor*).

Table 2: Proposed Clinical Population and Dosing Regimen

Patient Population	Dose	Regimen
Adults		
Treatment-naïve	50 mg	Once Daily
Treatment-experienced, integrase inhibitor-naïve	50 mg	Once Daily
Integrase inhibitor-resistant	50 mg	Twice Daily
Pediatrics		
Aged 12 years and older and weighing at least 40 kg	50 mg	Once Daily

2.7 REGULATORY BACKGROUND

IND 75,382 was received in the Division of Antiviral Products on 10/24/07. On 11/27/07 the sponsor was notified that it was safe to proceed with clinical trials.

3 STUDIES SUBMITTED

3.1 STUDIES REVIEWED

Secondary Pharmacology

Secondary Pharmacological Evaluation of the HIV Integrase Inhibitor GSK1349572B in Radioligand Binding and Enzyme Assays and Isolated Tissue Assays

Safety Pharmacology

Effects of ERC-349572 Sodium on Central Nervous System in Rats

Effects of ERC-349572 Sodium on Respiratory System in Rats

Effects of ERC-349572 Sodium on Ionic Current in Cells Expressing hERG Channels

Effects of ERC-349572 sodium on Cardiovascular System in Conscious Monkeys (Shionogi Study No. E-349572-SF-025-L)

Pharmacokinetics

Pharmacokinetics in Rats Following Single Administration of ERC-349572 (GSK1349572)

Pharmacokinetics in Dogs Following Single Administration of ERC-349572 (GSK1349572)

Pharmacokinetics of Pediatric Granule Formulations in Dogs

Pharmacokinetics in Monkeys Following Single Administration of ERC-349572 (GSK1349572)

Distribution

Preliminary in vitro protein binding of ERC-349572 (GSK1349572) in human, cynomolgus

monkey, dog and rat sera by equilibrium dialysis.

An in vitro study of plasma protein binding of GSK1349572 in human

An in vitro Study to Determine the Plasma Protein Binding of GSK1349572 in Human Plasma Containing EDTA or Heparin Anticoagulants

Preliminary cell permeability of GSK1349572 and interaction with P-glycoprotein (Pgp) in hMDR1-MDCK cells in the presence of bio-relevant buffers.

An In Vitro Investigation of the Passive and Absorptive Membrane Permeability of [¹⁴C] GSK1349572 in MDCKII-MDR1 Cells

An In Vitro Investigation of the Transport via Heterologously Expressed Human P-glycoprotein of [¹⁴C] GSK1349572 in MDCKII-MDR1 Cells Study Number: 08DMR028

An In Vitro Investigation into the Inhibition by GSK1349572 of Xenobiotic Transport via Human P-Glycoprotein, Heterologously Expressed in MDCKII Cells Study Number: 08DMR021

An In Vitro Investigation of the Transport via Heterologously Expressed Human Breast Cancer Resistance Protein of [¹⁴C] GSK1349572 in MDCKII-BCRP cells. Study Number: 11DMR004

An In Vitro Investigation into the Inhibition by GSK1349572 of Xenobiotic Transport via Human Breast Cancer Resistance Protein Heterologously Expressed in MDCKII Cells.

An In Vitro Investigation into the Inhibition of Estradiol-17-β-D-Glucuronide Transport by GSK1349572 in Human Membrane Vesicles Expressing the Multidrug Resistance Associated Protein-2 Transporter

An in vitro investigation into the inhibition of estradiol-17-β-D-Glucuronide Transport by GSK2832500A (GSK1349572 glucuronide metabolite) in Human Membrane Vesicles Expressing the Multidrug Resistance Associated Protein-2 Transporter

An In Vitro Investigation into the Inhibition by GSK1349572 of Xenobiotic Transport via Human OATP1B1 and OATP1B3

Assessment of GSK1349572 and co-administered compounds as inhibitors of human OCT2 mediated transport

IC₅₀ determination of GSK1349572 and assessment of GSK1349572 and co-administered compounds as inhibitors of human OCT2 mediated transport.

Determination of Inhibitory Potential of GSK1349572A on [¹⁴C]Metformin Uptake in HEK293-OCT1 Cells

[¹⁴C]GSK1349572: Quantitative Tissue Distribution of Test Substance-Related Material Using Whole Body Autoradiography Following Single Oral Administration of [¹⁴C]GSK1349572 (50 mg/kg) to Male Lister-Hooded (Partially Pigmented) Rats

[¹⁴C]GSK1349572: Placental Transfer and Lactal Excretion Following Administration of a Single oral Dose to Pregnant or Lactating Rats

Metabolism

Preliminary in vitro metabolic stability of GSK1349572 in rat, dog, cynomolgus monkey, and human liver S9, and in rat and human freshly isolated and cryopreserved hepatocytes

Determination of the Potential of GSK1349572 to Form Glutathione Adducts In Vitro

An In Vitro Investigation of the Potential for Metabolic Activation Following Incubation of [¹⁴C]GSK1349572 with Pooled Rat, Monkey or Human Liver Microsomes (GSK Study no. 07DMR124)

Preliminary Metabolite Identification Following Incubation of GSK1349572 in Cryopreserved Rat, Dog, Monkey and Human Hepatocytes

An in vitro Study to Investigate the Metabolism of [¹⁴C]GSK1349572 in Rat, Monkey and Human Hepatocytes (Study Number 07DMR121)

An In Vitro Investigation into the Human Oxidative Enzymology of [¹⁴C]GSK1349572

An assessment of the metabolic stability of GSK1349572 to glucuronidation by recombinant UGT1A1

An In Vitro Investigation of the Human Enzymes involved in Glucuronidation of [¹⁴C]GSK1349572 (GSK Study no. 08DMR067)

Preliminary Evaluation of Metabolic Production of Stereoisomers Following Incubation of GSK1349572 in Cryopreserved Rat, Dog, Cynomolgus Monkey, and Human Hepatocytes

In Vitro Cell Based Evaluation of GSK1349572B as an Activator of the Nuclear Receptor Rat PXR.

In Vitro Cell Based Evaluation of GSK1349572B as an Activator of the Nuclear Receptor Human PXR.

An In Vitro Evaluation of the Effect of GSK1349572 on mRNA Levels of Cytochrome P450 Genes in Cultured Human Hepatocytes.

A Preliminary Investigation into the In Vitro Inhibition of Human Cytochrome P450 Enzymes by GSK1349572.

An in vitro Investigation into the Inhibition of the Human Cytochrome P450 enzymes CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 by GSK1349572.

An In Vitro Investigation into the Inhibition of UDP-Glucuronosyltransferase 1A1 and 2B7 by GSK1349572 Study Number: 09DMR031

Isolation and Identification of Metabolites of [¹⁴C]GSK1349572 in Samples Obtained from the Isolated Perfused Rat Liver (IPRL) Model (GSK Study no. 07DMR120)

Investigation of the Metabolism of GSK1349572 in the Mouse Following a Single Oral Administration of [¹⁴C]GSK1349572 at 100 mg/kg (Study Number: 09DMR028)

Investigation of the Metabolism of GSK1349572 in Rat Following a Single Oral Administration of [¹⁴C]GSK1349572 at 50 mg/kg (GSK Study no. 08DMR017)

Evaluation of the Potential for Epimerization of GSK1349572 in Juvenile Rat Plasma (GSK Study no. 10DMR005)

Metabolite Profiling and Structural Characterization of GSK1349572 in Rat Milk Following a Single Oral Administration

Effects of ERC-349572 sodium on Hepatic Drug Metabolizing Enzymes in Two-Week Oral Toxicity Study in Rats

Quantitative Metabolic Profiling and Metabolite Identification in Plasma, Urine, Bile and Feces of Monkeys Administered [¹⁴C]GSK1349572 Orally as a Suspension (10 mg/kg) (GSK Study no. 08DMR054)

Elimination

Elimination of Radioactivity Following a Single Oral Administration of [¹⁴C] GSK1349572 to Male and Female Intact and Male Bile Duct Cannulated Mice (GSK Study no. 09DMR021)

Elimination of radioactivity following a single oral administration of [¹⁴C] GSK1349572 to male and Female Intact and Male Bile Duct Cannulated rats at a target dose level of 50mg/kg

Elimination of Radioactivity Following a Single Oral (10 mg/kg) Administration of [¹⁴C]GSK1349572 to Male Bile Duct-Cannulated Cynomolgus Monkeys ((b) (4) Study no. 650-098)

Elimination of Radioactivity Following a Single Oral (10 mg/kg) Administration of [¹⁴C]GSK1349572 to Male and Female Intact Cynomolgus Monkeys ((b) (4) study no. 7717-706)

Repeat-dose Studies

Preliminary Carcinogenicity Study (Gavage) of S-349572 Sodium in Mice for 2 Weeks

Preliminary carcinogenicity study (gavage) of S-349572 (GSK1349572) sodium in mice for 13 weeks

Two-Week Oral Toxicity Study of ERC-349572 sodium in Rats - Shionogi Study Number E-349572-TB-012-L

One-Month Oral Toxicity Study of S-349572 sodium in rats

6-Month Oral Toxicity Study of S-349572 Sodium in Rats (final report - previous interim report issued)

Two-Week Oral Toxicity Study of ERC-349572 sodium in Monkeys - Shionogi Study Number

E-349572-TF-029-L

One-Month Oral Toxicity Study of ERC-349572 sodium in Monkeys

Nine-Month Oral Toxicity Study of S-349572 Sodium in Monkeys Study #SBL055-074 (final report - previous interim report issued)

Genotoxicity

GSK1349572A: Bacterial Mutation Assay (Ames Test) with Salmonella typhimurium and Escherichia coli

GSK1349572A: In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus (GlaxoSmithKline Study Number MLA-580)

GSK1349572A: In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus

GSK1349572A: oral bone marrow micronucleus assay in rats

(b) (4) Bacterial Mutation Assay (Ames Test) with Salmonella typhimurium and Escherichia coli (screening study)

Bacterial mutation assay conducted (b) (4)

Reverse Mutation Test with Bacteria on DHEP, (b) (4) (Ames Test (b) (4))

(b) (4) In vitro mutation assay with L5178Y mouse lymphoma cells at the TK locus (screening study)

Carcinogenicity

Carcinogenicity Study (Gavage) of S-349572 sodium in Mice for 104 Weeks

Carcinogenicity Study (Gavage) of S-349572 sodium in Rats for 104 Weeks

Reproductive and Developmental Toxicity

Oral Study for Effects of S-349572 sodium on Fertility and Early Embryonic Development to Implantation in Rats

Dose Range-Finding Oral Study for Effects of S-349572 Sodium on Embryo-Fetal Development in Rats

Oral Study for Effects of S-349572 sodium on Embryo-Fetal Development in Rats

Oral study for effects of S349572 sodium on pre- and postnatal development, including maternal function in rats

Two-Week Oral Toxicity Study of S-349572 sodium in Non-Pregnant Rabbits (b) (4) Study No.: SG08062

Dose Range-Finding Oral Study for Effects of S-349572 sodium on Embryo-Fetal Development in Rabbits Study #SG08063

Oral Study for Effects of S-349572 sodium on Embryo-Fetal Development in Rabbits

Special Toxicology Studies

GSK1349572: Oral Dose Range Tolerability Toxicity Study in Juvenile Rats (GlaxoSmithKline Study No. D09072)

GSK1349572A: Oral Dose Range Study in Juvenile Rats (GlaxoSmithKline Study No. D09126)

GSK1349572A: Oral Toxicity Study in Juvenile Rats (GlaxoSmithKline Study No. G09229)

GSK1349572A: Local Lymph Node Assay in the Mouse Project Number: 1127/1834

GSK1349572B: Local Lymph Node Assay in the Mouse Project Number: 1127/1886

GSK1349572B: Determination of Skin Irritation Potential using the Skinethic Reconstituted Human Epidermal Model; Project Number: 1127/1884

Dermal Irritation Study of S-349572 Sodium in Rabbits

GSK1349572B: Determination of Eye Irritation Potential using an In Vitro Test Strategy (Project Number: 1127/1885)

Ocular Irritation Study of S-349572 Sodium in Rabbits

Immunotoxicity Study of S-349572 Sodium in Rats: Determination of Specific Antibody Formation Against T-cell Dependent Antigen

3.2 STUDIES NOT REVIEWED

GSK1349572B: Single-Dose Subcutaneous and Intramuscular Toxicokinetic Study in Rats (GlaxoSmithKline Study No. R42470)

GSK1349572B: Single-Dose Subcutaneous and Intramuscular Toxicokinetic Study in Rats (GlaxoSmithKline Study No. R42475)

GSK1349572B: Single-Dose Intramuscular Tolerability and Toxicokinetic Study in Male Rats

GSK1349572B: Single-Dose Intramuscular Tolerability and Toxicokinetic Study With Multiple Formulations in Male Rats Followed by 43 Day TK Sample Collection

Preliminary Oral Dose Toxicokinetics Study of MTS-0297994B in Dogs

Preliminary Single Oral Dose Toxicokinetic Study of ERC-349572 Sodium in Monkeys

Supplemental Oral Dose Toxicokinetic Study of S-349572 Sodium in Monkeys

GSK1349572: Single-Dose Oral, Subcutaneous or Intramuscular Toxicokinetic Study in Female Monkeys

3.3 PREVIOUS REVIEWS REFERENCED

Nonclinical studies, including safety pharmacology, ADME, repeat-dose toxicology, and genetic toxicology studies to support the NDA have been reviewed previously. The review is attached to this document as Appendix 1 and is summarized in the appropriate sections of this review.

4 PHARMACOLOGY

Dolutegravir inhibits HIV integrase by binding to the integrase active site and blocking the strand transfer step of retroviral deoxyribonucleic acid (DNA) integration which is essential for the HIV replication cycle.

4.1 PRIMARY PHARMACOLOGY

Please see the Clinical Microbiology review by Lisa Naeger, Ph.D. for a complete review of the pharmacology of dolutegravir.

4.2 SECONDARY PHARMACOLOGY

Dolutegravir was evaluated for possible interactions in in vitro enzyme, receptor, ion channel and transporter binding site assays and also isolated tissue assays. Significant binding (64% inhibition) of dolutegravir (10 μ M) was noted at the melanocortin (MC4) receptor. The MC4 receptor has been shown to be associated with food consumption and, therefore, body weight.

4.3 SAFETY PHARMACOLOGY

At 20 μ M DTG there was 16.1% inhibition of hERG channel tail current in HEK-293 cells expressing hERG cDNA. According to the sponsor's calculations the DTG concentration equates to 8.38 μ g/ml; or systemic exposures (AUC) of 201.1 μ g.h/ml. The in vitro effect is considered weak and no safety concerns were identified in clinical trials.

Dolutegravir did not produce acute blood pressure/heart rate effects in conscious telemetered male monkeys or respiratory or neurobehavioral effects in conscious male rats at doses up to 1000 and 500 mg/kg, respectively.

5 PHARMACOKINETICS/ADME/TOXICOKINETICS**5.1 PK/ADME**

The absorption, distribution, excretion, and metabolism of DTG have been studied in vitro and in vivo in animals.

ANIMAL PHARMACOKINETICS

In rats, there was no increase in systemic exposure (C_{max} and AUC₀₋₂₄) at doses above 500 mg/kg. In monkeys, AUC₀₋₂₄ increases were less than dose-proportional from 50 to 500 mg/kg, while C_{max} did not increase at doses above 125 mg/kg. Volume of distribution (V_d) at steady state and plasma clearance (Cl) were low (V_d/Cl for rat= 103/0.23, dog =352/2.2 and monkey=279/2.1 [V_d : ml/kg; Cl: ml/min/kg]) in all species examined. Dolutegravir half-lives ranged from 5.24 hours (dogs) to 6.18 hours (rats).

ABSORPTION

Oral bioavailability ranged from 24.9% (non-fasted monkeys) to 51.5% (fasted rats).

DISTRIBUTION

The *in vitro* protein binding of DTG in human, monkey, dog and rat sera was 99.3, 99.1, 95.4 and 99.9%, respectively.

The distribution and placental transfer of radioactivity following a single 50-mg/kg oral dose of [^{14}C]-DTG to timed-pregnant Sprague Dawley rats on approximately gestation day 18 was determined by using quantitative whole-body autoradiography. In timed-pregnant rats, radioactivity was rapidly and widely distributed to most dam and fetal tissues, with the highest values obtained from 2 to 10 hours postdose. The fetus and fetal organs had measurable concentrations of radioactivity through 24 hours postdose, with the exception of the fetal brain and fetal spinal cord, which had their last measurable concentration at 10 hours postdose. Drug-derived radioactivity was quantifiable at low levels through 10 hours in the maternal organs protected by the blood:brain barrier, and additionally at 24 hours in brain cerebrum.

Placental transfer of radioactivity was evident. The concentration in the whole fetus was 2.4 μg equivalents [^{14}C]-DTG /g at 2 hours postdose, and steadily increased to a value of 4.0 μg equivalents [^{14}C]-DTG /g at 10 hours postdose before decreasing to 1.3 μg equivalents [^{14}C]-DTG /g at 24 hours postdose.

The dam matrices with the highest concentrations of radioactivity at 2 hours postdose were blood, bile, placenta, kidney medulla, uterus, and lungs, with respective values of 33.2, 25.0, 22.0, 20.3, 20.3, and 19.1 μg equivalents [^{14}C]-DTG /g. The dam matrices with the highest concentrations of radioactivity at 24 hours postdose were blood, uterus, urinary bladder, amniotic sac, and placenta, with values of 9.8, 9.1, 7.5, 7.3, and 6.9 μg equivalents [^{14}C]-DTG /g, respectively.

The fetal matrices with the highest concentrations of radioactivity at 2 hours postdose were fetal blood, fetal myocardium, and fetal muscle, with values of 3.5, 2.9, and 2.8 μg equivalents [^{14}C]-DTG /g, respectively. The fetal matrices with the highest concentrations of radioactivity at 24 hours postdose were fetal bone marrow, fetal blood, and fetal muscle, with values of 5.2, 1.5, and 1.4 μg equivalents [^{14}C]-DTG /g, respectively.

METABOLISM

The comparative metabolic profile of DTG between nonclinical species and humans is presented in the Figure below (excerpted from sponsor).

Dolutegravir is primarily metabolized via UGT1A1 to form the ether glucuronide (dolutegravir glucuronide; M3). Other metabolic products included a glucose conjugate (M2) and an N-dealkylated product (M1).

Dolutegravir is the predominant circulating compound in plasma, and renal elimination of unchanged drug is low (<1% of the dose). The steady-state plasma metabolic profile of dolutegravir was similar to the single dose metabolic profile, indicating data obtained after single dose administration was an adequate predictor of the profile at steady-state. Exposures in the nonclinical metabolism studies adequately reflected exposures in the toxicity studies. No disproportionate human metabolites were noted.

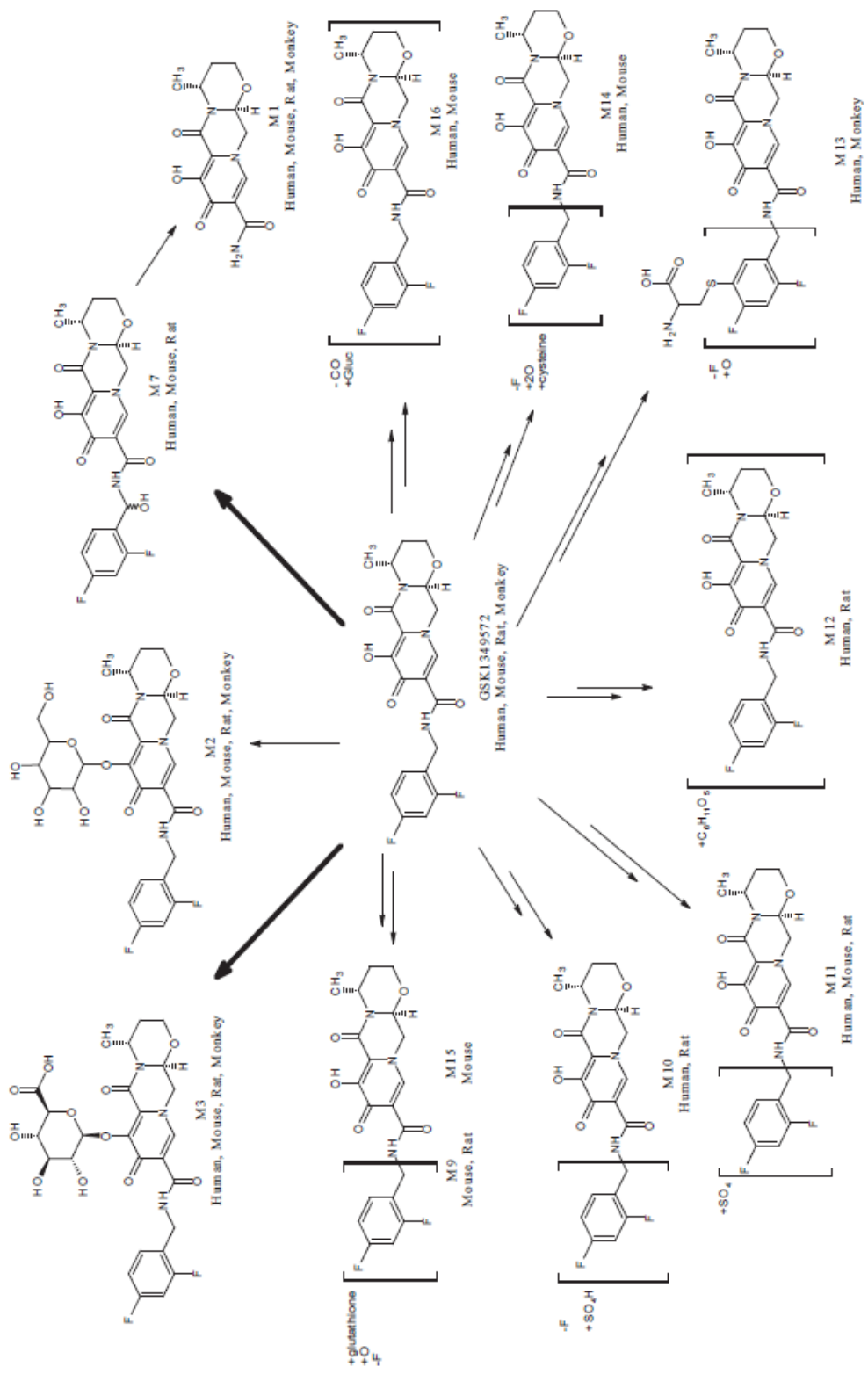
ELIMINATION

In all nonclinical species studies, an oral dose of dolutegravir was excreted primarily via the fecal route. Fecal excretion consisted primarily of unchanged DTG.

Lacteal excretion of DTG-associated radioactivity was assessed following administration of a single 50-mg/kg oral dose of [^{14}C]-DTG to Sprague Dawley rats at approximately 10 days postpartum. Radioactivity was detected in milk at the first time point of 1 hour postdose, with a mean concentration of 10 μg equivalents [^{14}C]-DTG /g. The concentration of radioactivity in milk steadily increased to a mean C_{max} value of 47.3 μg equivalents [^{14}C]-DTG /g at 8 hours postdose, and then decreased to a mean of 1.8 μg equivalents [^{14}C]-DTG /g at 24 hours postdose.

Following administration of radiolabeled DTG, excretion of radioactivity was essentially 100% in all species. The radiolabel location was metabolically stable with no notable sequestration or covalent binding of dolutegravir to plasma or excreta. Biliary excretion in animals accounted for the major portion of the absorbed dose and represented the predominant excretion route for dolutegravir glucuronide. Dolutegravir glucuronide likely undergoes enterohepatic circulation, resulting in recycling of DTG in plasma.

In vitro, DTG inhibited the renal organic cation transporter (OCT) 2 ($\text{IC}_{50} = 1.9 \mu\text{M}$), which provides a mechanistic basis for the non-pathological mild serum creatinine increases observed in clinical studies. As a weak inhibitor of UGT1A1, DTG has the potential to interfere with the conjugation of bilirubin which could result in a mild increase in total or unconjugated bilirubin on prolonged treatment with DTG.



Key: **Bolded arrows indicate the primary metabolic products in humans (M3 the predominant product, M7 a notable metabolite)**

6 GENERAL TOXICOLOGY

6.1 SINGLE-DOSE TOXICITY

Dolutegravir: Single Dose Oral Gavage non-GLP Toxicity Study in One Female Cynomolgus Monkey

Single oral gavage doses of Dolutegravir at 50, 125, 250 and 500 mg/kg were administered to fasted female cynomolgus monkeys (n= 1/group). There were no toxicologically significant findings.

6.2 REPEAT-DOSE TOXICITY

Study Title: Dolutegravir: 2-Week Oral Toxicity Study in Sprague Dawley Rats

Key Findings:

- The stomach is the target organ identified.
- The NOAEL is defined as 150 mg/kg (AUC =1445 µg.h/mL)

Rats were dosed with 0 (control), 50, 150 or 500 mg/kg/day. The stomach is the target organ identified. The sponsor's NOAEL = 500 mg/kg/day (C_{max}=116 µg/mL AUC_{0-24h}=1830 µg.h/mL). However, because of drug-induced gastric mucosal lesions, the Pharmacology/Toxicology Reviewer (Dr. KM Wu) defined the NOAEL as 150 mg/kg (AUC_{0-24h}=1445 µg.h/mL).

Systemic exposure (C_{max} and AUC_{0-24h}) to Dolutegravir were generally less than dose- proportional (ceiling/saturable effects) and no notable (>2-fold) sex-related differences in at any dose or sampling occasion were observed.

Dose (mg/kg/d ay)	Sex	C _{max} (ug/mL) Day 1	End of Study	AUC _{0-24h} (ug.h/mL) Day 1	End of Study
50	M/ F	58.5/ 75.4	65.7/ 95.6	881/ 1110	1040/ 1610
150	M /F	82.7/ 83.3	74.1/ 106	994 /1050	1150/ 1740
500	M /F	87.1/ 117	108 / 124	1360 / 1350	1710/ 1950

Study Title: 1-month Oral Toxicity Study of S-349572A (ERC-349572 sodium) in Rats**Key study findings:**

- The GI tract was identified as the target organ (system)
- The NOAEL is defined as the middle-high dose, 100 mg/kg, based on the finding of GI hemorrhage in high dose animals only (AUC 722.2 and 780.5 µg.h/mL in males and females, respectively).

Rats were administered 2, 10, 100 or 1000 mg/kg/day S-349572 via oral gavage for one month, and the study included a one month recovery period. Hemorrhage of the glandular stomach mucosa was noted in four high-dose (1000 mg/kg/day) animals (two males and two females). Slight edema, with eosinophilic infiltration, globule leukocytes, and/or mucous neck cells was present in the glandular stomach mucosa of some or all high dose animals. Similar findings, but in a smaller sub-set of animals, were seen in middle and low dose groups. The NOAEL is defined as the middle-high dose, 100 mg/kg, based on the finding of GI hemorrhage in high dose animals only (AUC 722.2 and 780.5 µg.h/mL in males and females, respectively).

Study no.:	S-349572-TB-043-L
Volume # and page #:	SN0086/Vol 1/page 78
Conducting laboratory:	Developmental Research Laboratories, Shionogi & Co., Ltd., 3-1-1, Futaba-cho, Toyonaka, Osaka 561-0825, Japan
Date of study initiation:	February 27, 2008
GLP compliance:	yes (Japanese)
QA report:	yes
Drug, lot #, and % purity:	A7Z001 (98.1%)

Methods

Doses:	control, 2, 10, 100, 1000 mg/kg/day
Species/strain:	Rat/Crl:CD(SD)
Number/sex/group:	10
Route, formulation, volume:	Oral gavage, 0.5% w/w% hydroxypropyl methylcellulose aqueous solution with 0.1% w/w% Tween 80 / Suspension, 10 mL/kg Homogeneity of the dose formulations were tested after 6 hours and on day 10 of dosing and found to be acceptable. Concentrations were confirmed in the first and last dose preparation.
Satellite groups used for toxicokinetics or recovery:	5/sex/dose (recovery/control and high dose) 4/sex/dose (toxicokinetics)
Age:	6 weeks
Weight:	males: 179.7 to 214 g females: 135.7 to 172.5 g

Results:

Mortality: Toxicity group animals were observed for morbidity/mortality and clinical signs twice daily (once daily on weekends or holidays). All dosed animals survived until the scheduled sacrifice.

Clinical signs: There were no remarkable findings.

Body weights: Body weight measurements were collected on Days 1 (first day of dosing), 6, 11, 16, 21, 26 and 31 (recovery day 1:R1), R6, R11, R16, R21, R26. There were no dose-related changes in body weight.

Food consumption: Food consumption was measured on Days 1 (first day of dosing), 6, 11, 16, 21, 26 and 31 (recovery day 1:R1), R6, R11, R16, R21, R26. There were no dose-related changes in food consumption.

Ophthalmoscopy: Ophthalmoscopy was performed on Days -5, 24, R25 (female) and R26 (male). There were no findings attributed to treatment with test article.

Hematology: Blood was collected from toxicity group animals at terminal necropsy for assessment of hematological parameters. There were no findings attributed to treatment with test article.

Clinical chemistry: Blood was collected from toxicity group animals at terminal necropsy for assessment of clinical chemistry parameters. There were no findings attributed to treatment with test article.

Urinalysis: Urine was collected on Days 117 and 179 of dosing, and at the end of the recovery period. An increase in protein excretion and an increase in specific gravity was noted in urine from high dose rats (both sexes), without correlative findings in clinical chemistry or histopathology.

Gross pathology: Necropsies were performed on animals one day after the end of the dosing period. Red focus (i.e., red spots) was noted in the GI tract of 3/10 males and 2/10 females from the high dose groups. This effect was not noted in recovery group animals.

Organ weights: The following tissues were weighed: Liver, Kidneys, Lungs (including bronchi), Heart, Spleen, Thymus, Adrenal glands, Pituitary gland, Submaxillary glands (including sublingual glands), Testes, Ventral prostate, Ovaries, Uterus, Brain. There were no findings attributed to treatment with test article.

Histopathology: Adequate Battery: yes
Peer review: yes

Test substance-related lesions were observed in the stomach at ≥ 100 mg/kg/day at the end of dosing period.

Mild hemorrhage in 2/10 per sex and pigment deposition in 1/10 males was noted in the glandular stomach at 1000 mg/kg/day. The finding of hemorrhage was considered to be toxicologically significant.

Findings in the glandular stomach included:

- increased mucous neck cells of 6/10 males and 8/10 females at 100 mg/kg/day, and 8/10 males and all females at 1000 mg/kg/day. Located in the neck region these cells secrete a soluble mucous only under vagal stimulation; i.e., not in the resting stomach.
- Eosinophil infiltration of 4/10 males and 6/10 females at 100 mg/kg/day and all animals at 1000 mg/kg/day,
- focal edema in the mucosa of 2/10 females at 100 mg/kg/day and 4/10 animals of both sexes at 1000 mg/kg/day,
- globule leukocyte infiltration into lamina propria of glandular stomach of 7/10 males and 9/10 females at 100 mg/kg/day and all animals at 1000 mg/kg/day were observed at the end of dosing period.

In the limiting ridge (i.e., in rats, a fold of the forestomach mucosa that separates the forestomach from the glandular stomachs),

- acanthosis of 1/10 per sex at 100 mg/kg/day and 4/10 per sex at 1000 mg/kg/day,
- mixed cellular infiltration of 8/10 males and 9/10 females at 100 mg/kg/day and 8/10 males and all females at 1000 mg/kg/day, and
- intercellular edema of epithelium in the limiting ridge of 5/10 males and 8/10 females at 100 mg/kg/day and 7/10 males and all females at 1000 mg/kg.

Similar gastrointestinal changes such as increased mucous neck cells in the glandular stomach and mixed cellular infiltration in the limiting ridge etc. were observed at 2 and 10 mg/kg/day, but the incidence was low and/or comparable with the control group. Therefore, it was not considered to be test substance-related findings.

In the recovery period, the incidence and severity of the above gastrointestinal findings at 1000 mg/kg/day were comparable with those of the control.

Toxicokinetics: The sampling points for the control and S-349572 sodium dosing groups were 1, 2, 4, 6, 10 and 24 hours after administration on Day 1, and prior to dosing and 1, 2, 4, 6, 10 and 24 hours after administration on Days 14 and 29. Systemic exposures increased proportionately with dose between 2 and 10 mg/kg, and in a less than dose-proportional manner at higher doses (see table below, *excerpted from sponsor*).

	<u>0 (Control)</u>		<u>2</u>		<u>10</u>		<u>100</u>		<u>1000</u>	
Daily Dose (mg/kg)	M: 15	F: 15	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 15	F: 15
Number of Animals										
Toxicokinetics										
Number of animals	4	4	4	4	4	4	4	4	4	4
C _{max} (µg/mL)										
Day 1	NC	NC	3.5	4.6	15.2	21.2	43.7	54.4	95.1	115.6
Day 14	NC	NC	4.0	9.2	18.2	25.1	61.5	60.7	107.9	109.1
Day 29	NC	NC	4.7	7.8	23.7	34.6	49.2	61.6	118.6	112.2
AUC _{0-24 hr} (µg·hr/mL)										
Day 1	NC	NC	39.3	60.0	219.6	278.4	692.9	775.1	1678.0	1614.6
Day 14	NC	NC	44.5	87.3	242.4	303.8	779.3	824.5	1665.9	1853.5
Day 29	NC	NC	53.0	81.7	274.2	378.4	722.2	780.5	1836.9	1737.4

REPEAT-DOSE TOXICITY

Study title: 6-month Oral Toxicity Study of S-349572 sodium in Rats

Key study findings:

- The GI tract (hemorrhage of the glandular stomach mucosa) was identified as the target organ.
- The NOAEL was defined as the middle dose, 50 mg/kg (AUC 607 and 922 µg.h/mL in males and females, respectively) based on stomach mucosal hemorrhage at the high dose.

Rats were administered 5, 50 or 500 mg/kg/day S-349572 via oral gavage for up to six months (the study included a 4-month interim sacrifice). The 500 mg/kg high dose was selected based on previous studies (two-week and 1-month) in rats that demonstrated saturated absorption kinetics at doses greater than 500 mg/kg (i.e., 1000 mg/kg). At the four-month interim sacrifice, rats (10 animals/sex/group) were sacrificed and toxicity parameters were evaluated. In addition, systemic exposure to test article was evaluated in 4 animals/sex/group.

By the end of six months of dosing, hemorrhage of the glandular stomach mucosa was noted in two high-dose (500 mg/kg/day) males. Slight thickening of the forestomach lining, slight edema, with eosinophilic infiltration, globule leukocytes, and/or mucous neck cells were present in the glandular stomach mucosa of all high dose

animals. In the middle dose groups (50 mg/kg/day), similar findings were noted, with the absence of thickening of the forestomach limiting ridge mucosa. In the low dose group (5 mg/kg/day), increased globule leukocytes in the glandular stomach submucosa were noted in one female. The NOAEL was defined as the middle dose, 50 mg/kg (AUC 607 and 922 µg.h/mL in males and females, respectively) based on stomach mucosal hemorrhage at the high dose.

Study no.:	SBL055-082 (Sponsor study no. S-349572-TF-055-L)
Volume #, and page #:	Vol 1/page 1
Conducting laboratory:	(b) (4)
Date of study initiation:	July 16, 2008
GLP compliance:	yes
QA report:	yes
Drug, lot #, and % purity:	A7Z001 (98.1%) and B86001 (98.9%) (test article supplied by Shionogi & Co., Ltd)

Methods

Doses:	control, 5, 50, 500 mg/kg/day
Species/strain:	Rat/Crl:CD(SD)
Number/sex/group:	10/12 (at 4-month and 6-month sacrifice, respectively)
Route, formulation, volume:	Oral gavage, 0.5% w/w% hydroxypropyl methylcellulose aqueous solution with 0.1% w/w% Tween 80 / Suspension, 10 mL/kg Concentrations of the dose formulations were tested on days 1, 120 and 180 of dosing and found to be acceptable. Homogeneity was confirmed on day 1 of dosing.
Satellite groups used for toxicokinetics or recovery:	4/sex/dose (+ 2 spare)
Age:	6 weeks
Weight:	45 to 115 g

Results:

Mortality: Toxicity group animals were observed for morbidity/mortality and clinical signs at least three times daily (once pre-dose and ~1 and 4 hours post-dose). All dosed animals survived until the scheduled sacrifice.

Clinical signs: Neither unilateral (one middle-dose male starting day 109 through end of dosing) and bilateral (one high-dose male starting day 110 through end of dosing) hindlimb swelling nor eyeball opacity in one high-dose male was considered to be related to test article administration. There were no other remarkable findings.

Body weights: Body weight measurements were collected weekly during dosing and recovery periods. There were no dose-related changes in body weight related to four or six months of test article administration.

Food consumption: Food consumption was measured weekly during dosing and recovery periods. There were no dose-related changes in food consumption related to test article administration.

<u>Ophthalmoscopy:</u>	Ophthalmoscopic examinations were performed at the end of four-month and six month treatment periods. There were no findings attributed to treatment with test article.
<u>Hematology:</u>	Blood was collected from toxicity group animals at terminal necropsy for assessment of hematological parameters. There were no findings attributed to treatment with test article.
<u>Clinical chemistry:</u>	Blood was collected from toxicity group animals at terminal necropsy for assessment of clinical chemistry parameters. There were no findings attributed to treatment with test article.
<u>Urinalysis:</u>	Urine was collected on Days 117 and 179 of dosing, and at the end of the recovery period. There were no findings attributed to treatment with test article.
<u>Gross pathology:</u>	Necropsies were performed on animals one day after the end of the four-month and six-month dosing periods. White focus was noted in the stomach of two high-dose males, one after four months and one after six months of treatment. Red focus was noted in one male and two females from the high dose groups after six months of dosing. Only slight hemorrhage was noted microscopically.
<u>Organ weights</u>	(See histopathology table for weighed tissues): There were no findings attributed to treatment with test article.
<u>Histopathology:</u>	Adequate Battery: yes Peer review: yes

Hemorrhage of the glandular stomach mucosa was noted in two high dose (500 mg/kg/day) males (one after four months of dosing and one after six months of dosing). After six months of dosing, slight thickening of the forestomach lining (4/12 males and 4/12 females) was noted. Slight edema, with very slight or slight eosinophilic infiltration (12/12 males and 11/12 females), and slight increases in globule leukocytes (12/12 males and 12/12 females), or mucous neck cells (10/12 males and 10/12 females) were noted in the glandular stomach mucosa of high dose animals.

In the middle dose (50 mg/kg/day) groups, very slight thickening of the forestomach lining (1/12 males), with very slight eosinophilic infiltration (4/12 males and 7/12 females), a very slight (2/12 males and 3/12 females) or slight (9/12 males and 9/12 females) increase in globule leukocytes, or mucous neck cells (4/12 males and 7/12 females) were present in the glandular stomach mucosa.

In one low dose male (5 mg/kg/day), a very slight increase in globule leukocytes was noted in the glandular stomach submucosa.

Toxicokinetics: Blood for determination of toxicokinetic parameters was collected on Day 1 (first) of Dosing: 1, 2, 4, 6, 10, and 24 hours after dosing (total 6 points), and on Days 30 and 120 of Dosing: Before dosing, and 1, 2, 4, 6, 10, and 24 hours after dosing (total 7 points).

Systemic exposures increased in a less than dose-proportional manner. Only a 2-fold increase in exposures was observed despite a 10-fold increase between middle (50 mg/kg) and high (500 mg/kg) doses (see table below, *excerpted from sponsor*).

Table 3: Toxicokinetics from 6-month Oral Toxicity Study of S-349572 sodium in Rats

AUC _{0-24hr} (µg·hr/mL)					
Dose Level (mg/kg/day)		0	5	50	500
Number of Animals		4	4	4	4
Male	Day 1	NE	88.8 ± 17.0	636.7 ± 129.0	1450.1 ± 141.6
	Day 30	NE	123.5 ± 34.4	708.7 ± 169.6	1300.1 ± 154.6
	Day 120	NE	126.7 ± 15.5	594.0 ± 137.1	1274.3 ± 180.0
	Day 180	NE	115.7 ± 19.0	606.9 ± 121.2	1337.6 ± 258.3
Female	Day 1	NE	138.4 ± 27.6	731.0 ± 122.8	1450.2 ± 339.7
	Day 30	NE	216.7 ± 22.8	883.2 ± 126.0	1508.4 ± 353.1
	Day 120	NE	267.2 ± 35.5	932.7 ± 117.9	1660.3 ± 226.2
	Day 180	NE	289.7 ± 60.3	921.6 ± 74.6	1777.1 ± 226.3

NE: Not Estimated

Histopathology inventory

Study	SBL055-082 (6-month oral gavage)	09-2119 (13-week oral gavage)
Species	Rat	Mouse
Adrenals	X*	X
Aorta	X	X
Bone Marrow smear	X	X**
Bone (femur)	X	X
Brain	X*	X*
Cecum	X	X
Cervix		X
Colon	X	X
Duodenum	X	X
Epididymis	X*	X*
Esophagus	X	X
Eye	X	X
Fallopian tube		X
Gall bladder	NA	X
Gross lesions	X	X
Harderian gland		X
Heart	X*	X*
Ileum	X	X
Injection site	NA	NA
Jejunum	X	X
Kidneys	X*	X*
Lachrymal gland	X	X
Larynx		
Liver	X*	X*

Lungs	X*	X*
Lymph nodes, cervical		
Lymph nodes mandibular	X	
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Optic nerves		
Ovaries	X*	X*
Pancreas	X	X
Parathyroid	X*	
Peripheral nerve	X	X
Pharynx		
Pituitary	X*	X
Prostate	X*	X*
Rectum	X	
Salivary gland		X*
Sciatic nerve	X	X
Seminal vesicles	X*	X
Skeletal muscle	X	X
Skin	X	X
Spinal cord	X	X
Spleen	X*	X*
Sternum		
Stomach	X	X
Testes	X*	X*
Thymus	X*	X*
Thyroid	X*	X
Tongue	X	
Trachea		X
Urinary bladder	X	X
Uterus	X*	X*
Vagina	X	X
Zymbal gland		

X, histopathology performed

*, organ weight obtained

** smears prepared, not examined

Study Title: Dolutegravir: 2-Week Oral Gavage Toxicity Study in Cynomolgus Monkeys

KEY FINDINGS:

- The liver, immune organs (lymph nodes, bone marrow, adrenal, kidney, and GI and hemotologic system are target organs identified.
- The NOAEL was defined as 100 mg/kg [Day 14 AUC = 192 ug.h/ml (males) and 187 ug.h/ml (females). However, per Dr. Wu's review, due to the large individual variation in systemic exposure, the lowest measured Day 1 AUC_{0-24h} (41 ug.h/ml) and C_{max} (5.75 ug/ml) at 100 mg/kg will define the NOAEL exposure.

STUDY NO.:	Study No. SG07030; E-34572- TF-029-L
REPORT NO.:	RD2007/01142/00
LABORATORY:	(b) (4)
STUDY INITIATION:	5/07
GLP:	yes (x) no ()
QA REPORT:	yes (x) no ()
LOT/PURITY:	R06001 (same as clinical batch)
FORMULATION:	vehicle = 0.5% w/w aqueous hydroxypropyl methylcellulose with 0.1% Tween 80
METHODS:	
DOSING:	0 (control), 100, 300 or 1000 mg/kg/day
SPECIES/STRAIN:	Cynomolgus monkeys
#/SEX/GROUP:	3
ROUTE	Oral gavage, 1/day
MORTALITY:	Checked at least once daily (mortality and moribundity)
CLINICAL SIGNS:	Daily
BODY WEIGHTS:	2/Weekly
FOOD CONSUMPTION:	2/Weekly
OPHTHALMOSCOPY:	Pretest, during Week 2
EKG:	At least weekly
HEMATOLOGY:	During Week 2
CLINICAL CHEMISTRY:	During Week 2
URINALYSIS:	At termination

NECROPSY &**HISTOPATHOLOGY:** At termination (including bone marrow smears)**RESULTS:**

MORTALITY: One female (1000 mg/kg/day) died on Day 13. This animal showed repeated daily emesis began on Day 2 and diarrhea on Day 5 with progression to decreased activity, lateral or crouching position, pale oral mucosa, and subnormal body surface temperature prior to death on Day 13.

CLINICAL SIGNS, BODY WEIGHTS & FOOD CONSUMPTION:

Hematology and clinical chemistry performed on this animal showed an increase (2X control) in fibrinogen, marked increases (3X control) in ALT and urea nitrogen and decreases (0.8 to 0.9X control) in Na and Cl. Liver and digestive tract were autolyzed, and histopathological findings that might cause death were not evident in the kidneys, heart or lungs. A direct cause of death was not determined. The sponsor speculated that the deteriorated GI effects (emesis, diarrhea, ulcer in colon) with changes in blood electrolytes contributed to the death.

All animals at 300 and 1000 mg/kg/day exhibited emesis and diarrhea (loose, muddy or watery stools) from initiation of dosing. Two males at 1000 mg/kg/day had decreased activity and lateral or crouching position from Day 13. In 100 mg/kg group, emesis was reported in 2/3 males and 1/3 females.

Persistent decreases (2-17%) in BW or BW gain occurred in 2/sex at 300 mg/kg/day and in 2 males and all females at 1000 mg/kg/day. Of these animals, 1 male and 2 females at 300 mg/kg/day and 2 males and 1 female (animal found dead on Day 13) at 1000 mg/kg/day had decreased food consumption.

EKG: Unremarkable.

OPHTHALMOLOGY: Unremarkable

HEMATOLOGY: Hematology findings included: decreases in the reticulocyte count (0.2 to 0.5-X control) in males and females at 300 and 1000 mg/kg; decreases in the platelet count (0.5 to 0.7-X control) and increases in fibrinogen (1.4 to 1.6- X control) in males and females at 1000 mg/kg; a decrease in the reticulocyte ratio (0.3 to 0.5-X control) and prolongation of APTT (1.1-X control) in males at 1000 mg/kg; and a decrease (0.9-X control) in the red blood cell count in females at 1000 mg/kg.

CLINICAL CHEMISTRY:

Clinical chemistry findings included: increases in ALT (2.3X control) in males at 300 mg/kg and in males and females at 1000 mg/kg (5 to 10X control); increases in AST (2X control), total bilirubin (2 to 3X control), urea nitrogen (1.6 to 1.8X control) and creatinine (1.2 to 1.5X control) and decreases in sodium, chloride (0.87 to 0.95X control) and the A/G ratio (0.7X control) in males and females at 1000 mg/kg; increases in triglycerides (1.9X control) in males at 1000 mg/kg; and a decrease (0.7X control) in total cholesterol in females at 1000 mg/kg.

URINALYSIS: Urinalysis showed decreases in the urinary volume (0.3 to 0.6X control) and Na/Cl excretion levels (0.2 to 0.3X control) in males and females and a decrease in K excretion level (0.3X control) in females at 1000 mg/kg.

GROSS PATHOLOGY:

Gross pathology revealed reddish spots in the stomach or colon, small thymus, brownish discoloration of the mesenteric lymph nodes and enlargement of the lymph nodes in the abdominal cavity in animals at 300 and 1000 mg/kg, including the animal found dead on Day 13, and whitish protrusion in the esophagus and reddish spots in the ileum at 1000 mg/kg. Decreases in the weight of the thymus were noted at 300 and 1000 mg/kg and increases in the weights of the liver and adrenals were noted at 1000 mg/kg.

Histopathology

GI: ≥ 300 mg/kg: Atrophy of the mucosal epithelium and cell debris from the crypts of the cecum, colon and rectum, hemorrhage in the mucosa of the colon, atrophy of acinar cells in the pancreas (+mononucleated infiltrate) and parotid glands (+atrophy of the mucosal epithelium and hemorrhage in the mucosa of the stomach were observed in males at 1000 mg/kg/day). The frequency of these findings were reported in 1/3-2/3 of MD and 2/3-3/3 of HD animals.

Liver: 1000 mg/kg/day: single cell necrosis, vacuolation of hepatocytes, and reduced P450 content (30%) in the liver. Liver organ weight increased was reported in both sexes of HD animals (30%-40%)

Kidney: 1000 mg/kg/day: dilatation of the tubules in the kidneys (both sexes).

Lymph Node/Thymus/Spleen:

Atrophy of the cortex of the thymus and decrease in lymphocytes in the paracortex of the mesenteric lymph nodes in monkeys at ≥ 300 mg/kg and in the submandibular lymph nodes in monkeys at 1000 mg/kg. At 1000 mg/kg, gelatinous bone marrow + hypocellularity, atrophy of the white pulp in the spleen were noted in males and females. In the bone marrow examinations, a decrease in the nucleated cell count was noted in one male at 1000 mg/kg.

Adrenal: ≥ 300 mg/kg: decrease in lipid droplets in zona fasciculata cells in the adrenals (MD 2/6; HD 4/6). Hypertrophy of the zona fasciculata occurred in 2/3 of 1000 mg/kg animals)

TOXICOKINETICS

Systemic exposures (C_{max} & AUC_{0-24h}) to Dolutegravir were less than dose- proportional and might be due to repeated emesis that resulted in a large degree of variability and some overlap in exposures between dose groups (pronounced at 300 and 1000 mg/kg/day.)

Table 4: Plasma concentrations and toxicokinetic parameters in Male Monkeys on Day 14 of dosing (excerpted from sponsor)

Group	Animal No.	Plasma concentration ($\mu\text{g/mL}$)							C_{max} ($\mu\text{g/mL}$)	T_{max} (h)	$\text{AUC}_{0-24\text{h}}^{\text{a}}$ ($\mu\text{g}\cdot\text{h/mL}$)
		Pre	0.5 h	1 h	2 h	4 h	8 h	24 h			
Control	01	N.D. [1]	N.D. [1]	0.0383 [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.C.	N.C.	N.C.
	02	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.C.	N.C.	N.C.
	03	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.C.	N.C.	N.C.
100 mg/kg	11	1.81 [1]	7.09 [1]	17.4 [1]	20.9 [1]	20.1 [1]	4.23 [1]	1.43 [1]	20.9	2	162
	12	1.32 [1]	10.5 [1]	25.1 [1]	28.3 [1]	23.3 [1]	6.57 [1]	1.43 [1]	28.3	2	214
	13	2.37 [1]	10.1 [1]	13.4 [1]	20.2 [1]	22.8 [1]	7.66 [1]	1.12 [1]	22.8	4	200
	Mean	1.83	9.23	18.6	23.1	22.1	6.15	1.33	24.0	2.7	192
	S.D.	0.53	1.86	5.9	4.5	1.7	1.75	0.18	3.8	1.2	27
	(N)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
300 mg/kg	21	1.87 [1]	5.13 [1]	20.7 [1]	26.6 [1]	22.8 [1]	13.3 [1]	1.62 [1]	26.6	2	273
	22	0.611 [1]	4.29 [1]	13.6 [1]	10.2 [1]	10.5 [1]	3.86 [1]	1.63 [1]	13.6	1	111
	23	3.24 [1]	14.0 [1]	14.1 [1]	23.2 [1]	25.0 [1]	7.01 [1]	1.85 [1]	25.0	4	213
	Mean	1.91	7.81	16.1	20.0	19.4	8.06	1.70	21.7	2.3	199
	S.D.	1.31	5.38	4.0	8.7	7.8	4.81	0.13	7.1	1.5	82
	(N)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
1000 mg/kg	31	3.26 [1]	8.94 [1]	24.9 [1]	22.6 [1]	20.9 [2]	9.43 [1]	0.955 [1]	24.9	1	223
	32	6.85 [1]	16.3 [1]	27.1 [1]	32.6 [2] ^b	35.2 [2]	36.7 [2] ^b	10.1 [1]	36.7	8	632
	33	1.50 [1]	3.72 [1]	5.86 [1]	9.03 [1]	16.9 [2]	13.2 [1]	4.21 [1]	16.9	4	237
	Mean	3.87	9.65	19.3	21.4	24.3	19.8	5.09	26.2	4.3	364
	S.D.	2.73	6.32	11.7	11.8	9.6	14.8	4.64	10.0	3.5	232
	(N)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)

[]: Dilution factor

N.C.: Not calculated

N.D.: Not detected, below the lower limit of quantification (0.03 $\mu\text{g/mL}$)^a The values of 0 hour were calculated using the pretreatment value.^b Reanalysis results with dilution were adopted since the results of initial analysis exceeded the upper limit of calibration curve (30 $\mu\text{g/mL}$).**Table 5: Plasma concentrations and toxicokinetic parameters in Female Monkeys on Day 14 of dosing (excerpted from sponsor)**

Group	Animal No.	Plasma concentration ($\mu\text{g/mL}$)							C_{max} ($\mu\text{g/mL}$)	T_{max} (h)	$\text{AUC}_{0-24\text{h}}^{\text{a}}$ ($\mu\text{g}\cdot\text{h/mL}$)
		Pre	0.5 h	1 h	2 h	4 h	8 h	24 h			
Control	41	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.C.	N.C.	N.C.
	42	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	0.0331 [1]	0.0529 [1]	N.D. [1]	N.C.	N.C.	N.C.
	43	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.D. [1]	N.C.	N.C.	N.C.
100 mg/kg	51	1.04 [1]	7.48 [1]	16.2 [1]	26.6 [1]	22.3 [1]	10.1 [1]	1.17 [1]	26.6	2	233
	52	1.47 [1]	6.41 [1]	10.3 [1]	18.6 [1]	25.3 [1]	6.51 [1]	2.38 [1]	25.3	4	199
	53	1.08 [1]	13.0 [1]	14.8 [1]	17.1 [1]	13.7 [1]	3.51 [1]	1.32 [1]	17.1	2	130
	Mean	1.20	8.96	13.8	20.8	20.4	6.71	1.62	23.0	2.7	187
	S.D.	0.24	3.54	3.1	5.1	6.0	3.30	0.66	5.2	1.2	52
	(N)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
300 mg/kg	61	2.52 [1]	12.3 [1]	16.2 [1]	23.9 [1]	25.2 [1]	14.8 [1]	4.77 [1]	25.2	4	317
	62	3.55 [1]	10.7 [1]	13.3 [1]	25.6 [1]	24.2 [1]	11.3 [1]	3.03 [1]	25.6	2	264
	63	2.31 [1]	10.4 [1]	12.0 [1]	18.5 [1]	19.2 [1]	11.2 [1]	2.35 [1]	19.2	4	231
	Mean	2.79	11.1	13.8	22.7	22.9	12.4	3.38	23.3	3.3	271
	S.D.	0.66	1.0	2.2	3.7	3.2	2.1	1.25	3.6	1.2	43
	(N)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
1000 mg/kg	71	5.14 [1]	12.9 [1]	12.5 [1]	22.1 [1]	26.5 [2]	14.8 [1]	1.74 [1]	26.5	4	292
	72 ^b										
	73	7.12 [1]	13.3 [1]	18.3 [1]	25.6 [1]	34.1 [2]	19.8 [1]	6.87 [1]	34.1	4	416
	Mean	6.13	13.1	15.4	23.9	30.3	17.3	4.31	30.3	4.0	354
	S.D.										
	(N)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)

[]: Dilution factor

N.C.: Not calculated

N.D.: Not detected, below the lower limit of quantification (0.03 $\mu\text{g/mL}$)^a The values of 0 hour were calculated using the pretreatment value.^b Died on Day 13 of dosing

CONCLUSION

Based on these results, the NOAEL of Dolutegravir was considered to be 100 mg/kg/day. The mean AUC value for the male 100 mg/kg dose group on Day 1 was 172 ug.h/ml (range 41 to 309 ug.h/ml). Day 14 systemic exposures at 100 mg/kg were 192 ug.h/ml (males) and 187 ug.h/ml (females). However, per the review by Dr. Wu, due to the large individual variation in systemic exposure, the lowest measured Day 1 AUC_{0-24h} (41 ug.h/ml) and C_{max} (5.75 ug/ml) at 100 mg/kg will define the NOAEL exposure.

Study title: One-Month Oral Toxicity Study of ERC-349572 sodium in Monkeys

Key study findings:

- The GI tract was identified as the target organ (system).
- The NOAEL was considered to be 50 mg/kg/day for both males and females: Corresponding exposures (AUC Day 30) were 111 and 153 µg.h/mL in males and females, respectively.

Cynomolgus monkeys were dosed with 0, 25, 50, 100 mg/kg via oral gavage for one month. Vomiting, diarrhea, decreased body weights and food consumption, inflammatory cells infiltration in the lamina propria, atrophy of the mucosal epithelium and cell debris from the crypts of the cecum, colon or rectum were considered to be test article related, but due to local effects rather than systemic exposures. Atrophy of the thymus and acinar cells in the pancreas were noted at 100 mg/kg but were considered to be related to the poor physical condition of the animals and not direct effects of the test article.

The no observed adverse effect level (NOAEL) of ERC-349572 was considered to be 50 mg/kg/day for both males and females under the experimental conditions of this study. Corresponding exposures (AUC Day 30) were 111 and 153 µg.h/mL in males and females, respectively.

Study no.:	SBL055-082
Volume # and page #:	EDR
Conducting laboratory and location:	SHIONOGI & CO., LTD. Developmental Research Laboratories Drug Safety Evaluation
Date of study initiation:	August 10, 2007
GLP compliance:	yes
QA report:	yes
Drug, lot #, and % purity:	ERC-349572 sodium; 02 (99.1%)

Methods

Doses:	0, 25, 50, 100 mg/kg
Species/strain:	Cynomolgus monkey
Number/sex/group or time point (main study):	5 (control and high dose) or 3 (low and middle doses)

Route, formulation, volume, and infusion rate:	Suspension; 0.5% HPMC w/ Tween 80; 10 mL/kg via nasogastric gavage
Satellite groups used for toxicokinetics or recovery:	2 (control and high dose) for 1 month recovery
Age:	2.5 - 3 years
Weight:	males: 2.15 to 3.35 kg females: 2.25 to 3.00 kg

Results:

Mortality: Animals were checked for morbidity/mortality three times daily during the dosing phase. All animals survived to scheduled necropsy.

Clinical signs: Clinical signs were assessed three times daily during the dosing phase. Males and females in the high dose (100 mg/kg) group exhibited vomiting/vomit and diarrhea (muddy or watery stools) from initiation of dosing and 1 male and 1 female in this group exhibited crouching position from Day 17 of dosing.

No clinical signs were noted after Day 4 of recovery.

Body weights: Body weights were recorded on Days -7, -4, 1, 4, 8, 11, 15, 18, 22, 25, 28 and 30 of dosing and Days 1, 4, 8, 11, 15, 18, 22, 25, 28 and 30 of recovery. Persistent decreases in body weights or body weight gain were noted in 2 males and 2 females in the 100 mg/kg group.

No appreciable body weight changes were noted in any animal during the recovery period.

Food consumption: Food consumption was assessed daily. The decreased body weights noted in a few animals (see above) correlated with decreased food consumption.

Ophthalmoscopy: Ophthalmologic examinations were performed pre-dosing and during week 4 of dosing. No test article related effects were noted.

EKG: Electrocardiography testing was performed on days pre-dosing and during week 4 of dosing, and during week 4 of the recovery period. No test article related effects were noted.

Hematology: Blood samples were collected via the femoral vein pre-dose and during Weeks 2 and 4 of dosing and Week 4 of recovery. Findings in high dose animals included decreases in reticulocytes (5-fold) and neutrophil (2-fold) and increased fibrinogen (2-fold) in one male, and decreases (~10%) in the red blood cell count in four females. These findings were considered to be secondary to the deteriorated physical condition of the animals.

No significant hematological findings were noted during week 4 of the recovery period.

Clinical chemistry: Blood samples were collected via the femoral vein pre-dose and during Weeks 2 and 4 of dosing and Week 4 of recovery. There were no toxicologically significant findings. Slight

changes in clinical chemistry parameters were considered to be secondary to the deteriorated physical condition of the animals.

No significant changes in clinical chemistry parameters were noted during week 4 of the recovery period.

Urinalysis: Urine was collected pre-dose and during Weeks 2 and 4 of dosing and Week 4 of recovery. Decreases in chloride excretion level were noted in males (~30%) and females (~60%) in the high dose group.

Gross pathology: No remarkable effects were noted.

Organ weights: The following organs were weighed at necropsy: Lungs, submandibular glands, pancreas, liver, heart, kidneys, testes, epididymes, prostate, seminal vesicles, ovaries, uterus, brain, spleen, thymus, and adrenals. No remarkable effects were noted.

Histopathology:

Adequate Battery: yes
Peer review: yes

Histopathological findings in the 100 mg/kg group included: inflammatory cells infiltration in the lamina propria of the cecum, colon and rectum in both sexes; cell debris from the crypts of the cecum and colon in males; atrophy of the thymus, acinar cells in the pancreas and mucosal epithelium of the cecum and colon in females.

These lesions in the cecum, colon and rectum were similar to those noted in the previously conducted 2-week study (Study No. SG07030) and were therefore considered to be treatment-related.

Atrophy of the thymus was observed in 2 females (slight in one and severe in the other) in the 100 mg/kg group. This finding was noted in animals with markedly decreased body weights and one monkey with severe atrophy of the thymus also exhibited atrophy of acinar cells in the pancreas. Therefore, these atrophic lesions were considered to be associated with malnutrition and not directly related to treatment with the test substance.

These changes were not noted in high dose animals following a one month recovery period.

Toxicokinetics:

Sampling time points were

Day 1: 0.5, 1, 2, 4, 8 and 24 hours post-dosing (6 time points)

Day 6: Pre-dosing and 0.5, 1, 2, 4, 8 and 24 hours post-dosing (7 time points)

Days 15 and 30: Pre-dosing and 0.5, 1, 2, 4, 8 and 24 hours post-dosing (7 time points)

Table 6: Toxicokinetic Parameters of ERC-349572 in Male Monkeys

Day	Dose mg/kg		AUC _{0-24hr} µg·hr/mL	C _{max} µg/mL	T _{max} hr	C _{24hr} µg/mL
1	25	Mean	60.31	8.70	1.7	0.43
		SD	12.34	0.36	0.6	0.08
		N	3	3	3	3
	50	Mean	72.87	9.64	1.7	0.68
		SD	8.30	1.00	0.6	0.40
		N	3	3	3	3
	100	Mean	99.00	12.11	1.6	0.57
		SD	23.66	1.88	0.5	0.23
		N	5	5	5	5
15	25	Mean	116.21	15.34	3.3	0.83
		SD	23.05	2.48	1.2	0.25
		N	3	3	3	3
	50	Mean	120.35	14.53	1.7	0.78
		SD	55.22	5.46	0.6	0.30
		N	3	3	3	3
	100	Mean	109.79	14.30	1.8	0.79
		SD	28.85	1.65	0.4	0.68
		N	5	5	5	5
30	25	Mean	108.30	13.92	2.0	0.56
		SD	29.62	3.43	0.0	0.23
		N	3	3	3	3
	50	Mean	111.32	14.45	2.0	0.89
		SD	28.32	3.27	0.0	0.35
		N	3	3	3	3
	100	Mean	148.41	15.95	2.2	1.66
		SD	102.87	4.71	1.1	1.79
		N	5	5	5	5

Table 7: Toxicokinetic Parameters of ERC-349572 in Female Monkeys

Day	Dose mg/kg		AUC _{0-24hr} µg·hr/mL	C _{max} µg/mL	T _{max} hr	C _{24hr} µg/mL
1	25	Mean	67.88	11.15	1.3	0.47
		SD	3.11	2.11	0.6	0.08
		N	3	3	3	3
	50	Mean	68.74	10.66	1.7	0.40
		SD	7.74	0.85	0.6	0.01
		N	3	3	3	3
	100	Mean	90.18	12.15	2.8	0.74
		SD	30.97	5.04	1.1	0.41
		N	5	5	5	5
15	25	Mean	111.60	18.83	1.7	0.60
		SD	19.69	3.70	0.6	0.24
		N	3	3	3	3
	50	Mean	104.17	16.00	2.0	0.42
		SD	37.54	5.54	0.0	0.09
		N	3	3	3	3
	100	Mean	128.93	18.19	2.4	0.84
		SD	31.69	4.56	0.9	0.30
		N	5	5	5	5
30	25	Mean	83.93	15.38	1.7	0.34
		SD	18.69	2.72	0.6	0.13
		N	3	3	3	3
	50	Mean	153.17	20.38	3.3	0.50
		SD	69.21	8.63	1.2	0.10
		N	3	3	3	3
	100	Mean	92.04	13.39	2.4	0.64
		SD	30.13	4.34	0.9	0.30
		N	5	5	5	5

Study title: Nine-Month Oral Toxicity Study of S-349572 sodium in Monkeys**Key study findings:**

- The GI tract was identified as the target organ system (local intolerance).
- The NOAEL was defined as the middle dose, 15 mg/kg, corresponding to day 270 AUC values of 37 and 41 $\mu\text{g}\cdot\text{hr}/\text{mL}$ in males and females, respectively.

Cynomolgus monkeys were dosed with 0, 3, 10, 15, or 50 (30) mg/kg via oral gavage for nine months. In the high dose group, GI-related clinical signs and deteriorating health of animals led to euthanasia or death of two male monkeys and a dose reduction on Day 70 from 50 mg/kg to 30 mg/kg. One high dose male and one high dose female were given a dose holiday from day 70 to day 85 (female) or day 70 to day 89 (male).

GI effects were considered to be due to local intolerance, and not a result of systemic exposure to test article. The NOAEL was defined as the middle dose, 15 mg/kg, (corresponding to day 270 AUC values of 37 and 41 $\mu\text{g}\cdot\text{hr}/\text{mL}$ in males and females, respectively) due to gastrointestinal intolerance with gross pathology and histopathology seen at the high dose (30/50 mg/kg).

Study no.:

SBL055-082

Volume # and page #:

vol. 1 p. 1

Conducting laboratory and location:

(b) (4)

Date of study initiation:

May 15, 2008

GLP compliance:

yes

QA report:

yes

Drug, lot #, and % purity:

A7Z001 (98.1%) and B86001 (98.9%)

Methods**Doses:**

0, 3, 10, 15, 50 (30)

Species/strain:

Cynomolgus monkey

Number/sex/group or time point (main study):

9 (control and high dose) or 7 (middle doses)

Route, formulation, volume, and infusion rate:

Suspension; 0.5% HPMC w/ Tween 80; 10 mL/kg via nasogastric gavage

Satellite groups used for toxicokinetics or recovery:

2 (control and high dose)

Age:

3 – 4 years

Weight:

males: 2.87 to 4.47 kg

females: 2.26 to 3.41 kg

Results:

Mortality: Animals were checked for morbidity/mortality three times daily during the dosing phase. One high dose male was sacrificed moribund on day 55 of dosing, and one high dose male died on day 59 of dosing. These deaths were considered to be related to local effects of Dolutegravir on the GI tract.

Clinical signs: Clinical signs were assessed three times daily during the dosing phase. The high dose male that was sacrificed moribund had diarrhea and/or loose stool from day 5 of dosing, and no stool on day 52. The high dose male that died on day 59 had diarrhea/loose stool from day 18 of dosing, and absent stool on day 55. Both of the animals exhibited excessive salivation from day 25 or 28 immediately after dosing. Additional clinical signs began on or after day 48 of dosing in the euthanized animal and day 50 in the animal that died. Observations included vomiting, decreased activity, abnormal position, suppressed response to stimulation with hypothermia and bradypnea.

Similar clinical signs (diarrhea and/or soft stool, continuous or sporadic salivation, vomiting, emaciation and decreased spontaneous activity) were noted in the surviving male (1) and female (1) high dose animals.

Diarrhea and/or loose stool were noted in one male in the middle (15 mg/kg/day) dose group (days 70 – 104).

There were no remarkable clinical signs in the 3 and 10 mg/kg/day dose groups.

Body weights: Body weights were recorded once weekly during the dosing phase. Decreased body weights were noted in both animals that did not survive to terminal sacrifice. In addition, one high dose male and one high dose female had lower body weights compared to pre-dosing values starting at day 42 (female) and on days 70 and 77 (male). No effects on body weight were noted in other dose groups, and no effects were noted at the end of the recovery phase.

Food consumption: Food consumption was assessed daily. The decreased body weights noted in a few animals (see above) correlated with decreased food consumption.

Ophthalmoscopy: Ophthalmologic examinations were performed pre-dosing and on days 23, 113, and 259 of dosing. No test article related effects were noted.

EKG: Electrocardiography testing was performed on days 22, 112, 172, and 258 of dosing, and on day 22 of the 30 day recovery period. No test article related effects were noted.

Hematology: Blood samples were collected via the femoral vein pre-dose and on days 30, 69, 120, 180, and 266 of dosing. There were no remarkable dose-related effects on hematology parameters.

Clinical chemistry: Increased AST (2.5X) and bilirubin (2.8X), increased BUN (12.5X) and creatinine (3.7X), and slight kidney dilatation of distal renal tubules and cellular and hyaline casts were noted in the moribund animal in the 50 mg/kg/day group (euthanized on Day 55). The findings were considered secondary to the moribund condition. There were no other remarkable dose-related effects on clinical chemistry parameters.

Urinalysis: Urine was collected on days 29, 119, 179, and 256 of dosing. No test article effects were noted.

Gross pathology: Red focus in the mucosa of the stomach was noted in one of four high dose females at the end of dosing and one of two high dose females at the end of recovery.

Organ weights: The following organs were weighed at necropsy: Lungs, submandibular glands, pancreas, liver, heart, kidneys, testes, epididymes, prostate, seminal vesicles, ovaries, uterus, brain, spleen, thymus, and adrenals. No remarkable effects were noted.

Histopathology:

Adequate Battery: yes
Peer review: yes

At the end of dosing and recovery, multifocal mononuclear cell infiltration and hemorrhage in the lamina propria, multifocal erosion, and multifocal epithelial regeneration correlated with red focus in the stomach mucosa seen macroscopically.

Toxicokinetics:

Sampling time points were (Day 1): 0.5, 1, 2, 4, 8, and 24 hours after dosing; (Days 30, 69, 83 (30 mg/kg dose group), 99 (1 female in 30 mg/kg dose group), 103 (1 male in 30 mg/kg dose group), 120, 180 and 270): pre-dose and 0.5, 1, 2, 4, 8, and 24 hours after dosing. The time to maximum concentration (T_{max}) was approximately two hours. Increases in C_{max} and AUC were less than dose-proportional, with no significant sex differences.

Table 8: Systemic Exposure Following Nine-Month of Oral Administration of S-349572 sodium in Monkeys

AUC _{0-24hr} (µg·hr/mL)		Dose Level (mg/kg/day)					
		0	3	10	15	30 ^{a)}	50 ^{a)}
Male	Day 1	NE (9)	15.24 ± 3.93 (7)	30.71 ± 7.59 (7)	46.42 ± 9.92 (7)	NS	62.86 ± 12.78 (9)
	Day 30	NE (9)	19.12 ± 4.00 (7)	32.01 ± 8.95 (7)	42.62 ± 9.23 (7)	NS	67.25 ± 13.10 (9)
	Day 69	NE (9)	17.88 ± 6.79 (7)	31.34 ± 5.27 (7)	46.23 ± 10.79 (7)	NS	75.14 ± 29.78 (7)
	Day 83	NE (9)	NS	NS	NS	49.24 ± 9.87 (6)	NS
	Day 120	NE (9)	16.30 ± 6.00 (7)	36.65 ± 7.09 (7)	45.25 ± 9.85 (7)	58.36 ± 10.32 (7)	NS
	Day 180	NE (6)	18.95 ± 8.25 (4)	31.61 ± 10.84 (4)	40.62 ± 11.21 (5)	62.67 ± 12.21 (5)	NS
	Day 270	NE (6)	18.87 ± 6.36 (4)	32.28 ± 9.06 (4)	36.67 ± 4.30 (5)	61.65 ± 18.93 (5)	NS
Female	Day 1	NE (9)	15.30 ± 7.62 (7)	34.48 ± 6.63 (7)	30.59 ± 6.90 (7)	NS	63.42 ± 13.56 (9)
	Day 30	NE (9)	17.24 ± 5.64 (7)	39.06 ± 13.34 (7)	51.32 ± 9.22 (7)	NS	66.47 ± 28.33 (9)
	Day 69	NE (9)	15.62 ± 4.97 (7)	44.85 ± 11.78 (7)	63.81 ± 16.08 (7)	NS	66.11 ± 25.17 (9)
	Day 83	NE (9)	NS	NS	NS	44.86 ± 15.51 (8)	NS
	Day 120	NE (9)	16.51 ± 6.25 (7)	51.00 ± 21.63 (7)	75.31 ± 17.53 (7)	68.60 ± 24.45 (9)	NS
	Day 180	NE (6)	14.45 ± 4.45 (4)	38.54 ± 15.92 (4)	41.68 ± 3.59 (5)	43.50 ± 6.04 (6)	NS
	Day 270	NE (6)	15.46 ± 3.37 (4)	37.65 ± 12.23 (4)	40.88 ± 10.62 (5)	61.70 ± 19.88 (6)	NS

Figures in parentheses were expressed number of animal

a): Dose level of 50 mg/kg/day from Day 1 to 69, 30 mg/kg/day from Day 70 until termination

NE: Not Estimated

NS: No Sample

Study title: Preliminary Carcinogenicity Study (gavage) of S-349572 sodium in Mice for 13 weeks

Key study findings

- The stomach was identified as a target organ in mice.
- The NOAEL was defined as 500 mg/kg based on signs of stomach irritation and/or inflammation. Corresponding systemic exposures were 1010 µg·hr/mL and 1300 µg·hr/mL in males and females, respectively.

Mice were administered 10, 50, 500, or 1500 mg/kg/day S-349572 via oral gavage for 13-weeks. At the high dose, alkaline phosphatase and aspartate aminotransferase were increased (< 2-fold) without histopathological correlate. Microscopically, increased mucous neck cells were noted in high dose animals, suggestive of stomach irritation or inflammation.

Study no.: 09-2119
Volume #, and page #: Vol 1/page 1
Conducting laboratory: (b) (4)

Date of study initiation: April 28, 2009
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity: B86001 (98.9%) (test article supplied by Shionogi & Co., Ltd)

Methods

Doses: control, 10, 50, 500, 1500 mg/kg/day
Species/strain: Mouse/CD-1 (ICR)BR
Number/sex/group: 10
Route, formulation, volume: Oral gavage, 0.5% w/w% hydroxypropyl methylcellulose aqueous solution with 0.1% w/w% Tween 80 / Suspension, 10 mL/kg. Stability was confirmed by analysis of the low and high dose formulations on days 4 and 7 after preparation. Concentrations were found to be acceptable. Homogeneity was confirmed prior to initiation of the study.

Satellite groups used for toxicokinetics or recovery: 18/sex/dose/timepoint (9 controls)
Age: 7 weeks
Weight: Male 18.8 to 36.0 g Female 14.9 to 27.7 g

Protocol deviations: Dose confirmation assays during week 13 indicated that Group 2 and Group 3 doses were 81.3 and 86.7% of nominal, respectively, greater than the $\pm 10\%$ deviation allowed by the protocol. Day 1 and Day 28 assays were within the allowable range. Toxicokinetic parameters were determined using the assay results for week 13 Groups 2 and 3.

Results:

Mortality: Toxicity group animals were observed for morbidity/mortality and clinical signs twice daily. Physical examinations were conducted weekly. There were two unscheduled deaths in the toxicokinetic animals, one middle-high dose male (day 18) and one high dose female (day 28).

Clinical signs: There were no clinical signs related to test article administration.

Body weights: Body weight measurements were collected weekly during dosing. There were no dose-related changes in body weight related to test article administration.

Food consumption: Food consumption was measured weekly during dosing. There were no dose-related changes in food consumption related to test article administration.

Hematology: Blood was collected from toxicity group animals at terminal necropsy for assessment of hematological and clinical chemistry parameters. There were no findings attributed to treatment with test article.

Clinical chemistry: Blood was collected from toxicity group animals at terminal necropsy for assessment of clinical chemistry parameters. Several changes (increases) to clinical chemistry parameters were noted in high dose animals, although all changes were < 2-fold compared to controls (see table below, *excerpted from sponsor*).

Table 9: Clinical Chemistry Changes in Mice

Dose	Males		Females			
mg/kg/day	ALKP	TBILI	AST	K	ALT	ALKP
1500	1.47x	1.44x	1.45x	1.11x	1.78x	1.14x

ALKP: Alkaline Phosphatase; TBILI: Total Bilirubin; AST: Aspartate Aminotransferase;
K: Potassium; ALT: Alanine Aminotransferase.

Gross pathology: Necropsies were performed on animals one day after the end of the dosing. There were no macroscopic findings.

Organ weights (See histopathology table for weighed tissues): There were no findings attributed to treatment with test article.

Histopathology: Adequate Battery: yes
Peer review: yes

In the glandular mucosa of the stomach, mucous neck cells with occasional mucosal and/or submucosal eosinophilic and lymphocytic infiltrates seen in high dose males and females were indicative of adaptation to irritation.

Table 10: Test Article-related Microscopic Findings

	M		F	
S-349572 sodium mg/kg/day	0	1500	0	1500
No. Examined	10	10	10	10
Stomach:				
<i>Increased mucous neck cells, glandular mucosa</i>				
Minimal	0	6	0	7
Slight	0	2	0	2
Total Incidence	0	8	0	9

Toxicokinetics: Blood for determination of toxicokinetic parameters was collected on Day 1, 28 and during Week 13 of Dosing: 1, 2, 4, 6, 10, and 24 hours after dosing. Toxicokinetic parameters are presented in the table below (*excerpted from sponsor*).

Table 11: Toxicokinetic Parameters of Dolutegravir following Oral Administration to Mice

Day	Sex	Group	Nominal S-349572 Dose ^a (mg/kg/day)	Actual S-349572 Dose (mg/kg/day)	AUC _{0-24h} (µg•h/mL)	C _{max} (µg/mL)	t _{max} (h)
1	Male	2	10	9	211	16.0	4
		3	50	47	477	43.9	4
		4	500	488	923	77.3	6
		5	1500	1472	1440	109	1
	Female	2	10	9	212	19.3	1
		3	50	47	528	53.7	4
		4	500	488	1110	88.3	4
		5	1500	1472	1420	114	1
28	Male	2	10	9	193	19.7	6
		3	50	46	634	51.0	6
		4	500	476	1040	83.4	4
		5	1500	1542	1250	89.3	2
	Female	2	10	9	270	24.6	4
		3	50	46	602	65.3	4
		4	500	476	1280	89.4	4
		5	1500	1542	1410	116	2
85	Male	2	10	8	257	18.5	1
		3	50	43	653	52.7	4
		4	500	458	1010	82.1	4
		5	1500	1406	1320	103	1
	Female	2	10	8	256	28.0	1
		3	50	43	740	62.6	4
		4	500	458	1300	109	2
		5	1500	1406	1350	118	1

^aAnimals were dosed S-349572 sodium (correction factor = 1.07)

7 GENETIC TOXICOLOGY

Dolutegravir was not mutagenic or genotoxic in the *in vitro* bacterial reverse mutation (Ames) assay, the *in vitro* mammalian forward mutation (mouse lymphoma) assay, and the *in vivo* micronucleus test in mice (see Appendix 1 for review by Dr. KM Wu).

8 *CARCINOGENICITY*

Study title: Carcinogenicity Study (Gavage) of S-349572 Sodium in Mice for 104 Weeks

Study no.:	09-2177
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11 March 2010 (Date Study Director signed the Protocol)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-349572 sodium, lot B86001 (98.9%), lot 091001 (100%) as supplied
CAC concurrence:	On February 2, 2010 the CAC recommended doses of 0 (water), 0 (vehicle), 7.5, 25 and 500 mg/kg/day by oral gavage, based on saturation of absorption and concern over GI effects over the long term. Dose spacing is based on AUC.

Key Study Findings

- S-349572 administered orally to mice for up to 2 years had no effect on survival.
- There were no S-349572- related clinical signs or effects on body weight, food consumption and hematology.
- There were no neoplastic or nonneoplastic findings attributed to S-349572.
- The no observed adverse effect level (NOAEL) for non-neoplastic findings after chronic oral administration was the high dose of 500 mg/kg/day.
- The steady state (Day 182) AUC_{0-24h} values for the high dose groups were 953 and 1210 µg.h/mL for males and females, respectively, which were approximately 20-fold the mean estimated steady-state human AUC_{0-24h} of 54 µg.h/mL (50 mg qd to adults), respectively.

Adequacy of Carcinogenicity Study

The doses used for this study were appropriately selected based on saturation of absorption.

Appropriateness of Test Models

The mouse is an animal model commonly utilized in carcinogenicity studies. A historical data base is available for comparative evaluation, including a previous 13-week preliminary study of S-349572 sodium.

Evaluation of Tumor Findings

No tumor incidences in male or female mice satisfied the appropriate criteria to be described as statistically significant.

Methods

Doses:	0 (water), 0 (0.5% HPMC/0.1% Tween 80), 7.5, 25, or 500 mg/kg/day
Frequency of dosing:	Once per day
Dose volume:	10 mL/kg/day
Route of administration:	Gavage
Formulation/Vehicle:	0.5% HPMC/0.1% Tween 80
Basis of dose selection:	The high dose, 500 mg/kg/day, was chosen based on saturation of absorption between 500 mg/kg/day and 1500 mg/kg/day, and concern for gastrointestinal intolerance (observed in rats and monkeys) which may have manifested over time.
Species/Strain:	Albino Mice Crl:CD-1(ICR)BR
Number/Sex/Group:	65/sex/group
Age:	Approximately 5 weeks
Animal housing:	Animals were single-, pair or triple-housed in elevated, stainless steel, wire mesh cages during the first week of the stabilization period and individually housed thereafter.
Paradigm for dietary restriction:	None
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Toxicokinetic animals (20/sex/Groups 1 and 2; 45/sex/Groups 3-5)
Deviation from study protocol:	Deviations noted were unlikely to affect the validity of study findings.

Observations and Results

Mortality

No test article-related early mortality occurred. There were no statistically significant differences in overall survival of water or vehicle control vs. test article-treated animals.

The percentage of animals surviving at termination is shown in the following table.

Table 12: Percentage of Animals Surviving at Terminal Sacrifice.^a

Dose (mg/kg/day)	0	0	7.5	25	500
Treatment	Water	Vehicle	S-349572	S-349572	S-349572
Males	46.2	44.6	56.9	40.0	40.0
Females	30.8	41.5	35.4	35.4	29.2

^aInitial on-test number of animals = 65/sex/group; terminal sacrifice commenced Week 105 for males and Weeks 102-103 for females.

By both trend and pairwise comparison, there were no statistically significant differences in survival in males and females dosed at 7.5, 25 and 500 mg/kg/day in comparison with water or vehicle

control group. Reduced survival (20) of females at 500 mg/kg/day resulted in cessation of dosing at Week 101. Reduced survival (20) of Group 1 females (water control) resulted in early sacrifice of all surviving females on Weeks 102-103 of the study. All surviving males were euthanized at the end of the study (Week 105). Among the animals whose cause of death was determined, there were no major differences between the different groups. Overall, the most common causes of death were lymphoreticular/hematopoietic neoplasms (males and females).

Table 13: Major Causes of death in found dead or unscheduled sacrifice mice dosed orally with S-349572-sodium for up to 24 months

Dose of S-349572 (mg/kg)	Males					Females				
	0*	0**	7.5	25	500	0*	0**	7.5	25	500
Total number of animals examined	35	36	28	39	39	45	39	42	42	46
Lymphoreticular/Hematopoietic neoplasm	11	13	6	9	7	23	13	9	13	15
Lung neoplasia	4	1	2	4	4	1	2	3	4	2
Undetermined	7	3	6	9	8	3	10	10	6	16
Skin Ulceration/Inflammation	4	10	5	3	6	1	2	4	5	1
Liver neoplasia	4	1	2	3	1	0	0	0	0	0

*water control
**vehicle control

Clinical Signs

There were no test article-related clinical signs.

Body Weights

There were no test article-related effects on body weights.

Feed Consumption

There were no test article-related effects on food consumption.

Gross Pathology

There were no test article-related macroscopic findings. All macroscopic findings occurred sporadically or at a similar incidence in the water control, vehicle control and test article-treated groups. The findings in all groups were generally typical of the background findings expected in this age and strain of mouse.

Histopathology

Peer Review

All protocol-required tissues from 6 males and 6 females in the control groups and 6 males and 6 females in the 500 mg/kg/day groups were examined from survived animal microscopically. The animal numbers are shown below. Additionally, all tumors were reviewed for all animals to confirm the diagnosis and the terminology.

Neoplastic

No tumor incidences in male or female mice satisfied the appropriate criteria to be described as statistically significant.

Nonneoplastic findings

There were no non-neoplastic findings that were considered to be associated with administration of the test article.

Toxicokinetics

Following repeated oral administration of 7.5, 25 and 500 mg/kg/day of S-349572 to male and female mice, the AUC_{0-24h} and C_{max} values of S-349572 on Day 182 were comparable to values on Day 26 (see table below, excerpted from sponsor).

Table 14: Plasma Toxicokinetic Parameters of S-349572 in Male and Female Mice on Days 26 and 182 following Oral (Gavage) Administration.

Day	Sex	Group	S-349572 Dose (mg/kg/day)	AUC _{0-24h} (&g·h/mL)	C _{max} (&g/mL)	T _{max} (h)	t _{1/2} (h)
26	Male	3	7.5	176	13.4	1	3.94
		4	25	579	40.8	4	3.98
		5	500	1180	77.7	6	4.13
	Female	3	7.5	235	20.3	2	4.07
		4	25	565	44.4	1	4.65
		5	500	1300	94.7	1	4.58
182	Male	3	7.5	148	14.5	2	4.85
		4	25	327	27.4	4	4.17
		5	500	953	71.9	1	4.73
	Female	3	7.5	157	16.6	4	4.55
		4	25	494	43.3	6	5.19
		5	500	1210	94.5	2	4.12

No accumulation of S-349572 was observed after repeated oral administration of S-349572 from Day 26 to Day 182 in male and female mice at the examined dose levels. No marked gender differences in exposure to S-349572 were observed on Days 26 and 182 after oral administration of 7.5, 25 and 500 mg/kg/day of S-349572.

Dosing Solution Analysis

Dose confirmation analyses were performed for each formulation (including controls) during weeks 1, 4, 13, 26, 39, 52, 65, 78, 91, and 103, in duplicate. The concentrations of duplicate samples were within –10% of each other and the mean concentrations were within –10% of nominal concentrations, which are both within protocol specified criteria.

Stability of the dosing formulations for Group 3 (0.75 mg/mL) and Group 5 (50 mg/mL) concentrations, under storage conditions used in this study, were determined at time points of 8 and 14 days (at 2-8 °C), and 24 hours (at room temperature). Results showed that the concentrations at each analysis interval were within $\pm 10\%$ of the initial concentrations (Time 0), which are within protocol specified criteria.

Study title: Carcinogenicity Study (gavage) of S-349572 sodium in Rats for 104 Weeks

Study no.:	09-2178
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	01 March 2010 (Date Study Director signed the Protocol)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	S-349572 sodium, lot B86001 (98.9%), lot 091001 100.1%
CAC concurrence:	The CAC met to discuss this protocol on February 2, 2010. The Committee recommended doses of 0 (water), 0 (vehicle), 2, 10, and 50 mg/kg/day by oral gavage, based on MTD (GI irritation and hemorrhage of the glandular stomach mucosa in males at 500 mg/kg/day) and saturation of absorption.

Key Study Findings

- S-349572 administered orally by gavage to rats at doses of 2, 10 and 50 mg/kg/day for up to 2 years had no effect on survival and was not carcinogenic.
- The no observed adverse effect level (NOAEL) for non-neoplastic findings after chronic oral administration was the high dose of 50 mg/kg/day.
- The steady state (Day 182) AUC_{0-24h} values for the high dose groups were 713 and 1140 µg.h/mL for males and females, respectively, which were 13-fold and 21-fold the mean estimated steady-state human AUC_{0-24h} of 54 µg.h/mL (50 mg qd to adults), respectively.

Adequacy of Carcinogenicity Study

The doses used for this study were appropriately selected based on MTD and saturation of absorption.

Appropriateness of Test Models

CD Sprague Dawley rats are an appropriate animal model.

Evaluation of Tumor Findings

No tumor incidences satisfied the appropriate criteria to be described as statistically significant.

Methods

Doses:	0 (water) or 0 (0.5% HPMC/0.1% Tween 80), 2, 10, or 50 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg/day
Route of administration:	Oral gavage
Formulation/Vehicle:	Solution/Aqueous 0.5 w/w% hydroxypropyl methylcellulose (HPMC) solution with 0.1 w/w% Tween 80 (0.5% HPMC/0.1% Tween 80)
Basis of dose selection:	In the 6-month study in rats, the difference in exposure levels between the 500 mg/kg/day dose group and the 50 mg/kg/day dose group was as little as 2 fold; therefore, based on a dosing period of 104 weeks for this study a high dosage of 50 mg/kg/day was chosen as the maximally tolerated dose.
Species/Strain:	Rats/Sprague-Dawley
Number/Sex/Group:	65
Age:	Approximately 7 weeks
Animal housing:	Animals were pair housed in elevated, stainless steel, wire mesh cages during the first week of the stabilization period and individually housed thereafter.
Paradigm for dietary restriction:	Not applicable
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Toxicokinetics
Deviation from study protocol:	Due to the dose formulation batch size requirements necessitating the preparation of 2 batches, single samples from each batch were sampled and analyzed rather than the protocol required duplicate samples from a single batch.

The Week 1 homogeneity and dose concentration did not meet the protocol acceptance criteria ($\pm 10\%$ of the nominal concentration) and was therefore reformulated.

The Group 3, 14 day refrigerated stability analysis concentration (82.3%) was not within the protocol specified criteria of within $\pm 10\%$ of the initial Group 3 concentration (98.8%).

Observations and Results

Mortality

The percentage of animals surviving at termination is shown in the following table (excerpted from sponsor).

Table 15: Percentage of animals surviving at terminal sacrifice. ^a

Dose (mg/kg/day)	0	0	2	10	50
Treatment	Water	Vehicle	S-349572	S-349572	S-349572
Males	38.5	33.8	33.8	38.5	26.2
Females	30.8	41.5	23.1	30.8	26.2

^aInitial on-test number of animals = 65/sex/group; terminal sacrifice commenced Week 104 for males; females were euthanized Week 95.

Mortality was slightly higher in females dosed at 2 and 50 mg/kg/day but survival rates were not dose-dependent and the changes were considered unrelated to test article administration. Reduced survival in females resulted in cessation of dosing on Weeks 88 at 50 mg/kg/day, Week 90 at 2 mg/kg/day and Week 94 at 10 mg/kg/day. All surviving females in Groups 1 through 5 were euthanized on Week 95 of the study.

All males were euthanized beginning Week 104. Among the animals whose cause of death was determined, there were no major differences between dose groups. Overall, the most common causes of death were pituitary tumor (males and females) and mammary gland tumors (females) (see table below, excerpted from sponsor).

Table 16: Primary causes of death.

	Male					Female				
S-349572 (mg/kg)	0	0	2	10	50	0	0	2	10	50
No. unscheduled decedents	40	43	43	40	48	45	38	50	45	48
Pituitary Gland Neoplasms	11	11	16	14	17	30	33	37	30	39
Mammary Gland Neoplasms	0	0	0	0	0	5	3	7	7	3

Clinical Signs

There were no test article-related clinical observations. Clinical signs that were observed are considered common incidental findings in aging rats and unrelated to test article administration.

In both males and females, the overall incidence of palpable masses was similar in test article-treated groups to the water and vehicle control groups.

Body Weights

There were no test article-related effects on body weights.

Feed Consumption

There were no test article-related effects on food consumption.

Gross Pathology

Macroscopic findings were independent of dose, sporadic or due to biologic variation and were not related to S-349572 administration.

Histopathology

Peer Review

All protocol-required tissues from 6 males and 6 females in the control groups and 6 males and 6 females in the 50 mg/kg/day groups were examined microscopically. The animal numbers are shown below. Additionally, all tumors and all incidents of pancreatic islet cell hyperplasia were reviewed for all animals to confirm the diagnoses and the terminology.

Neoplastic

No tumor incidences in male or female rats satisfied the appropriate criteria to be described as statistically significant.

Non Neoplastic

There were no non-neoplastic findings that were considered to be associated with administration of the test article.

Toxicokinetics

Values of the selected toxicokinetic parameters for S-349572 are summarized in the table below (excerpted from sponsor).

Table 17: Values of Plasma Toxicokinetic Parameters of S-349572 in Male and Female Rats on Days 28 and 182 following Oral (Gavage) Administration of S-349572

Day	Sex	Group	S-349572 Dose (mg/kg/day)	AUC _{0-24h} ^a (μg·h/mL)	C _{max} (μg/mL)	T _{max} (h)
28	Male	3	2	101	7.29	2
		4	10	348	24.7	2
		5	50	841	57.6	4
	Female	3	2	114	8.78	1
		4	10	495	33.4	1
		5	50	1170	87.0	4
182	Male	3	2	100	7.71	1
		4	10	340	21.3	6
		5	50	713	40.9	4
	Female	3	2	279	20.4	1
		4	10	731	40.4	1
		5	50	1140	68.5	1

^aAUC_{last} = AUC_{0-24h}

With few exceptions, the AUC_{0-24h} and C_{max} values of S-349572 on Day 182 were comparable to those values on Day 28 after repeated oral administration of 2, 10 and 50 mg/kg/day of S-349572 to male and

female rats. In general, the AUC_{0-24h} and C_{max} values were comparable in males and females (\leq 3-fold difference).

Dosing Solution Analysis

Analysis of dose formulations confirmed that the preparation procedure used for this study produced homogeneous mixtures. Stability of the dosing formulations, under storage conditions used in this study, was determined at time points of 24 hours at room temperature and up to 8 and 14 days refrigerated. Analyses conducted during the treatment period confirmed that dose solutions of appropriate concentration were administered.

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Study title: Dose Range-finding Oral Study for Effects of S-349572 sodium on Embryo-Fetal Development in Rabbits.

Key study findings

- 1000 mg/kg was identified as the appropriate high dose for the pivotal embryo-fetal development study in rabbits.

In this dose range-finding study, female rabbits were dosed from Days 6-18 of gestation, as this period corresponds with implantation to closure of the hard palate of rabbit fetuses and is appropriate for evaluation of the potential adverse effects of the test article on pregnant animals and embryo-fetal development in rabbits. There was an apparent dose-related increase in post-implantation loss (2, 4, 5, 7, in control, low, middle and high dose animals), but the difference was not statistically significant. As in the two-week study in non-pregnant rabbits, 1000 mg/kg was considered to be the appropriate high dose for the pivotal embryo-fetal development study in rabbits.

Study no.:	S-349572-TF-060-L
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 28, 2008
GLP compliance:	yes (b) (4)
QA report:	yes
Drug, lot #, and % purity:	S-349572 sodium, A7Z002, 99.1%

Methods

Doses:	100, 300, 1000 mg/kg
Species/strain:	Rabbits/Kbl:JW/Female
Number/sex/group:	4-5/group
Route, formulation, volume, and infusion rate:	Oral gavage, 0.5 w/w% hydroxypropyl methylcellulose in 0.1 w/w% Tween 80 aqueous solution/Suspension, 5 mL/kg
Parameters and endpoints evaluated:	Gross pathology; evidence of implantation; number of corpora lutea

Results

Mortality: One dam in the middle dose group delivered prematurely and was euthanized.

Clinical signs: Rabbits were examined for clinical signs twice daily during the dosing period. Scant or no feces for 1, 2, 2 and 4 dams and scant or no urine for 0, 2, 2 and 4 dams were observed in the control, 100, 300 and 1000 mg/kg groups, respectively. These signs were associated with decreased food consumption.

Body weight: Statistically significant decreases in body weight gain were noted in the 1000 mg/kg group on Days 7, 8 and 14 of gestation and were considered to be treatment-related.

Feed Consumption: Statistically significant decreases in food consumption were noted in the 1000 mg/kg group on Days 7 and 8 of gestation as compared to the control group. Since all dams in this group exhibited markedly decreased food consumption during the dosing period, the decreases were considered to be treatment-related. Markedly decreased food consumption was evident in 2 dams each in the 100 and 300 mg/kg groups. However, no statistically significant differences from the control group were noted in these groups and the relationship between the decreases and the test article administration was unclear.

Necropsy: No gross lesions were evident in any dam.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

There was an apparent dose-related increase in post-implantation loss (2, 4, 5, 7, in control, low, middle and high dose animals), but the difference was not statistically significant. There were no statistically significant differences between the control and any treated group in the number of corpora lutea, implantations or live fetuses, pre-implantation loss or sex ratio (males/total), body weights or placental weights of live fetuses, indicating no treatment-related effects on these parameters.

Study title: Oral Study for Effects of S-349572 sodium on Embryo-Fetal Development in Rabbits.

Key study findings

- Suppressed body weight gain, decreased food consumption and scant or no feces/urine associated with the decreased food consumption were noted in the 1000 mg/kg group and were considered to be toxicologically significant.
- The no observed adverse effect levels of S-349572 were considered to be 200 mg/kg/day (GD18 AUC = 15 µg.h/mL) for maternal general toxicity and 1000 mg/kg/day (GD18 AUC = 30 µg.h/mL) for maternal reproductive function and embryo-fetal development.

The post-implantation loss in the 1000 mg/kg group in this study was relatively high (14.7%), but the incidence rate fell within the range of historical control values, and was not considered to be treatment related. Suppressed body weight gain, decreased food consumption and scant or no feces/urine associated with the decreased food consumption were noted in the 1000 mg/kg group and were considered to be toxicologically significant. There were no treatment-related effects on the viability, growth or external, visceral or skeletal morphology of fetuses at any dose level. The no

observed adverse effect levels of S-349572 were considered to be 200 mg/kg/day (GD18 AUC = 15 µg.h/mL) for maternal general toxicity and 1000 mg/kg/day (GD18 AUC = 30 µg.h/mL) for maternal reproductive function and embryo-fetal development.

Study no.: S-349572-TF-065-L
Conducting laboratory and location: (b) (4)
Date of study initiation: October 29, 2008
GLP compliance: yes (b) (4)
QA report: yes
Drug, lot #, and % purity: S-349572 sodium, B86001, 98.9%

Methods

Doses: 40, 200, 1000 mg/kg
Species/strain: Rabbits/Kbl:JW/Female
Number/sex/group: 18-20/group (20, 20, 18, and 19 for control, low, middle and high doses)
Route, formulation, volume, and infusion rate: Oral gavage, 0.5 w/w% hydroxypropyl methylcellulose in 0.1 w/w% Tween 80 aqueous solution/Suspension, 5 mL/kg
Design: Dams were dosed between GD6 and 18, and C-sections were performed on GD29.
Parameters and endpoints evaluated: the numbers of corpora lutea, implantations and live and dead fetuses and body weights, placental weights, sex and external, visceral and skeletal morphology of live fetuses.

Results

Mortality: No dams died or became moribund throughout the study period. One dam in the 40 mg/kg group aborted.

Clinical Signs/Body Weight/Feed Consumption: Suppressed body weight gain, decreased food consumption and scant or no feces/urine associated with the decreased food consumption were noted in the 1000 mg/kg group and were considered to be toxicologically significant.

<u>Daily Dose (mg/kg)</u>			<u>0 (control)</u>	<u>40</u>	<u>200</u>	<u>1000</u>
<u>Dams</u>	Body Weights (%) ^b	G7	3.65 kg	0	0	-2
		G19	3.88 kg	-1	-2	-4
	Body Weight Gain from G6	G7	-0.01 kg	0.00 kg	-0.03 kg	-0.10 kg **
		G19	0.22 kg	0.21 kg	0.13 kg *	0.03 kg **
	Food Consumption (%) ^b	G7	201 g/day	+4	-14	-47 **
		G19	199 g/day	0	-7	-18

Dunnett's Test * - $p < 0.05$ ** - $p < 0.01$

Toxicokinetics: Blood samples were collected at 0.5, 1, 2, 4, 8 and 24 hours post-dosing on the first day of dosing (Day 6 of gestation) and pre-dosing (approx. 24 hours post-dosing on Day 17 of

gestation) and 0.5, 1, 2, 4, 8 and 24 hours post-dosing on the last day of dosing (Day 18 of gestation). The mean C_{max} values increased less than dose proportionally between the 40 and 1000 mg/kg groups. The mean AUC_{0-24hr} values increased dose proportionally between the 40 and 200 mg/kg groups, while those values increased less than dose proportionally between the 200 and 1000 mg/kg groups. No obvious repeated dosing effects were observed in the toxicokinetic parameters. See table below (*excerpted from sponsor*).

Table 18: Toxicokinetic Parameters for Dolutegravir following Oral administration to Rabbits

Dose Level (mg/kg/day)		0	40	200	1000
Number of Animals		3	3	3	3
AUC_{0-24hr} ($\mu g \cdot hr/mL$)	Day 6 of gestation	NE	2.1 \pm 0.1	15.6 \pm 1.5	36.8 \pm 14.3
	Day 18 of gestation	NE	2.6 \pm 0.3	14.5 \pm 6.1	30.1 \pm 7.0
C_{max} ($\mu g/mL$)	Day 6 of gestation	NE	0.8 \pm 0.1	1.5 \pm 0.3	2.3 \pm 0.4
	Day 18 of gestation	NE	1.3 \pm 0.1	1.7 \pm 0.3	2.1 \pm 0.6
T_{max} (hr)	Day 6 of gestation	NE	0.5 \pm 0.0	1.2 \pm 0.8	3.5 \pm 4.0
	Day 18 of gestation	NE	0.7 \pm 0.3	0.7 \pm 0.3	0.8 \pm 0.3
C_{24hr} ($\mu g/mL$)	Day 6 of gestation	NE	NE	NE	1.1 \pm 1.2
	Day 18 of gestation	NE	NE	NE	0.9 \pm 0.2

NE: Not Estimated

Necropsy: No gross lesions were evident in any dam.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

The post-implantation loss in the 1000 mg/kg group in this study was relatively high (14.7%). According to the test facility, the post-implantation loss in the negative control groups (31 groups) in the embryo-fetal developmental studies in the same strain of rabbits conducted at the test facility in the years 1999-2008 ranged from 4.1% to 19.2%. Therefore, the increased post-implantation loss in this group was within the range of spontaneous occurrence and was considered not to be treatment-related.

No treatment-related effects were evident on the viability, growth or external, visceral or skeletal morphology of fetuses at any dose level.

Study title: Oral Study for Effects of S-349572 Sodium on Fertility and Early Embryonic Development to Implantation in Rats

Key study findings

- Based on the lack of general toxicology and reproductive (fertility and early embryonic development) toxicology findings in rats, the no observed adverse effect level (NOAEL) of S-349572 sodium was considered to be 1000 mg/kg/day. Toxicokinetic parameters were not assessed.

The purpose of this study was to evaluate the potential adverse effects of S-349572 sodium on mating, fertility and early embryonic development in rats. Rats were administered S-349572 sodium at dose levels of 0 (control), 100, 300 and 1000 mg/kg/day via oral gavage from 28 days prior to mating, throughout the mating period, until 1 day prior to necropsy (63-66 days in total) for males and from 14 days prior to mating, throughout the mating period, until Day 7 of gestation for females (up to 42 total days of dosing).

Study no.:	S-349572A-TF-063-L
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 1, 2008
GLP compliance:	yes (b) (4)
QA report:	yes
Drug, lot #, and % purity:	S-349572 sodium, B86001, 98.9%

Methods

Doses:	0, 100, 300, 1000 mg/kg
Species/strain:	Rat/Crl:CD(SD)
Number/sex/group:	20 rats/group
Route, formulation, volume, and infusion rate:	Oral gavage, 0.5 w/w% hydroxypropyl methylcellulose in 0.1 w/w% Tween 80 aqueous solution 10 mL/kg
Study design:	Once daily gavage 28 days prior to mating, throughout the mating period, until 1 day prior to necropsy (63-66 days in total) for males and from 14 days prior to mating, throughout the mating period, until Day 7 of gestation for females (up to 42 total days of dosing).
Parameters and endpoints evaluated:	sperm concentration and motility and Caesarean-sectioning observations.

Results

Mortality: Rats were observed twice daily for morbidity/mortality. All animals survived to scheduled necropsy.

Clinical signs: No significant clinical observations were noted.

Body weight: Body weights were recorded twice weekly. There were no treatment-related effects on body weight.

Feed Consumption: Feed consumption was recorded on days that body weights were measured. There were no treatment-related effects on food consumption.

Necropsy: There were no test article-related findings.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): No test article-related effects were observed in mating or fertility parameters.

Study title: Oral Study for Effects of S-349572 sodium on Embryo-Fetal Development in Rats

Key study findings

- No significant toxicology
- The NOAEL is defined as 1000 mg/kg/day for dams and embryo-fetal development: Corresponding exposures (AUC) on gestation day 17 were 2032 µg.h/mL.

S-349572 sodium was orally administered to pregnant rats on Gestation Days 6 to 17 at doses of 0, 100, 300 or 1000 mg/kg/day to detect adverse effects of S-349572 sodium on pregnant dams and embryo-fetal development in rats. The systemic exposure of S-349572 to pregnant rats was also evaluated. Based on the lack of test article related results, it is concluded that S-349572 sodium had no adverse effects on dams and fetal viability, teratogenicity and intrauterine growth at doses as high as 1000 mg/kg/day in this study. Therefore, the no observed adverse effect level of S-349572 sodium under conditions of this study is estimated to be 1000 mg/kg/day for dams and embryo-fetal development: Corresponding exposures (AUC) on gestation day 17 were 2032 µg.h/mL.

Study no.:

S-349572-TB-062-L

Conducting laboratory and location:

(b) (4)

Date of study initiation:

September 17, 2008

GLP compliance:

yes (b) (4)

QA report:

yes

Drug, lot #, and % purity:

S-349572 sodium, B86001, 98.9%

Methods

Doses:

0, 100, 300, 1000 mg/kg

Species/strain:

Rat/Crl:CD(SD)

Number/sex/group:

20 rats/group

Route, formulation, volume, and infusion rate:

Oral gavage, 0.5 w/w%
hydroxypropyl methylcellulose in 0.1 w/w%
Tween 80 aqueous solution 10 mL/kg
Once daily gavage dosing on gestation days 6 to 17

Study design:

Parameters and endpoints evaluated:

Clinical signs, body weight, food consumption and Caesarean-sectioning observations.

Results

Mortality: Rats were observed twice daily for morbidity/mortality. All animals survived to scheduled necropsy.

Clinical signs: No significant clinical observations were noted.

Body weight: Body weights were recorded twice weekly. There were no treatment-related effects on body weight.

Feed Consumption: Feed consumption was recorded on days that body weights were measured. There were no treatment-related effects on food consumption.

Necropsy: There were no test article-related findings.

Fetal parameters: No test article-related effects were observed in fetal viability, sex ratio, fetal body weight, placental weight, external morphology, gross findings of placentae of live fetuses, visceral and skeletal morphology and degree of skeletal ossification.

Toxicokinetics

Table 19: Toxicokinetic Parameters for Dolutegravir following Oral administration to Pregnant Rats

Gestation Day	Dose level mg/kg/day		AUC _{0-24hr} (µg·hr/mL)	C _{max} (µg/mL)	T _{max} (hr)	C _{24hr} (µg/mL)
Day 6	100	Mean	948.7	64.1	3.0	14.4
		SD	203.7	10.4	1.2	5.3
		N	4	4	4	5
	300	Mean	1096.4	74.0	4.0	11.8
		SD	129.9	10.8	0.0	3.1
		N	5	5	5	5
	1000	Mean	1840.9	138.5	2.0	32.2
		SD	340.8	10.0	0.0	23.9
		N	5	5	5	5
Day 17	100	Mean	1251.8	78.0	5.6	21.8
		SD	238.0	14.7	3.0	5.7
		N	5	5	5	5
	300	Mean	1409.2	82.2	3.8	22.2
		SD	228.1	16.4	2.3	8.0
		N	5	5	5	5
	1000	Mean	2031.8	108.9	4.8	48.7
		SD	278.4	8.8	3.0	23.1
		N	5	5	5	5

Study title: Oral Study for Effects of S-349572 sodium on Pre- and Postnatal Development, Including Maternal Function, in Rats

Key Study Findings

- Noted effects included suppressed body weight gain and decreased food consumption in dams (F0) and decreased body weights in the subsequent generation (F1) at 1000 mg/kg.
- NOAELs were considered to be 50 mg/kg/day for maternal general toxicity (F0) and for development of the subsequent generation (F1).
- The NOAEL was defined as 1000 mg/kg/day for maternal reproductive functions (F0) such as maintenance of pregnancy, delivery and nursing.

F0 dams:

Female rats were administered 0, 5, 50, or 1000 mg/kg S-349572 sodium from Day 6 of gestation to Day 20 of lactation. No death occurred in any dam (F0). Suppressed body weight gain and decreased food consumption were noted in dams (F0) in the 1000 mg/kg group during the early stages of the lactation period. No treatment-related effects were noted at clinical observation or gross pathology or on the duration of gestation, gestation index, delivery or nursing behavior of any dam (F0).

F1 offspring:

Decreased body weights were noted in the subsequent generation (F1) in the 1000 mg/kg group from pre-weaning until adolescence. No treatment-related toxic effects were noted on any other parameters in this group.

Study no.:	GlaxoSmithKline Study No. SGI0306
Study report location:	Electronic (eCTD SN 0265)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 15, 2011
GLP compliance:	Yes (b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	S-349572 sodium (S-349572B), lot no. 091001, 101.1%

Methods

Doses: 0, 5, 50, 1000 mg/kg
 Frequency of dosing: Once daily
 Route of administration: Oral
 Formulation/Vehicle: 0.5 w/w% hydroxypropyl methylcellulose in 0.1 w/w% Tween 80 aqueous solution/Suspension
 Species/Strain: Rats/Crl:CD(SD)/Female
 Number/Sex/Group: 0 (22 pregnant), 5 (22), 50 (21), 1000 (20)

Observations and Results

F₀ Dams

Effects were limited to decreased food consumption and body weights during lactation.

<u>Daily Dose (mg/kg)</u>		<u>0 (control)</u>	<u>5</u>	<u>50</u>	<u>1000</u>
<u>F₀ Females:</u>	No. Pregnant	22	22	21	20
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	—	—	—	—
	Necropsy Observations	—	—	—	—
	Gestation Body Weights (% ^a)	G20 396 g	+2	+2	-1
	Body Weight Gains from G6	G20 119 g	121 g	123 g	116 g

—: No noteworthy findings

G: Gestation day

L: Lactation day

a - For controls, group means are shown. For treated groups, percent differences from controls are shown.

Statistically significant decreases in the body weight gain from values at Day 0 of lactation were noted in the 1000 mg/kg group on Days 4 and 7 of lactation as compared to the control group, indicating suppressed body weight gain during the lactation period. These changes correlated with statistically significant decreases in food consumption were noted in the 1000 mg/kg group on Days 4 and 7 of lactation as compared to the control group, indicating effects of treatment with the test article.

<u>Daily Dose (mg/kg)</u>		<u>0 (control)</u>	<u>5</u>	<u>50</u>	<u>1000</u>
<u>F₀ Females:</u>	Lactation Body Weights (% ^a)	L4 310 g	+4	+2	-3
	Lactation Body Weights (% ^a)	L7 322 g	+2	+1	-2
	Lactation Body Weights (% ^a)	L21 311 g	+1	+1	+2
	Body Weight Gains from L0	L4 18 g	21 g	14 g	-4 g **
	Body Weight Gains from L0	L7 29 g	30 g	25 g	10 g **
	Body Weight Gains from L0	L21 19 g	15 g	13 g	13 g
	Gestation Food Consumption (% ^a)	G20 24.1 g/day	+2	+2	-3
	Lactation Food Consumption (% ^a)	L4 28.7 g/day	+5	+5	-14 *
	Lactation Food Consumption (% ^a)	L7 45.3 g/day	-4	-2	-8 *
	Lactation Food Consumption (% ^a)	L21 67.9 g/day	-5	-3	-6
	Mean Duration of Gestation (days)	21.8	21.9	21.8	22.0
	No. Delivery (Gestation Index, %)	22 (100)	22 (100)	21 (100)	20 (100)
	Abnormal Parturition	—	—	—	—

F₁ Generation

Effects were limited to decreased body weights in offspring.

<u>F₁ Litters:</u>	No. Litters Evaluated	22	22	21	20
<u>(Pre-weaning)</u>	No. of Total Litter Losses	1	1	0	0
	Mean No. Pups/Litter	14.3	14.0	14.5	14.3
	Mean No. Liveborn Pups/Litter	13.9	13.5	14.2	13.7
	External Malformations (No. of Pup)	0	0	0	1 ^b

—: No noteworthy findings G: Gestation day L: Lactation day Dunnett's Test * - $p < 0.05$ ** - $p < 0.01$

a - For controls, group means are shown. For treated groups, percent differences from controls are shown.

Statistical significance is based on actual data (not on the percent differences).

b - Multiple malformations (meningocele and absent eye bulge)

Statistically significant decreases were noted in the body weights of female offspring (F₁) in the 1000 mg/kg group at 11, 14, 18 and 21 days of age as compared to the control group. In addition, body weights tended to decrease in male offspring (F₁) in the same group at the same intervals, though not statistically significant. All changes in body weights were considered to be treatment-related.

<u>Daily Dose (mg/kg)</u>	<u>0 (control)</u>	<u>5</u>	<u>50</u>	<u>1000</u>
<u>F₁ Litters:</u>	No. Dead Newborns	9	11	6
<u>(Pre-weaning)</u>	Mean % Postnatal Survival to L4	94.0	94.5	99.4
	Mean % Postnatal Survival to Weaning	100	100	100
	Mean Pup Body Weights (g) L0 Male	6.53	6.63	6.66
	Mean Pup Body Weights (g) L0 Female	6.14	6.30	6.24
	Mean Pup Body Weights (g) L11 Male	29.1	29.4	29.3
	Mean Pup Body Weights (g) L11 Female	28.0	28.3	28.1
	Mean Pup Body Weights (g) L14 Male	37.8	38.1	37.7
	Mean Pup Body Weights (g) L14 Female	36.5	36.6	36.3
	Mean Pup Body Weights (g) L18 Male	48.6	48.2	48.0
	Mean Pup Body Weights (g) L18 Female	47.0	46.3	46.1
	Mean Pup Body Weights (g) L21 Male	61.4	61.1	60.5
	Mean Pup Body Weights (g) L21 Female	59.1	58.7	57.9
	Mean Pup Sex Ratios (% Males)	48.1	54.0	49.7
	Pup Clinical Signs	—	—	—
	Physical Development ^c	—	—	—
	Early Behavior ^d	—	—	—
	Pup Necropsy Observations	—	—	—

—: No noteworthy findings L: Lactation day Dunnett's Test * - $p < 0.05$ ** - $p < 0.01$

c - Pinna unfolding, growth of abdominal hair, eruption of upper incisor and eyelid opening

d - Back righting and negative geotaxis

<u>Daily Dose (mg/kg)</u>		<u>0 (control)</u>	<u>5</u>	<u>50</u>	<u>1000</u>
<u>F₁ Males:</u>	No. Evaluated Post-weaning (R, B)	42 (21, 21)	42 (21, 21)	42 (21, 21)	40 (20, 20)
<u>(Post-weaning)</u>	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	—	—	—	—
	Necropsy Observations	—	—	—	—
	Body Weights (g) 28 Days of Age	103	104	102	98
	Body Weights (g) 35 Days of Age	163	166	163	156
	Body Weights (g) 42 Days of Age	232	237	233	222
	Body Weights (g) 70 Days of Age	442	451	449	432
	Sensory Function 4 Weeks of Age ^e	—	—	—	—
	Open Field Test 5 Weeks of Age	—	—	—	—
	Conditioned Avoidance Response ^f	—	—	—	—
	Preputial Separation	—	—	—	—
	Mean No. Days Prior to Mating	3.6	3.3	2.6	3.2
	No. Mated (% Copulation) ^g	20 (95.2)	20 (95.2)	21 (100)	19 (100)
	No. Fertile Males (% Fertility) ^g	20 (100)	19 (95.0)	21 (100)	19 (100)
<u>F₁ Females:</u>	No. Evaluated Post-weaning (R, B)	42 (21, 21)	42 (21, 21)	42 (21, 21)	38 (19, 19)
<u>(Post-weaning)</u>	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	—	—	—	—
	Necropsy Observations	—	—	—	—

<u>Daily Dose (mg/kg)</u>		<u>0 (control)</u>	<u>5</u>	<u>50</u>	<u>1000</u>
<u>F₁ Females:</u>	Body Weights (g) 28 Days of Age	93	93	92	89 *
<u>(Post-weaning)</u>	Body Weights (g) 35 Days of Age	139	139	137	133 *
	Body Weights (g) 42 Days of Age	174	175	174	166 *
	Body Weights (g) 70 Days of Age	261	262	259	250
	Sensory Function 4 Weeks of Age ^e	—	—	—	—
	Open Field Test 5 Weeks of Age	—	—	—	—
	Conditioned Avoidance Response ^f	—	—	—	—
	Opening of Vagina	—	—	—	—
	Mean Days of Estrous Cycle	4.3	4.7	4.1	4.0
	No. Mated (% Copulation) ^g	20 (95.2)	20 (95.2)	21 (100)	19 (100)
	No. Pregnant Females (% Fertility) ^g	20 (100)	19 (95.0)	21 (100)	19 (100)
	Gestation Body Weights (g) G13	365	362	378	348
	Mean No. Corpora Lutea	16.4	16.4	18.0	16.8
	Mean No. Implantations	15.4	15.5	16.2	15.8
	Mean % Pre-implantation Loss	5.5	5.0	8.6	5.8

F₂ Generation

There were no statistically significant differences from the control group in the number of corpora lutea, implantations or live embryos or pre- or post-implantation loss (%) in any treated group.

<u>F₂ Litters:</u>	Mean No. Live Conceptuses/Litter	14.6	14.7	15.5	14.8
	No. Dead Conceptuses ^h	16	15	15	18
	Mean % Post-implantation Loss	5.2	5.1	4.2	6.2

—: No noteworthy findings

G: Gestation day

Dunnett's Test * - $p < 0.05$

e - Visual placing response, pupillary reflex, Preyer's reflex and pain response

f - 7-8 Weeks of Age

g - Results during the 1st (14 days) and 2nd (7 days) mating periods

h - Total of early deaths after implantation, unformed embryos and formed embryos with placenta

10 SPECIAL TOXICOLOGY STUDIES

Study title: DolutegravirA: Oral Toxicity Study in Juvenile Rats

Key study findings: Juvenile rats were administered Dolutegravir (0.5, 2 or 75 mg/kg/day) via oral gavage from Day 4 to 66 postpartum (pp). The deaths of two male pups dosed with 75 mg/kg/day were considered to be test article-related. Additional test article-related effects included decreased body weights primarily in high dose females, and degeneration/regeneration of nasal mucosa across dose groups resulting from direct contact with expelled test article. Those findings are considered to be local effects and not related to systemic exposure to Dolutegravir. The no observed adverse effect level (NOAEL) in juvenile rats was considered to be 2 mg/kg/day. Systemic exposures (AUC_[0-24]) on Day 13 pp were 303 µg.h/mL and 316 µg.h/mL in males and females, respectively. On Day 32 pp the AUC_[0-24] were reduced to 85.7 µg.h/mL and 93.3 µg.h/mL in males and females, respectively.

Study no.:	G09229
Volume #, and page #:	vol. 1 p. 1
Conducting laboratory and location:	GlaxoSmithKline (GSK) Safety Assessment 709 Swedeland Road King of Prussia, PA 19406 USA
Date of study initiation:	25 Jan 2010
GLP compliance:	yes
QA report:	yes
Drug, lot #, and % purity:	DolutegravirA, B87002 (99.6%)

Methods

Doses:	0.5, 2 or 75 mg/kg/day
Species/strain:	Rat/[CrI:CD(SD)]
Number/sex/group or time point (main study):	50 pups/sex/group
Route, formulation, volume, and infusion rate:	Suspension; 0.5% HPMC w/ 0.1% Tween 80; 5 mL/kg via oral gavage
Satellite groups used for toxicokinetics or recovery:	see table below (<i>excerpted from sponsor</i>)

Table 20: Group Designations (table excerpted from sponsor)

Group	# of Litters/ Group	Litter Size		Total # of Pups/Group		Dose (mg/kg/day)	Dose Volume (mL/kg)
		Male	Female	Male	Female		
1	10	5	5	50	50	0	5
2	10	5	5	50	50	0.5	5
3	10	5	5	50	50	2	5
4	10	5	5	50	50	75	5
5	2	5	5	10	10	0 mg/kg/day cyclosporine	10
6	2	5	5	10	10	20 mg/kg/day cyclosporine	10

Note: Dose levels for Groups 1 through 4 are expressed as parent compound, GSK1349572. The vehicle for Groups 1 to 4 was the same. Groups 5 and 6 are the positive control TDAR subset, the vehicle for Groups 5 and 6 was be the same. TDAR subset animals in Groups 1 through 4 and all animals in Groups 5 and 6 received a single dose of 6 mg/kg KLH on Day 45 pp.

Age:

Day 4 to 66 postpartum

Table 21: Observations (table excerpted from sponsor)

Procedure	Frequency	
	Groups 1 through 4	Groups 5 and 6
Viability check	Daily	Daily during dosing
Daily observations	At least once prior to dosing, and once immediately after dosing each group, and again 1 to 2 hours after dosing Note: Clinical signs (e.g. scabs, broken nails, and broken teeth) that have a low probability of resolving during the course of a given day may only be recorded once a day. Existing clinical signs will be monitored daily until the condition is resolved.	At least once prior to dosing, and once immediately after dosing each group, and again 1 to 2 hours after dosing Note: Clinical signs (e.g. scabs, broken nails, and broken teeth) that have a low probability of resolving during the course of a given day may only be recorded once a day. Existing clinical signs will be monitored daily until the condition is resolved.
Detailed clinical observations	Day 21, 28, 66 and 67 pp	Day 21 and 35 pp
Body weight ¹	Daily	Day 7, 14, 21, and 28 pp; and Daily during dosing
Food consumption ¹	Day 28, 34, 41, 48, 55, 62 and 66 pp	Day 28, 34, 41, 48 and 55 pp
Vaginal Perforation ¹	Day 27 pp until evident	Day 27 pp until evident
Balano-Preputial Skinfold Separation ¹	Day 40 pp until evident	Day 40 pp until evident
Immunology: TDAR	KLH administration: Day 45 pp Anti-KLH IgM evaluation: Day 50 pp Anti-KLH IgG evaluation: Day 59 pp	KLH administration: Day 45 pp Anti-KLH IgM evaluation: Day 50 pp Anti-KLH IgG evaluation: Day 59 pp
Immunology: T and B cell enumeration and T cell V beta usage	Day 67 pp	----
Hematology	Day 67 pp	----
Coagulation	Day 67 pp	----
Clinical chemistry	Day 67 pp	----
Urinalysis	Day 67 pp	----
Toxicokinetics	Day 13 and 32 pp	----
Terminal body weight	Day 67 pp	----
Method of euthanasia	Exsanguination after administration of isoflurane for main study necropsy subset Exsanguination after administration of carbon dioxide for toxicokinetic subset animals (on Day 13 and 32 pp) and TDAR subset animals (on Day 60 pp)	Exsanguination after administration of carbon dioxide (on Day 60 pp)
Necropsy ²	Day 67 pp	----
Organ weights	Day 67 pp	----
Bone marrow smears ³	Necropsy	----
Tissue collection	Necropsy	----
Long bone measurement ⁴	Post-necropsy	----
Tissue processing and exam	Post-necropsy	----
Stage-dependent evaluation of spermatogenesis	Post-necropsy	----

Results:

Mortality: Two male pups dosed with 75 mg/kg/day Dolutegravir (GSK572) were found dead, one on day 12 pp and one on day 17 pp. There were no clinical observations, or macroscopic or microscopic findings, and a cause of death was not determined. Due to the two deaths occurring only in high dose group, and given that there were deaths in a previous dose range study (albeit at higher doses), the possible relationship to treatment could not be excluded. Therefore these preweaning deaths are considered test article related. Also, there was one death in the control group that occurred during the toxicokinetic sampling procedure on Day 32 pp.

Clinical signs: Sporadic cases of rats expelling small amounts of test article by mouth following oral gavage were noted across dose groups. This observation was not considered to be due to test article toxicity, as there was no relationship between incidence and dose.

Body weights:

There were test article-related effects on mean body weight at 75 mg/kg/day in males and females during the preweaning periods and in females during the post weaning period. Over the preweaning treatment period (Day 4 and 21 pp), mean body weight gain was decreased (~0.9X control mean gain for males and females. (see table below, *excerpted from sponsor*). Food consumption was not affected during the post weaning period, and growth (i.e., length of long bone) was not affected. Thus, the body weight effect at this dose level is interpreted to be an effect of general toxicity of the compound and not an effect on developmental growth.

GSK1349572 Daily Dose (mg/kg) ^a	0 (Control)		0.5		2		75	
	M	F	M	F	M	F	M	F
Group Composition (10 litters/group each litter 5 pups/sex/litter)	50	50	50	50	50	50	50	50
Juveniles Assigned to Study on Day 3 pp, treatment initiated on Day 4 pp for all subset groups:								
Subset I - Main Study Necropsy Day 67 pp, nonlittermates	10	10	10	10	10	10	10	10
Subset II -TDAR, Day 50 and 59 pp, nonlittermates	10	10	10	10	10	10	10	10
Subset III - Toxicokinetic Evaluation on Day 13 pp	20	20	20	20	20	20	20	20
Subset IV - Toxicokinetic Evaluation on Day 32 pp	10	10	10	10	10	10	10	10
Subset I: Main Study Necropsy (Day 67 pp)								
Mortality ^b (found dead, one on Day 12 pp and one on Day 17 pp)	0	0	0	0	0	0	2 ^b	0
Clinical Observations of early decedents							none	
Macroscopic Observations of early decedents							none	
Clinical Observations	-	-	-	-	-	-	-	-
Body Weight Gain (g) ^c								
Day 4 to 13 pp	19.59g	18.96g	1.03X	1.05X	1.02X	1.01X	1.05X	0.98X
Day 13 to 21 pp	27.76g	25.84g	1.00X	1.03X	0.97X	1.04X	0.73X*	0.77X*
Day 4 to 21 pp (preweaning period)	47.35g	44.80g	1.01X	1.04X	0.99X	1.03X	0.86X*	0.86X*
Body Weight (g) ^c								
Day 16 pp	42.03g	40.21g	1.00X	1.03X	1.00X	1.01X	0.93X*	0.91X*
Day 19 pp	50.01g	47.70g	1.02X	1.04X	0.98X	1.01X	0.90X*	0.90X*
Day 21 pp	58.45g	55.24g	1.01X	1.04X	0.99X	1.03X	0.88X*	0.88X*
Day 28 pp	102.7g	95.97g	1.01X	1.01X	1.02X	1.03X	0.95X	0.89X*
Day 42 pp	242.2g	184.2g	1.0X	0.98X	0.98X	1.00X	0.98X	0.90X*
Day 66 pp (end of treatment period)	459.6g	282.3g	1.00X	0.93X	0.99X	0.95X	1.03X	0.91X

Food consumption: There was no effect of test article on food consumption.

Physical Development: There was no effect on days to balano-preputial separation (separation of prepuce from the glans penis) or vaginal opening.

Hematology: There were no remarkable findings.

Clinical chemistry: There were no remarkable findings, including coagulation parameters.

Urinalysis: Excreted urine volume appeared to be greater in high dose males (33.39 ± 11.28 mL) than controls (24.10 ± 6.64 mL), although the difference was not found to be statistically significant.

Gross pathology: There were no test article-related macroscopic findings.

Organ weights: The following organs were weighed at necropsy: liver, heart, kidneys, testes, prostate, ovaries, brain, spleen, thymus, and adrenals. In addition, femur length (as an indicator of growth) was recorded. No remarkable effects on organ weights or femur length were noted.

Histopathology: Adequate Battery: yes
Peer review: yes

Microscopic findings included degeneration/regeneration of the olfactory and/or respiratory epithelium of the nasal cavity of male (≥ 0.5 mg/kg/day) and female (75 mg/kg/day) rats. The histopathology was considered secondary to expelling of the gavaged material rather than a direct test article effect and is consistent with a local irritation effect.

Spermatogenesis: Stage-dependent qualitative evaluation of spermatogenesis was conducted. There were no remarkable findings.

Toxicokinetics: Sampling time points were (Day 1): 0.5, 1, 2, 4, 8, and 24 hours after dosing; (Days 30, 69, 83 (30 mg/kg dose group), 99 (1 female in 30 mg/kg dose group), 103 (1 male in 30 mg/kg dose group), 120, 180, and 270): pre-dose and 0.5, 1, 2, 4, 8, and 24 hours after dosing.

The systemic exposure of Dolutegravir was lower on Day 32 pp compared to Day 13 pp (see table below, *excerpted from sponsor*). The systemic exposure of Dolutegravir generally increased in approximate proportion to the increase in dose when the dose was increased from 0.5 to 2 mg/kg/day, but the increase was less than proportional when the dose increased to 75 mg/kg/day. The 150-fold increase in dose resulted in a 17-to 93-fold increase in systemic exposure.

Table 22: Toxicokinetic Parameters for Dolutegravir following Oral Administration in Juvenile Rats (table excerpted from sponsor)

Dose (mg/kg/day)	Male				Female			
	Mean AUC ^a (0-24) (µg.h/mL)		Mean C _{max} ^a (µg/mL)		Mean AUC ^a (0-24) (µg.h/mL)		Mean C _{max} ^a (µg/mL)	
	Day 13pp	Day 32pp	Day 13pp	Day 32pp	Day 13pp	Day 32pp	Day 13pp	Day 32pp
0.5	92.0	9.90	4.55	1.50	86.5	27.1	4.69	2.41
2	303	85.7	15.2	7.71	316	93.3	16.4	7.46
75	1540	917	88.0	69.9	1549	1044	85.4	77.4

n = 3/timepoint/dose, with the exception of the 8 hour timepoint on Day 13 pp following 75 mg/kg/day, for which n =4.

a. Composite parameters were derived from mean plasma concentration data.

Immunotoxicity Assessment: There were no test article-related effects on immunologic competence when measured on Day 67 pp as measured by T cell dependent antibody response (TDAR), and no effects on lymphocyte subsets (T cells, both CD4 and CD8 subsets, and B cells) and CD4 or CD8 T cell receptor Vβ usage in peripheral blood.

Special Toxicology Study

Study title: Immunotoxicity Study of S-349572 Sodium in Rats: Determination of Specific Antibody Formation against T-cell Dependent Antigen

Key study findings: Rats were administered 10, 100 or 1000 mg/kg S-349572 by oral gavage for one month. Effects of test article on T-cell dependent antibody response were assessed. There were no effects of test article administration on T-cell dependent antibody response.

Study no.:	S-349572-TB-064-L
Volume #, and page #:	SN 0073/vol. 1 p. 267
Conducting laboratory and location:	Developmental Research Laboratories, Shionogi & Co., Ltd., 3-1-1, Futaba-cho, Toyonaka, Osaka 561-0825, Japan
Date of study initiation:	September 10, 2008
GLP compliance:	yes (Japanese)
QA report:	yes
Drug, lot #, and % purity:	Dolutegravir sodium salt, B86001 (98.9%)

Methods

Doses:	10, 100, 1000 mg/kg/day
Species/strain:	Rat/[CrI:CD(SD)]
Number/sex/group or time point (main study):	10/sex/group

Route, formulation, volume, and infusion rate:

Suspension; 0.5% HPMC w/ 0.1%
Tween 80; 10 mL/kg via oral gavage
4 weeks

Age:

Results:

Mortality: Rats were observed twice daily (once daily on weekends and holidays) for morbidity and mortality. There were no early deaths.

Clinical signs: There were no significant clinical signs.

Body weights: Body weights were recorded every three days. There were no significant effects on body weight.

Gross pathology: Thymus, spleen and adrenal glands were examined macroscopically. There were no test article-related effects.

Organ weights: The following organs were weighed at necropsy: spleen, thymus, and adrenals. Spleen weights were increased in high dose (1000 mg/kg) males, without histopathological correlate.

Histopathology: Spleens were examined microscopically. No test article-related findings were noted.

Immunotoxicity Assessment: Hemocyanin, Keyhole limpet (KLH) was used as an antigen challenge. IgM class anti-KLH antibody titers were determined by ELISA. No statistically significant difference was observed in the anti-KLH antibody titer between each of the S-349572 sodium dosing groups and the control group in both males and females.

Local Toxicity

Dermal

Slight erythema was noted in two of three rabbits at 24 hrs, but the erythema disappeared by 48 hours. The test article is classified as causing mild irritation under the conditions of this study.

Ocular

The test article was classified as slightly irritating under the conditions of an ocular irritation study.

11 INTEGRATED SUMMARY AND SAFETY EVALUATION

Repeat dose studies were performed in mice (up to 13 weeks), rats (up to 6 months), and nonhuman primates (up to 9 months). *In vitro* and *in vivo* genotoxicity studies were conducted. Two-year oral carcinogenicity studies were conducted in mice and rats. The reproductive and developmental toxicity studies included fertility studies in male and female rats, embryo-fetal developmental studies in rats and rabbits, and a peri- and postnatal developmental study in rats. Dolutegravir toxicity was also assessed in juvenile rats.

In animal species assessed in the nonclinical toxicology program, oral bioavailability ranged from 24.9% (non-fasted monkeys) to 51.5% (fasted rats). Parent drug was the primary compound in plasma following dolutegravir administration. No metabolites were present at concentrations greater than 10% of parent. *In vivo*, dolutegravir is primarily metabolized to an ether glucuronide conjugate (M3) that was the main metabolite in mice, rats and humans. In monkeys, the dolutegravir glucuronide (M3) and glucose (M2) conjugates were present at approximately equal concentrations. Dolutegravir glucuronide formation occurs through oxidation catalyzed by cytochrome P450 (CYP) 3A followed by conjugation catalyzed by uridine diphosphate-glucuronosyl transferase (UGT) 1A1. In mice, rats, monkeys and humans, oxidative defluorination with subsequent glutathione or cysteine addition was present indicating the formation of a reactive metabolite, specifically an electrophilic arene oxide intermediate. Except in mice, these products were a small fractional part of the overall clearance. Reactive metabolites are a concern due to potential mutagenic and carcinogenic properties. Negative findings with DTG in both genotoxicity assays and carcinogenicity studies suggest there is minimal concern in this case. Reactive metabolites have also been associated with hepatic and/or renal toxicity of some drugs. With DTG there were no significant liver or kidney toxicity findings in chronic nonclinical studies at systemic exposures several fold greater than the anticipated human exposures. In some hepatic cases observed in clinical trials, DTG related liver injury could not be ruled out.

In all nonclinical species studies, an oral dose of DTG was excreted primarily in feces and consisted primarily of unchanged DTG. Biliary excretion was the predominant excretion route for dolutegravir glucuronide. Both the glucuronide and glucose conjugates were detected in bile but not feces, suggesting that these DTG conjugates are deconjugated in the intestine to reform DTG, which is then reabsorbed into the systemic circulation (i.e., enterohepatic circulation).

In vitro, DTG inhibited the renal organic cation transporter (OCT) 2 ($IC_{50} = 1.9 \mu M$), which provides a mechanistic basis for the mild serum creatinine increases observed in clinical studies. As a weak inhibitor of UGT1A1, DTG has the potential to interfere with the conjugation of bilirubin.

No adverse effects of DTG on the cardiovascular, respiratory, or central nervous systems were noted during safety pharmacology studies. Dolutegravir did not produce acute effects on blood pressure or heart rate parameters in conscious telemetered male monkeys at doses up to 1000 mg/kg, or respiratory or neurobehavioral effects in conscious male rats at doses up to 500 mg/kg.

At 20 μM DTG (8.38 $\mu g/ml$; $AUC=201.1 \mu g.h/ml$) there was 16.1% inhibition of hERG channel tail current in HEK-293 cells expressing hERG cDNA. The effect is considered weak and not a safety concern.

A summary of systemic exposures, related NOAELs, and margins of exposure (ratio of nonclinical: clinical exposures) is presented in tabular form below. Given that the primary toxicity is local GI effects (rather than effects associated with systemic exposure) safety margins calculated using systemic exposure comparisons can be considered conservative. Less conservative safety margins for DTG may be calculated using dose (mg/kg). Using the nonhuman primate example, the NOAEL for the nine month monkey study (15 mg/kg/day) is 15X and 8X the human mg/kg equivalent dose (based on a 50 kg human) for a 50 mg QD and BID dose, respectively.

Table 23: Safety Margins from Nonclinical Toxicology Studies

	Study Type	Dose (mg/kg)	AUC μg*hr/mL		Margin (Ratio of Animal to Human Exposure) ^a (QD/BID dosing)		Margin (Ratio of Animal to Human Dose) ^b (QD/BID dosing)
			Male	Female	Male	Female	
Rat	2 week	150 (NOAEL)	1445		27/19		150/75
	1 month	100 (NOAEL)	722	780	13/10	14/10	100/50
	6 month	50 (NOAEL)	607	922	11/8	17/12	50/25
	Fertility/ Teratogenicity	1000 (NOAEL)		2032		38/27	
	Fetal Development	50 (NOAEL)	1040	1610	19/14	30/21	50/25
	Carcinogenicity	50	713	1140	13/10	21/15	
	Juvenile	2 (NOAEL)	303	316	6/4	6/4	
Rabbit	Embryofetal development	200 (maternal NOAEL)		15		0.3/0.2	
		1000 (reproductive / embryofetal development NOAEL)		30		0.6/0.4	
Mice	13 week	500 (NOAEL)	1010	1300	19/13	24/17	
	Carcinogenicity	500	953	1210	18/13	22/16	
NHP	2 week	100 (NOAEL)	192	187	4/3	3/2	100/50
	1 month	50 (NOAEL)	111	153	2/1	3/2	50/25
	9 month	15 (NOAEL)	37	41	0.7/0. 5	0.8/0.5	15/8

^a Safety margins based on systemic exposure comparisons. In HIV-infected adult subjects dosed with Dolutegravir at 50 mg once or twice daily the mean systemic exposures were 54 and 75 μg*hr/mL, respectively.

^b Given that the primary toxicity is local GI effects (rather than effects associated with systemic exposure) safety margins calculated using systemic exposure comparisons can be considered conservative. Less conservative safety margins for DTG may be calculated using the human mg/kg equivalent dose (1 mg/kg or 2 mg/kg for the QD and BID dose, respectively, based on a 50 kg human).

In the two week study in monkeys, one female in the high dose (1000 mg/kg) group died on Day 13 of dosing. This animal had exhibited emesis and diarrhea daily since the beginning of dosing. The animal's death was considered to be secondary to effects of DTG on the GI system, and subsequent effects on blood electrolytes. This animal's systemic exposure (AUC_{0-24}) on Day 1 was 277 $\mu\text{g}\cdot\text{h}/\text{mL}$. Gender-mean Day 14 exposure (AUC_{0-24}) at 1000 mg/kg/day was 360 $\mu\text{g}\cdot\text{h}/\text{mL}$, which corresponds to ~7X or ~5X above the expected human exposure for a 50 mg QD or BID dose, respectively.

In the nine month study in monkeys, two males from the high dose (50 mg/kg) group died or were euthanized on days 59 and 55, respectively. Clinical signs included emesis and diarrhea, with weight loss, suggesting gastrointestinal intolerance.

The following organs or organ systems were identified as targets of DTG toxicity:

Gastrointestinal

Gastrointestinal toxicity related to DTG administration was observed across species and in subchronic and chronic studies during the nonclinical development program. The GI toxicity is believed to be the result of a local drug effect at the mucosal surface of the gut following oral dosing, rather than systemic toxicity. This conclusion is supported by the fact that animals with GI effects had comparable systemic exposures to animals at lower dose levels which were not affected.

In a four week study in rats, effects in the high dose (1000 mg/kg) group included hemorrhage in the lamina propria of the stomach mucosa. The finding was not seen in high dose animals following a 4 week recovery period, suggestive of recovery. The NOAEL in the four week rat study was 100 mg/kg/day, based on the GI hemorrhage. Exposure at 100 mg/kg/day was 752 $\mu\text{g}\cdot\text{h}/\text{mL}$ (males and females combined), which corresponds to ~14X or ~10X above the expected human exposure for a 50 mg QD or BID dose, respectively.

In a six month (26 week) study in rats, hemorrhage in the glandular stomach mucosa was seen in tissue from one male from the high dose (500 mg/kg) group after 17 weeks of dosing, and in one high dose group male after 26 weeks of dosing. Hemorrhage was not seen following a four week recovery period, suggestive of recovery. The NOAEL was 50 mg/kg/day. When males and females were considered together, exposure at 50 mg/kg/day was 765 $\mu\text{g}\cdot\text{h}/\text{mL}$, which corresponds to ~14X or ~10X above the expected human exposure for a 50 mg QD or BID dose, respectively.

In monkeys, the most sensitive species, clinical signs of GI toxicity included vomiting, diarrhea, and associated mortality. In a two week study in monkeys, microscopic evidence of GI irritation included epithelial atrophy and mucosal hemorrhage in the stomach and lower GI tract (cecum, colon and/or rectum) in monkeys given ≥ 300 mg/kg/day. The NOAEL was 100 mg/kg/day. Exposure at 100 mg/kg/day was 190 $\mu\text{g}\cdot\text{h}/\text{mL}$, which corresponds to ~4X or 3X above the expected human exposure for a 50 mg QD or BID dose, respectively.

In a four week study in monkeys, clinical signs of vomiting, diarrhea, and body weight loss were attributed to DTG administration. Histopathological changes of the GI tract occurred in the 100 mg/kg dose group and included inflammatory cell infiltration (slight) in the lamina propria of the cecum, colon and rectum in both sexes; slight cell debris from the crypts of the cecum and colon in

males; and atrophy of the mucosal epithelium of the cecum and colon. The NOAEL was 50 mg/kg/day, corresponding to exposures of 132 $\mu\text{g}\cdot\text{h}/\text{mL}$ (males and females combined), which are ~2X above the expected human exposure for a 50 mg QD or BID dose.

In a nine month study in monkeys, two males from the high dose (50 mg/kg) group died or were euthanized on days 59 and 55, respectively. Clinical signs included emesis and diarrhea, with weight loss, suggesting gastrointestinal intolerance. Microscopic findings included slight mononuclear cell infiltration and hemorrhage in the lamina propria in the cecum and colon.

Also in monkeys, GI intolerance at the initial high dose of 50 mg/kg led to a decrease in dose to 30 mg/kg on day 70. Clinical signs associated with 50/30 mg/kg DTG administration included abnormal feces associated with decreased food consumption and decreased body weight. After nine months of dosing, microscopic findings were noted in one female in the 50/30 mg/kg group and included multifocal mononuclear cell infiltration in stomach tissue and slight hemorrhage in the lamina propria, along with very slight multifocal erosions, and multifocal epithelial regeneration. Although less severe, multifocal mononuclear cell infiltration and very slight hemorrhage in the lamina propria and multifocal epithelial regeneration in the stomach were observed in one female from the high dose group following a four week recovery period.

Exposures at end of study for the two affected females were lower compared to the other animals in this dose group ($\text{AUC}_{0-24} = 43.5$ to $48.8 \mu\text{g}\cdot\text{h}/\text{mL}$ versus gender mean for 50/30 mg/kg/day group of $61.7 \mu\text{g}\cdot\text{h}/\text{mL}$) and overlapped with exposures at 15 mg/kg/day (AUC_{0-24} range = 25.8 to $54.0 \mu\text{g}\cdot\text{h}/\text{mL}$). This observation is consistent with a local GI toxicity as opposed to a systemic effect.

The NOAEL for the 38 week dosing period was 15 mg/kg/day which corresponds to systemic exposures 0.7X the human exposure for a 50 mg QD dose and corresponds to 0.5X the human exposure for a 50 mg BID dose.

Liver

Liver toxicity that was considered to be related to administration of DTG was seen only in the two week study in monkeys. In males dosed with 1000 mg/kg, findings associated with liver toxicity included hepatocellular single cell necrosis and diffuse hepatocellular hypertrophy and/or vacuolation. Clinical chemistry changes included transient ALT increases at ≥ 300 mg/kg/day, increased AST, bilirubin, and triglycerides at 1000 mg/kg/day and decreased total cholesterol at 1000 mg/kg/day. The NOAEL was 100 mg/kg/day. Exposure at 100 mg/kg/day was $190 \mu\text{g}\cdot\text{h}/\text{mL}$, which corresponds to ~4X or ~3X above the expected human exposure for a 50 mg QD or BID dose, respectively.

In the nine month study in monkeys, signs of liver toxicity were restricted to a moribund animal from the high dose group that was euthanized on day 55. Clinical chemistry changes suggestive of liver damage included increased AST (2.5X) and bilirubin (2.8X). The findings associated with liver toxicity in the nine month study were considered secondary to the moribund condition of the animal. Exposure at the NOAEL (15 mg/kg/day) was $39 \mu\text{g}\cdot\text{h}/\text{mL}$, which corresponds to ~0.7X or ~0.5X the expected human exposure for a 50 mg QD or BID dose, respectively. No treatment related adverse effects on liver were observed in rats in studies up to 26 weeks.

Bone marrow and lymph nodes

In the two week study in monkeys, findings associated with bone marrow toxicity in animals in the high dose (1000 mg/kg) group included hypocellular and/or gelatinous bone marrow and atrophy of the white pulp in the spleen. In monkeys dosed with 300 mg/kg or 1000 mg/kg, findings associated with bone marrow and/or lymph node toxicity included a decrease in the paracortical lymphocytes of the submandibular and/or mesenteric lymph nodes. Decreased reticulocytes, RBCs, and platelets and increased APTT occurred in monkeys given 1000 mg/kg/day and are believed to correlate with the microscopic bone marrow changes. The NOAEL in the 2 week study was 100 mg/kg/day. Exposure at 100 mg/kg/day was 190 µg.h/mL, which corresponds to ~4X or ~3X above the expected human exposure for a 50 mg QD or BID dose, respectively.

No treatment related adverse effects on bone marrow or lymph nodes were observed in rats in studies up to 26 weeks.

A series of *in vitro* and *in vivo* genotoxicity tests have shown DTG to be free of genotoxic potential. Dolutegravir did not demonstrate a potential for allergic or delayed sensitization reactions. Dolutegravir was slightly/mildly irritating in skin and ocular model systems. Based on spectrophotometric assessment, DTG has a potential for phototoxicity. No ocular toxicity has been noted in nonclinical studies and no phototoxicity signals have been noted in clinical trials.

Dolutegravir carcinogenicity was assessed in two year bioassays in CD-1 mice and Sprague Dawley rats. No drug-related tumors were observed in mice. Systemic exposures at the high dose (500 mg/kg/day) were 18X and 13X (males), and 22X and 16X (females) the human exposure for a 50 mg QD dose and a 50 mg BID dose, respectively. Likewise, no drug-related tumors were observed in rats at exposures 13X and 10X (males), and 21X and 15X (females) the human exposure for a 50 mg QD dose and a 50 mg BID dose, respectively.

Dolutegravir did not affect male or female fertility in rats at doses up to 1,000 mg/kg/day, corresponding to systemic exposures approximately 30 times greater than human exposures at the 50 mg BID clinical dose. Oral administration of DTG to pregnant rats at doses up to 1,000 mg/kg daily from days 6 to 17 of gestation did not elicit maternal toxicity, developmental toxicity, or teratogenicity. The high dose corresponded to exposures approximately 38X and 27X exposures at the recommended clinical doses of 50 mg QD and BID, respectively.

The potential for adverse effects of DTG on pregnant/lactating female rats and on the development of the conceptuses and offspring was assessed at doses up to 1000 mg/kg. Dolutegravir was administered orally to female rats from Day 6 of gestation to Day 20 of lactation, the days that correspond to the period from implantation to weaning of rats. Decreased food consumption and body weight were noted in high dose (1000 mg/kg) F0 dams during lactation, and the NOAEL was considered to be 50 mg/kg for maternal toxicity in F0 dams. In the evaluation of parameters for maternal reproductive function (F0) (duration of gestation, gestation index, delivery and nursing behavior), no adverse effects were observed in the 1000 mg/kg group and NOAEL was concluded to be 1000 mg/kg/day. In the subsequent generation (F1), decreased body weights were noted in the 1000 mg/kg group from pre-weaning until adolescence. The NOAEL was defined as 50 mg/kg for F1 generation development, corresponding to systemic exposures greater than 14X the expected human exposures for a 50 mg BID dose.

Oral administration of DTG to pregnant rabbits at doses up to 1,000 mg/kg daily, approximately 0.6X and 0.4X the human exposures at the recommended 50 mg QD and BID doses, respectively, from days 6 to 18 of gestation did not elicit developmental toxicity or teratogenicity. In rabbits, maternal toxicity (decreased food consumption, scant/no feces/urine, suppressed body weight gain) was observed at 1,000 mg/kg.

In a juvenile rat study, the deaths of two male pups dosed with the high dose (75 mg/kg/day) were considered to be test article-related. Systemic exposures at 75 mg/kg/day were approximately 10-fold higher than systemic exposures at the NOAEL. Other test article-related effects were limited to decreased body weights primarily in high dose females, and degeneration/regeneration of nasal mucosa across dose groups resulting from direct contact with expelled test article. Both findings are considered to be local effects and not related to systemic exposure to DTG. Due to the deaths at 75 mg/kg, the NOAEL in juvenile rats was considered to be 2 mg/kg/day, corresponding to systemic exposures on Day 13 postpartum that were approximately 6X and 4X human exposures at the recommended clinical doses of 50 mg QD and BID, respectively.

Immunologic competence was assessed in the juvenile rat study and a one month repeat dose study. In juvenile rats (Day 67 postpartum) there were no test article-related effects on T cell dependent antibody response (TDAR), and no effects on lymphocyte subsets (T cells, both CD4 and CD8 subsets, and B cells) and CD4 or CD8 T cell receptor V β usage in peripheral blood. In mature rats, there were no effects of test article administration on T-cell dependent antibody response following administration of 10, 100 or 1000 mg/kg DTG for one month.

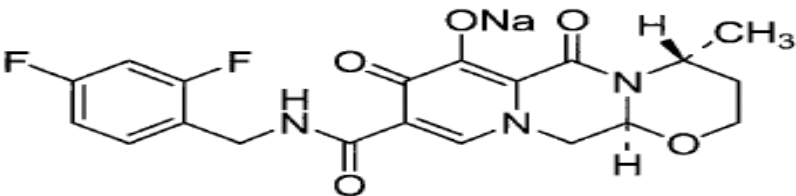
12 APPENDIX/ATTACHMENTS

Appendix 1
Original IND Pharmacology/Toxicology Review

PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND NUMBER: 75,382
REVIEW NUMBER: 1
SEQUENCE NUMBER/DATE: 000/Oct.-26, 2007
INFORMATION TO SPONSOR: Yes (x) No ()
SPONSOR: GlaxoSmithKline Research Triangle Park, NC 27709
MANUFACTURER: Same as above

REVIEWER NAME: Kuei-Meng Wu
DIVISION NAME: DAVDP
HFD No.: HFD-530
REVIEW COMPLETION: 11/28/07
DRUG: DolutegravirA (sodium salt); Dolutegravir [i.e., DolutegravirB (parent, free acid)]
CHEMICAL NAME: Sodium (4R,9aS)-5-Hydroxy-4-methyl-6,10-dioxo-3,4,6,9,9a,10-hexahydro-2H-1-oxa-4a,8adiazanthracene-7-carboxylic acid 2,4-difluorobenzylamide
FORMULA/MW: DolutegravirA: C₂₀H₁₈F₂NaO₅ MW=441.36

STRUCTURE: 
RELATED INDs/NDAs: Pre-IND 75382
DRUG CLASS: Antiviral- HIV Integrase Inhibitor
INDICATION: Treatment of HIV Infection
CLINICAL FORMULATION: 10, 25, 50, 100, 200 & 400 mg powder (DolutegravirA (b) (4) drug substance) DolutegravirB (free acid) as a suspension (Dolutegravir Dosing Suspension) for oral administration.
Excipients: (b) (4)
ROUTE OF ADMINISTRATION Oral

DISCLAIMER: TABULAR AND GRAPHICAL INFORMATION IS MODIFIED FROM SPONSOR'S SUBMISSION UNLESS STATED OTHERWISE.

INTRODUCTION	<p>The proposed IND involves an HIV integrase inhibitor, Dolutegravir, that has not been tested in humans. (b) (4)</p> <p>This IND proposed for an oral single-dose clinical trial in healthy volunteers. The pharm/tox portion of the document is evaluated in this review.</p>
MECHANISM OF MICROBIOLOGICAL ACTION:	<p>Dolutegravir inhibits HIV integrase catalyzed viral DNA strand transfer ($IC_{50} \approx 2.7-12.6$ nM.) It has been tested in several in vitro assays against the HIV-1 strains Ba-L and NL432 in human peripheral blood mononuclear cell ($IC_{50} \approx 0.5$ nM), against the HIV-1 strain IIB in MT-4 cells and in the pseudo-HIV assay. Dolutegravir's overall anti-HIV activity is claimed to be similar to that seen against wild type HIV-1 virus, against 19 of 20 HIV integrase mutants containing single mutations and 9 of 11 mutants containing 2 or more mutations.</p>
PROPOSED CLINICAL PROTOCOL	<p>The proposed first clinical study, ING 111207, is a Phase I, randomized, alternating panel, placebo-controlled, double-blind study to determine the safety, tolerability, and pharmacokinetic profile of Dolutegravir after single doses in healthy subjects. The projected single escalating doses of Dolutegravir will range from a starting dose of 10 mg to a maximum dose of 400 mg (i.e. 10, 25, 50, 100, 200, 400 mg qd), dependent upon exposure and safety.</p>
PREVIOUS CLINICAL EXPERIENCE	<p>None.</p>

(1) GENERAL TOXICITY STUDIES

STUDY TITLE:	Dolutegravir: Single Dose Oral Gavage non-GLP Toxicity Study in One Female Cynomolgus Monkey
KEY FINDINGS:	None (up to 500 mg/kg).
STUDY NO.:	E-34572- TF-008-R; Report# RD2007/0118400
VOLUME/PAGE:	5/p255
LABORATORY:	Shionogi and Co., Ltd.
STUDY	5/2004
INITIATION:	
GLP:	yes () no (x)
QA REPORT:	Yes () no (x)
METHODS:	Single oral gavage doses of Dolutegravir at 50, 125, 250 and 500 mg/kg were administered to fasted female cynomolgus monkeys (n= 1/group)(vehicle = 0.5% w/w aqueous hydroxypropyl methylcellulose with 0.1% Tween 80). The following were

RESULTS:	evaluated: clinical observations, body weights, food consumption, hematology, clinical chemistry and toxicokinetic analysis. TK was performed following each single dose. No adverse effects on body weight, clinical observations or clinical pathology occurred in monkeys at single oral doses of up to 500 mg/kg.
STUDY TITLE:	Dolutegravir: 2-Week Oral Toxicity Study in Sprague Dawley Rats
KEY FINDINGS:	The stomach is the target organ identified. The sponsor's NOAEL = 500 mg/kg/day (Cmax=116 ug/mL AUC _{0-24h} =1830 ug.h/mL). However, because of drug-induced gastric mucosal lesions, the NOAEL should be 150 mg/kg (AUC _{0-24h} =1445 ug.h/mL).
STUDY NO.:	E-34572- TB-012
REPORT NO.:	RD2007/01140/00
VOLUME/PAGE:	Volume 6, Page 1
LABORATORY:	Shionogi and Co., Ltd.
STUDY INITIATION:	5/07
GLP:	yes (x) no () QA
REPORT:	yes (x) no ()
LOT/PURITY:	MTS-0297994B-07
VEHICLE:	0.5% w/w aqueous hydroxypropyl methylcellulose with 0.1% Tween 80
DOSING:	0 (control), 50, 150 or 500 mg/kg/day
SPECIES/STRAIN:	Sprague-Dawley Rat/ CrI:CD (SD)IGS BR
#/SEX/GROUP:	10 (4 for TK)
ROUTE:	Oral gavage, 1/day
METHODS:	
MORTALITY:	Checked at least once daily (mortality and moribundity)
CLINICAL SIGNS:	Daily
BODY WEIGHTS:	2/Weekly
FOOD CONSUMPTION:	2/Weekly
OPHTHALMO-SCOPY:	Pretest, during Week 2
HEMATOLOGY:	during Week 2
CLINICAL CHEMISTRY:	during Week 2
URINALYSIS:	at termination
NECROPSY & HISTO-PATHOLOGY:	at termination (including stage-dependent evaluation of spermatogenesis)
RESULTS:	
MORTALITY:	None.
CLINICAL SIGNS:	Unremarkable.
BODY WEIGHTS:	Unremarkable.
FOOD CONSUMPTION:	Unremarkable.

CONSUMPTION:	
OPHTHALMOLOGY:	Unremarkable.
HEMATOLOGY:	Unremarkable.
CLINICAL CHEMISTRY:	Unremarkable.
URINALYSIS:	There were statistically significant increases in urine specific gravity in males given 500 mg/kg/day and in females given 50 and 500 mg/kg/day (no renal histopathology finding).
HISTOPATHOLOGY:	Stomach is the only target organ in histopathology: Mild increased mucous neck cells, eosinophil infiltration to submucosa and focal edema in the glandular stomach occurred in males and females (500 mg/kg/day 10/10 males, 7/10 females). The gastric mucosal lesions were judged by the sponsor to be treatment-related irritation.

Repeat Dose Toxicity			Study No. E-349572-TB-012-L							
Daily Dose (mg/kg)	0 (Control)		50		150		500			
Number of Animals	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10	M: 10	F: 10		
Urinalysis										
Number examined ^{a)}	9	10	9	10	10	10	10	10		
Specific gravity	1.019	1.021	1.036	1.039*	1.032	1.036	1.047**	1.046**		
Hematology										
Number examined ^{a, b)}	9	10	8	10	10	10	10	10		
Eosinophil ($\times 10^3/\mu\text{L}$)	-	0.05	-	0.07	-	0.09*	-	0.07		
Blood Chemistry										
Number examined ^{a, b)}	9	10	8	10	10	10	10	10		
Creatinine (mg/dL)	0.26	-	0.28	-	0.30**	-	0.29*	-		
Total bilirubin (mg/dL)	-	0.06	-	0.05	-	0.05	-	0.05*		
Sodium (mmol/L)	-	142	-	142	-	143	-	143*		
Organ Weight ^{c)} (%)	-	-	-	-	-	-	-	-		
Gross Pathology	-	-	-	-	-	-	-	-		
Histopathology										
Stomach: eosinophil infiltration, glandular stomach	Mild	4	0	N.E.	N.E.	2	5	9	5	
Stomach: increased mucous cell, glandular stomach	Mild	3	0	N.E.	N.E.	1	2	5	4	
Stomach: edema, glandular stomach	Mild	0	2	N.E.	N.E.	3	2	7	4	

Key: Cumulative Chi-squares test with Dunn-Sidak adj.: # - $p < 0.05$ N.E.: Not examined
 No noteworthy findings. The first day of dosing is Day 1 Dunnett's parametric multi. t-test: * - $p < 0.05$, ** - $p < 0.01$
 a) One male in the control group was excluded from statistical analysis due to abnormally high levels in AST, ALT and LD with incidental liver lesions. One male in the 50 mg/kg group was excluded from statistical analysis due to accidental loss of teeth.
 b) Samples of another male in the 50 mg/kg group were not collected due to a technical error.
 c) Both absolute and relative weights differed from controls in the direction indicated.

TOXICOKINETICS Systemic exposure (C_{\max} and AUC_{0-24h}) to Dolutegravir were generally less than dose-proportional (ceiling/saturable effects) and no notable (>2 -fold) sex-related differences in at any dose or sampling occasion were observed.

Dose (mg/kg/day)	Sex	C_{\max} (ug/mL) Day 1	End of Study	AUC_{0-24h} (ug.h/mL) Day 1	End of Study
50	M F	58.5 75.4	65.7 95.6	881 1110	1040 1610
150	M F	82.7 83.3	74.1 106	994 1050	1150 1740
500	M F	87.1 117	108 124	1360 1350	1710 1950

CONCLUSION The sponsor considered the NOAEL to be 500 mg/kg/day ($C_{\max}=116$ ug/mL AUC_{0-24h})

=1830 ug.h/mL). However, because of gastric mucosal lesions observed (see histopathology sponsor's table above), the NOAEL should be 150 mg/kg (AUC_{0-24h} =1445 ug.h/mL).

STUDY TITLE:	Dolutegravir: 2-Week Oral Gavage Toxicity Study in Cynomolgus Monkeys
KEY FINDINGS:	The liver, immune organs (lymph nodes, bone marrow, adrenal, kidney, and GI and hemotologic system are target organs identified. The sponsor's NOAEL= 100 mg/kg [AUC _{0-24h} (41 ug.h/ml) and Cmax (5.75 ug/ml)].
STUDY NO.:	Study No. SG07030; E-34572- TF-029-L
REPORT NO.:	RD2007/01142/00
VOLUME/PAGE:	
LABORATORY:	Shionogi and Co., Ltd.
STUDY	5/07
INITIATION:	
GLP:	yes (x) no ()
QA REPORT:	yes (x) no ()
LOT/PURITY:	R06001 (same as clinical batch)
FORMULATION/VEHICLE:	vehicle = 0.5% w/w aqueous hydroxypropyl methylcellulose with 0.1% Tween 80
METHODS:	
DOSING:	0 (control), 100, 300 or 1000 mg/kg/day
SPECIES/STRAIN:	Cynomolgus monkeys
#/SEX/GROUP:	3
ROUTE	Oral gavage, 1/day
TOXICOKINETICS	
MORTALITY:	Checked at least once daily (mortality and moribundity)
CLINICAL SIGNS:	Daily
BODY WEIGHTS:	2/Weekly
FOOD	2/Weekly
CONSUMPTION:	
OPHTHALMO-SCOPY:	Pretest, during Week 2
EKG:	At least weekly
HEMATOLOGY:	During Week 2
CLINICAL	During Week 2
CHEMISTRY:	
URINALYSIS:	At termination
NECREOPSY & HISTO-PATHOLOGY::	At termination (including bone marrow smears)
RESULTS:	
MORTALITY:	One female (1000 mg/kg/day) died on Day 13. This animal showed repeated daily emesis began on Day 2 and diarrhea on Day 5 with progression to decreased activity, lateral or crouching position, pale oral mucosa, and subnormal body surface temperature prior to death on Day 13.

	Hematology and clinical chemistry performed on this animal showed an increase (2X control) in fibrinogen, marked increases (3X control) in ALT and urea nitrogen and decreases (0.8 to 0.9X control) in Na and Cl. Liver and digestive tract were autolyzed, and histopathological findings that might cause death were not evident in the kidneys, heart or lungs. A direct cause of death was not determined. The sponsor speculated that the deteriorated GI effects (emesis, diarrhea, ulcer in colon) with changes in blood electrolytes contributed to the death.
CLINICAL SIGNS, BODY WEIGHTS & FOOD CONSUMPTION:	All animals at 300 and 1000 mg/kg/day exhibited emesis and diarrhea (loose, muddy or watery stools) from initiation of dosing. Two males at 1000 mg/kg/day had decreased activity and lateral or crouching position from Day 13. In 100 mg/kg group, emesis was reported in 2/3 males and 1/3 females.
EKG:	Persistent decreases (2-17%) in BW or BW gain occurred in 2/sex at 300 mg/kg/day and in 2 males and all females at 1000 mg/kg/day. Of these animals, 1 male and 2 females at 300 mg/kg/day and 2 males and 1 female (animal found dead on Day 13) at 1000 mg/kg/day had decreased food consumption.
OPHTHALMOLOGY:	Unremarkable.
HEMATOLOGY:	Unremarkable
	Hematology findings included: decreases in the reticulocyte count (0.2 to 0.5-X control) in males and females at 300 and 1000 mg/kg; decreases in the platelet count (0.5 to 0.7-X control) and increases in fibrinogen (1.4 to 1.6- X control) in males and females at 1000 mg/kg; a decrease in the reticulocyte ratio (0.3 to 0.5-X control) and prolongation of APTT (1.1-X control) in males at 1000 mg/kg; and a decrease (0.9-X control) in the red blood cell count in females at 1000 mg/kg.
CLINICAL CHEMISTRY:	Clinical findings included: increases in ALT (2.3-X control) in males at 300 mg/kg and in males and females at 1000 mg/kg (5 to 10-X control); increases in AST (2-X control), total bilirubin (2 to 3-X control), urea nitrogen (1.6 to 1.8-X control) and creatinine (1.2 to 1.5-X control) and decreases in sodium, chloride (0.87 to 0.95-X control) and the A/G ratio (0.7-X control) in males and females at 1000 mg/kg; increases in triglycerides (1.9-X control) in males at 1000 mg/kg; and a decrease (0.7-X control) in total cholesterol in females at 1000 mg/kg.
URINALYSIS:	Urinanalysis showed decreases in the urinary volume (0.3 to 0.6-X control) and Na/Cl excretion levels (0.2 to 0.3-X control) in males and females and a decrease in K excretion level (0.3-X control) in females at 1000 mg/kg.
GROSS PATHOLOGY:	Gross pathology revealed reddish spots in the stomach or colon, small thymus, brownish discoloration of the mesenteric lymph nodes and enlargement of the lymph nodes in the abdominal cavity in animals at 300 and 1000 mg/kg, including the animal found dead on Day 13, and whitish protrusion in the esophagus and reddish spots in the ileum at 1000 mg/kg. Decreases in the weight of the thymus were noted at 300 and 1000 mg/kg and increases in the weights of the liver and adrenals were noted at 1000 mg/kg.
HISTO-PATHOLOGY (SEE SPONSOR'S TABLE)	≥300 mg/kg: Atrophy of the mucosal epithelium and cell debris from the crypts of the cecum, colon and rectum, hemorrhage in the mucosa of the colon, atrophy of acinar cells

IN APPENDIX):	GI	in the pancreas (+mononucleated infiltrate) and parotid glands (+atrophy of the mucosal epithelium and hemorrhage in the mucosa of the stomach were observed in males at 1000 mg/kg/day). The frequency of these findings were reported in 1/3-2/3 of MD and 2/3-3/3 of HD animals.																																							
	Liver	1000 mg/kg/day: single cell necrosis, vacuolation of hepatocytes, and reduced P450 content (30%) in the liver. Liver organ weight increased was reported in both sexes of HD animals (30%-40%)																																							
	Kidney	1000 mg/kg/day: dilatation of the tubules in the kidneys (both sexes).																																							
	Lymph Node/Thymus/Spleen	Atrophy of the cortex of the thymus and decrease in lymphocytes in the paracortex of the mesenteric lymph nodes in monkeys at ≥300 mg/kg and in the submandibular lymph nodes in monkeys at 1000 mg/kg. At 1000 mg/kg, gelatinous bone marrow + hypocellularity, atrophy of the white pulp in the spleen were noted in males and females. In the bone marrow examinations, a decrease in the nucleated cell count was noted in one male at 1000 mg/kg.																																							
	Adrenal	≥300 mg/kg: decrease in lipid droplets in zona fasciculata cells in the adrenals (MD 2/6; HD 4/6). Hypertrophy of the zona fasciculata occurred in 2/3 of 1000 mg/kg animals)																																							
TOXICOKINETICS	Systemic exposures (Cmax & AUC0-24h) to Dolutegravir were less than dose-proportional and might be due to repeated emesis that resulted in a large degree of variability and some overlap in exposures between dose groups (pronounced at 300 and 1000 mg/kg/day.)																																								
		<table><tr><th>Dose mg/kg</th><th>Sex</th><th>Cmax (ug/mL) Day 1</th><th>End of Study</th><th>Cmax (ug/mL) Day 1</th><th>End of Study</th></tr><tr><td rowspan="2">100</td><td>M</td><td>21.3</td><td>24.0</td><td>172</td><td>192</td></tr><tr><td>F</td><td>18.9</td><td>23.0</td><td>150</td><td>187</td></tr><tr><td rowspan="2">300</td><td>M</td><td>30.9</td><td>21.7</td><td>324</td><td>199</td></tr><tr><td>F</td><td>17.5</td><td>23.3</td><td>142</td><td>271</td></tr><tr><td rowspan="2">1000</td><td>M</td><td>27.5</td><td>26.2</td><td>358</td><td>364</td></tr><tr><td>F</td><td>20.9</td><td>30.3</td><td>237</td><td>354</td></tr></table>	Dose mg/kg	Sex	Cmax (ug/mL) Day 1	End of Study	Cmax (ug/mL) Day 1	End of Study	100	M	21.3	24.0	172	192	F	18.9	23.0	150	187	300	M	30.9	21.7	324	199	F	17.5	23.3	142	271	1000	M	27.5	26.2	358	364	F	20.9	30.3	237	354
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1000	M	27.5	26.2	358	364																																				
	F	20.9	30.3	237	354																																				
CONCLUSION	Based on these results, the NOAEL of Dolutegravir was considered to be 100 mg/kg/day. Due to the large individual variation in systemic exposure, the lowest measured AUC0-24h (41 ug.h/ml) and Cmax (5.75 ug/ml) at 100 mg/kg will define the NOAEL exposure.																																								

(2) GENOTOXICITY STUDIES

STUDY TITLE:	Mutagenicity Testing of Dolutegravir with <i>Salmonella typhimurium</i> TA1535, TA1537, TA98 and TA100 and <i>Escherichia coli</i> WP2 uvrA.
KEY FINDINGS:	NEGATIVE.
STUDY NO:	V27467; WD2007/00514/00
LABORATORY:	(b) (4)
GLP:	Yes
BATCH NO.	R06001 (same as clinical batch)
INITIATION DATE:	6/07
METHODS:	Dolutegravir was evaluated for mutagenic potential in separate Standard Ames tests.
STRAINS:	<i>Salmonella typhimurium</i> tester strains TA1535, TA1537, TA98 and TA100 and

Escherichia coli strain WP2uvrA(pKM101). Two assays were performed for each tester strain both in the presence and absence of Aroclor-induced rat liver S9-mix together with appropriate vehicle (dimethylsulphoxide; DMSO) and positive controls (2-nitrofluorene, sodium azide, 9-aminoacridine, 4-nitroquinoline-1 oxide, benzo[a]pyrene and 2-aminoanthracene).

DOSAGE 5-849 µg /plate (limited by solubility in dimethyl sulphoxide)

RESULTS: The data for the vehicle controls were within the acceptable ranges. No evidence of bactericidal activity was observed. No clear increases in revertant colonies were seen at concentrations up to 849 µg/plate in either the presence or absence of S9-mix.

CONCLUSION: Dolutegravir was not genotoxic in the bacterial mutation assay when tested either in the presence or absence of S9-mix.

STUDY TITLE: **Mutagenicity testing of Dolutegravir with L5178Y tk^{+/−} mouse lymphoma cells, Forward mutation assay.**

KEY FINDINGS: NEGATIVE.

STUDY NO: V27468; Report: WD2007/005150/00

LABORATORY: (b) (4)

GLP: Yes

DATE: 6/07

BATCH NO. R06001 (same as clinical batch)

METHODS: The potential of Dolutegravir to induce non-lethal gene mutations and chromosome damage was assessed in vitro in L5178Y (TK^{+/−}) mouse lymphoma. Three independent experiments were performed: two in which cells were treated for 3 hours in the presence and absence of Aroclor-induced rat liver S9-mix, and a third in which cells were treated for 24 hours in the absence of S9-mix. All assays included appropriate vehicle (DMSO) and positive controls (methyl methanesulphonate and benzo[a]pyrene).

RESULTS: The maximum test concentrations examined for the 3- and 24-hour treatment period were limited to 85 µg/ml (in the presence and absence of S9-mix) by cytotoxicity. There were no clear increases in mutant frequency in any of the experiments compared with the concurrent vehicle controls.

CONCLUSION: Dolutegravir was not genotoxic in the mouse lymphoma L5178Y (TK^{+/−}) test system either in the presence or absence of S9-mix.

STUDY TITLE: **Micronucleus Assay in Rats Following Oral Dosing with Dolutegravir**

KEY FINDINGS: NEGATIVE.

STUDY NO: R27469, Report No. WD2007/00513/00

LABORATORY: GSK, UK

GLP: Yes

INITIATION DATE: 6/07

BATCH NO. R06001 (same as clinical batch)

METHODS: The potential of Dolutegravir to induce structural chromosome damage and aneuploidy in polychromatic erythrocytes was investigated in a rat bone marrow micronucleus assay. Dolutegravir was administered orally by gavage to male Sprague-Dawley rats (n=6/group) at doses of 0 (vehicle), 50, 150 or 500 mg/kg/day, for 2 consecutive 24 hour periods]. The vehicle used was 0.5% w/w aqueous hydroxypropyl methylcellulose with

	0.1% Tween 80. In addition to the vehicle control group, an untreated (undosed) control group comprising 6 male rats was included due to the non-standard nature of the vehicle. A positive control group (n=5 males) received a single oral dose of cyclophosphamide at 20 mg/kg. All animals were killed 24 hours following their final dose and femoral bone marrow smears were prepared (including the untreated control group), stained with acridine orange and analyzed by fluorescence microscopy.
RESULTS:	The data for the concurrent vehicle control were within the range determined from laboratory historical data. The positive control (cyclophosphamide) induced a clear unequivocal increase in the frequency of micronuclei. Therefore, the performance of the vehicle and positive control was consistent with a valid assay. No significant increase in micronucleated polychromatic erythrocytes was observed at any of the doses tested when compared to the concurrent vehicle control.
	The highest dose tested was the dose producing maximum systemic exposure, (C _{max} and AUC), of 87.1 ug/mL and 1360 ug.h/mL, respectively (TK not performed but was based on the Day 1 data from the rat 2-week oral toxicity study).
CONCLUSIONS:	Dolutegravir did not induce micronuclei in male rats in an in vivo bone marrow micronucleus assay following oral administration of doses up to 500 mg/kg/day
OVERALL CONCLUSIONS:	Overall, Dolutegravir was shown not to be mutagenic or clastogenic in the three tests presented above.

(3) REPRODUCTIVE TOXICITY STUDIES

Not yet performed.

(4) SECONDARY PHARMACOLOGY STUDIES

SAFETY PHARMACOLOGY RECEPTOR BINDING EFFECTS:	Secondary pharmacological profile has been evaluated by the sponsor in the following non-GLP secondary pharmacology studies, with its results summarized below.
	Dolutegravir was evaluated for possible interactions with 16 enzyme assays and 65 physiological receptor, ion channel and transporter binding sites and 12 isolated tissue assays. Dolutegravir at 10 uM did not significantly affect (defined as 250%) 80 of the 81 in vitro assays, and at 100 uM did not significantly affect any of the 12 tissue assays, except that a 64% inhibition of binding in the melanocortin (MC4) receptor was observed (related to body weight and food consumption).
hERG/QT	There is a 16.1% inhibition of hERG channel tail current in HEK-293 cells stably transfected with hERG cDNA at 20 uM (=8.38ug/ml; AUC=201.1 ug.h/ml). The effect is considered weak and should have no safety concern.
BP/HR, CNS & RESPIRATORY EFFECTS	Dolutegravir did not produce acute blood pressure/heart rate effects in conscious telemetered male monkeys or respiratory or neurobehavioural effects in conscious male rats at doses up to 1000 and 500 mg/kg, respectively.

(5) ANIMAL ADME STUDIES

ANIMAL PHARMACOKINETICS:	The absorption, distribution, excretion, and metabolism of Dolutegravir have been studied in vitro and in vivo in animals. A summary of the significant findings from these studies is presented below:
ABSORPTION:	Oral bioavailability ranged from 24.9% (non-fasted monkeys) to 51.5% (fasted rats) and half-life ranged from 5.24 hours (dogs) to 6.18 hours (rats).
RAT AND DOG TOXICOKINETICS	In rats, there was no increase in systemic exposure (C _{max} and AUC ₀₋₂₄) at doses above 500 mg/kg. In monkeys, AUC ₀₋₂₄ increases were less than dose-proportional from 50 to 500 mg/kg, while C _{max} did not increase at doses above 125 mg/kg. Volume of distribution at steady state and plasma clearance were low (V _d /Cl for rat= 103/0.23, dog =352/2.2 and monkey=279/2.1 [V _d : ml/kg; Cl: ml/min/kg]) in all species examined.
PROTEIN BINDING	The in vitro protein binding of Dolutegravir in human, monkey, dog and rat sera was 99.3, 99.1, 95.4 and 99.9%, respectively.
METABOLISM:	No metabolites were detected after incubation of the drug in rat, dog, monkey and human hepatocyte cultures, neither any significant of conversion of Dolutegravir to its enantiomer or two diastereomers were reported. The drug did not inhibit CYP 2D6, 2C9, 2C19 or 3A4 (in vitro data). However, dose-dependent decreases in total CYP content and CYP3A, CY12C11 and CY12B1 activities were observed in male rat liver samples obtained from a 14 day toxicity study. No changes in CYP activities were noted in female rats or in male and female monkeys.

(6) RISK ASSESSMENT BASED ON NON-CLINICAL TOXICOLOGY STUDIES ON Dolutegravir

≈ The toxicology portion of this IND included repeat-dose GLP studies up to two weeks in both rats and monkeys, three GLP mutagenicity studies, secondary pharmacology and ADME studies. Overall preclinical information submitted is considered adequate in support of the human trial proposed in this IND.

≈ Potential drug-induced toxicities and target organs of toxicity reported in this IND are as following:

- ^{3/4} **LETHAL DOSE:** ≥1000 mg/kg 2-week dog.
- ^{3/4} **LIVER TOXICITY (ENZYME ELEVATIONS):** ALT↑ (≥300 mg/kg 2-week dog[m]), ALT/AST↑/bilirubin↑ (1000 mg/kg 2-week dog study).
- ^{3/4} **LIVER TOXICITY (STRUCTURAL INJURY):** hepatocellular vacuolation/necrosis (1000 mg/kg 2-week dog study).
- ^{3/4} **THYMUS AND LYMPHOID TOXICITY:** thymus and mesenteric lymph node atrophy (≥300 mg/kg 2-week dog study); splenic white pulp atrophy/↓nucleated cell in bone marrow (1000 mg/kg 2-week dog study).
- ^{3/4} **KIDNEY TOXICITY:** renal tubule dilation (1000 mg/kg 2-week dog study).
- ^{3/4} **GI TOXICITY:** emesis/diarrhea, atrophy and mucosal hemorrhage of colon/rectum, atrophy of acinar cell of pancreas/parotid glands (≥300 mg/kg, 2-week dog study; stomach mucosal lesions at 1000 mg/kg); gastric mucosa glandular lesion (≥500 mg/kg, 2-week rat study).

^{3/4} **ADRENAL TOXICITY:** ↓fat droplets in zona fasciculata (≥300 mg/kg 2-week dog); zona fasciculata hypertrophy (1000 mg/kg 2-week dog study).

^{3/4} **HEMATOTOXICITY:** ↓reticulocyte (≥300 mg/kg males, 2-week dog study; 1000 mg/kg females) APTT/↓RBC/↓platelet (1000 mg/kg 2-week dog study).

**MARGIN OF SAFETY
USING BODY
SURFACE
CONVERSION**

Because of the lack of human pharmacokinetic data, the margin of safety will be estimated based on the body surface conversion method. The first table below summarizes NOAEL values and their exposures.

	NOAEL mg/kg	AUC (µg.h/mL)	HED mg/kg
14-Day Rat	500 (sponsor)	1830	79.4
	150 (FDA)	1445	23.8
14-Day Monkey	100	41	55.6

The following table provides margin of safety using the human equivalent dose (HED) comparisons:

Human Dose mg/person	Human Dose (mg/kg)	Safety Factor (14-Day Rat HED=79mpk, GSK)	Safety Factor (14-Day Rat HED=24mpk FDA)	Safety Factor (14-Day monkey HED=56)
10	0.2	474.0	144.0	336.0
25	0.4	189.6	57.6	134.4
50	0.8	94.8	28.8	67.2
100	1.7	47.4	14.4	33.6
200	3.3	23.7	7.2	16.8
400	6.7	11.9	3.6	8.4

Based on sponsor's target concentration (in vitro 41 ng/ml x 10=0.4 ug/ml), the margin of safety appears to be:

Target AUC=9.6 ug.h/ml) (Target conc: 0.4 ug/mlx24h =9.6ug.h/ml)	AUC=1830 at 500 mpk (14-Day Rat, GSK)	AUC=1445 at 150 mpk (14- Day Rat, FDA)	AUC=41 at 100 mpk (14- Day monkey)
Margin of safety->	191	151	4.3

**CONCLUSIONS &
REGULATORY
ACTIONS**

By using the body surface conversion method, the safety margins ranges from 4-12 (by using rat data as a reference) or approximately 8.4 (dog data) for the intended maximal dose proposed at 400 mg/day, and >100 for the lowest dose proposed (10 mg/day).

Because (1) Dolutegravir will be administered only for a single dose, and (2)

**COMMENTS TO
SPONSOR**

the primary relevant target organs of toxicity can be effectively monitored for its function, this IND is considered safe and the proposed protocol should be allowed to proceed.

General Comments

The Agency does not concur with rat's NOAEL chosen by the sponsor in the Investigator's Brochure, as stomach toxicity (mucosal lesions) occurred at 500 mg/kg in rats were evident and not dismissable.

Specific Pharm/Tox Requests

1. The sponsor should perform immunotoxicity testing according to guidance documents in the FDA website.
2. Please incorporate recovery group in future longer term toxicity studies to provide information on reversibility of significant drug induced toxicities.
3. The sponsor should provide a Gantt chart showing progression of the animal toxicity study plan, especially the reproductive toxicity studies, in reference to future human trials on Dolutegravir.

Appendix 2
Executive Carcinogenicity Assessment Committee
February 26, 2013 Meeting Minutes

Executive CAC

Date of Meeting: February 26, 2013

Committee: David Jacobson-Kram, Ph.D., DABT, OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Whitney Helms, Ph.D., DHOT, Alternate Member Hanan
Ghantous, Ph.D., DABT, DAVP Team Leader Mark
Seaton, Ph.D., DABT, DAVP Presenting Reviewer

Author of Draft: Seaton

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #204-790

Drug Name: Dolutegravir

Sponsor: ViiV Pharmaceuticals

Background:

The new drug application (NDA) package for dolutegravir (DTG) has been submitted to the Agency. The applicant is seeking marketing approval for the drug as a treatment for HIV infection in combination with other antiretroviral agents in adults and children). The proposed dosage for DTG is 50 mg once daily in treatment naïve and treatment experienced INI- naïve, HIV-infected adults and INI- naïve children 12 to 18 years old, weighing ≤ 40 kg. The DTG dose is 50 mg twice daily in INI-resistant subjects. The applicant has conducted two-year carcinogenicity studies in mice and rats. The two studies are presented below.

Mouse Carcinogenicity Study

Dolutegravir carcinogenicity in CD-1 mice was assessed at doses of 0 (water), 0 (0.5% HPMC/0.1% Tween 80 in water), 7.5, 25 and 500 mg/kg/day by oral gavage. Dose selection was based on saturation of absorption and concern over GI effects over the long term. Dose spacing was based on AUC. In female mice, the difference in survival between the high dose group animals and the vehicle control animals was statistically significant ($p = 0.0321$). No drug-related tumors were observed in mice. Systemic exposures at the 7.5, 25 and 500 mg/kg/day doses were 3x, 6x, and 18x in males, and 3x, 9x, and 22x in females than in humans at the recommended clinical dose of 50 mg QD, based on AUC.

Rat Carcinogenicity Study

Dolutegravir carcinogenicity in Sprague Dawley rats was assessed at doses of 0 (water), 0 (0.5% HPMC/0.1% Tween 80 in water), 2, 10, and 50 mg/kg/day by oral gavage. Dose selection was based on MTD (GI irritation and hemorrhage of the glandular stomach mucosa in males at 500 mg/kg/day) and saturation of absorption. The survival rate across the dose groups was similar to control. No drug-related tumors were observed in rats. Systemic exposures at the 2, 10, and 50 mg/kg/day doses were 2x, 6x, and 13x in males, and 5x, 14x, and 21x in females than in humans at the recommended clinical dose of 50 mg QD, based on AUC.

Executive CAC Recommendations and Conclusions:

Mouse:

- The Committee agreed that the study was adequate, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms.

Rat:

- The Committee agreed that the study was adequate, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\

- /Division File, DAVP
- /HGhantous, DAVP
- /Seaton, DAVP
- /Mosaddegh, DAVP
- /ASeifried, OND IO

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electronic record that was signed
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signature.

/s/

ADELE S SEIFRIED
02/27/2013

DAVID JACOBSON KRAM
02/28/2013

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/s/

MARK J SEATON
05/06/2013

HANAN N GHANTOUS
05/08/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 204-790 Applicant: ViiV Healthcare

Stamp Date: 12/17/12

Drug Name: Dolutegravir NDA/BLA Type: NME

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
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 <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

**IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION
FILEABLE?** Yes

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

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

None

Reviewing Pharmacologist	Date
--------------------------	------

Team Leader/Supervisor	Date
------------------------	------

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

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/s/

MARK J SEATON
01/28/2013

HANAN N GHANTOUS
01/28/2013