CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

209394Orig1s000

NON-CLINICAL REVIEW(S)

Comments on NDA209394 Glecaprevir/Pibrentasvir

From: A. Jacobs, AD

Date 5/15/17

1. I concur that there no pharm-tox approval issues
2. I have conveyed some other comments, including suggestions for labeling to the reviewer and supervisor, and they will address them as appropriate.

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/s/

ABIGAIL C JACOBS
05/15/2017

FDEPARTMENT 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:	209394
Supporting document/s:	1
Applicant's letter date:	12/14/2016
CDER stamp date:	12/14/2016
Product:	Glecaprevir/Pibrentasvir Glecaprevir: ABT-493 or A-1282576 and Pibrentasvir: ABT-530 or A-1325912
Indication:	Treatment of chronic hepatitis C virus (HCV) infection
Applicant:	AbbVie, Inc.
Review Division:	Division of Antiviral Products
Reviewer:	Ilona G. Bebenek, Ph.D., DABT
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
Division Director:	Debra Birnkrant, M.D.
Project Manager:	Alicia Moruf, PharmD

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1 Executive Summary

1.1 Introduction

The applicant is seeking marketing approval for Glecaprevir/Pibrentasvir; Gle/Pib, consisting of: glecaprevir, a nonstructural (NS) protein 3/4A protease inhibitor and pibrentasvir an inhibitor of the NS5A protein. Gle/Pib would be marketed as a treatment of chronic HCV genotype 1-6 infection in adults, including those with cirrhosis, who are either treatment-naïve or previously treated with interferon, pegylated interferon (pegIFN) and/or ribavirin. The proposed daily dosages for the components of Gle/Pib are three Gle 100 mg/Pib 40 mg tablets QD with food (total daily dose of 300 mg/120 mg Gle/Pib). The applicant has submitted a complete nonclinical package consisting of studies in mice, rats, rabbits, monkeys and dogs.

1.2 Brief Discussion of Nonclinical Findings

Glecaprevir (Gle): Gle was not associated with clinically relevant adverse effects on cardiovascular, neurological, or respiratory endpoints evaluated in safety pharmacology studies. Clinically relevant adverse effects were also not observed in pivotal repeat-dose general toxicology studies; clinically relevant non-adverse effects included elevated ALT and GGT levels in dogs; increases in both ALT and GGT were observed in clinical trials. Gle was not considered genotoxic based on negative results in the in vitro bacterial mutation assay, in vitro mammalian chromosome aberration assay, and in vivo rat micronucleus assay. Because Gle was not genotoxic and will be administered to humans for only up to 16 weeks, carcinogenicity studies were not required. There were no clinically relevant adverse effects on male or female fertility, embryofetal development, or pre/post-natal development in rats.

Pibrentasvir (Pib): Pib was not associated with clinically relevant adverse effects on cardiovascular, neurological, or respiratory endpoints evaluated in safety pharmacology studies. Clinically relevant adverse effects were also not observed in pivotal repeat-dose general toxicology studies. Pib was not considered genotoxic based on negative results in the in vitro bacterial mutation assay, in vitro mammalian chromosome aberration assay, and in vivo rat micronucleus assay. Because Pib was not genotoxic and will be administered to humans for only up to 16 weeks, carcinogenicity studies were not required. There were no clinically relevant adverse effects on male or female fertility in mice, embryofetal development in mice or rabbits, or pre/post-natal development in mice.

No significant toxicological findings were noted in a four-week combination study with Gle and Pib conducted in rats.

1.3 Recommendations

1.3.1 Approvability

The submitted nonclinical data is sufficient to support marketing application approval.

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies are recommended.

1.3.3 Labeling

The Sponsor's proposed product label is under review.

2 Drug Information

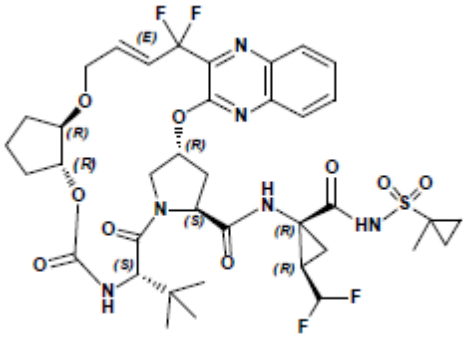
2.1 Drug

Generic Name	Glecaprevir
Code Name	ABT-493; A-1282596

Chemical Name: (3aR,7S,10S,12R,21E,24aR)-7-tert-butyl-N-[(1R,2R)-2-(difluoromethyl)-1-{[(1-methylcyclopropyl)sulfonyl] carbamoyl}cyclopropyl]-20,20-difluoro-5,8-dioxo- 2,3,3a,5,6,7,8,11,12,20,23,24a-dodecahydro-1H,10H-9,12-methanocyclopenta[18,19][1,10,17,3,6] trioxadiazacyclononadecino[11,12-b]quinoxaline-10-carboxamide

Molecular Formula/Molecular Weight	C38H46F4N6O9S/ 838.87

Structure or Biochemical Description



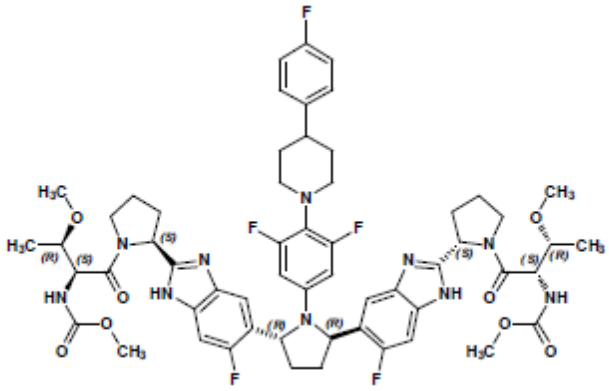
Pharmacologic Class	Nonstructural protein 3/4A (NS3/4A) protease inhibitor (PI).
Generic Name	Pibrentasvir

Code Name ABT-530; A-1325912

Chemical Name: Methyl {(2S,3R)-1-[(2S)-2-{5-[(2R,5R)-1-{3,5-difluoro-4-[4-(4 fluorophenyl)piperidin-1-yl]phenyl}-5-(6-fluoro-2-{(2S)-1-[N-(methoxycarbonyl)-O-methyl-Lthreonyl] pyrrolidin-2-yl}-1H-benzimidazol-5-yl)pyrrolidin-2-yl]-6-fluoro-1Hbenzimidazol-2-yl}pyrrolidin-1-yl]-3-methoxy-1-oxobutan-2-yl}carbamate

Molecular Formula/Molecular Weight C57H65F5N10O8/ 1113.18

Structure or Biochemical Description



Pharmacologic Class Nonstructural protein 5A (NS5A) inhibitor.

2.2 Relevant INDs, NDAs, BLAs and DMFs

AbbVie evaluated these direct acting agents (DAAs) for chronic hepatitis C under the following INDs: IND 116169 (ABT-493) and IND 116170 (ABT-530)

2.3 Drug Formulation

Gle/Pib Film-Coated Tablets, 100mg/40mg, are immediate release bilayer tablets containing 100 mg Gle in one layer and 40 mg Pib in the other layer.

Table 1 Composition of Gle/Pib Film-Coated Tablets 100 mg/40 mg

(b) (4)

Table 2 Total Excipient Quantities per Tablet

Component	Amount per Tablet (mg)
(b) (4)	
Copovidone, Type K 28	(b) (4)
Vitamin E (Tocopherol) Polyethylene Glycol Succinate (TPGS)	
Colloidal Silicon Dioxide (b) (4)	
Propylene Glycol Monocaprylate, Type II	
Croscarmellose Sodium	
Sodium Stearyl Fumarate	
(b) (4)	

2.4 Comments on Novel Excipients

Toxicology data (bacterial mutation assay, 28-day oral rat study and EFD study in rat) are provided for Capryol 90 (propylene glycol monocaprylate), an excipient in the glecaprevir/pibrentasvir film-coated tablets. These data demonstrate the low level of toxicity of Capryol 90 and provide support that the maximal daily intake of (b) (4) mg is not expected to present a human health risk. A summary of these studies is located under Special Toxicology Studies in Appendix 1.

Tocopherol (Vitamin E) will be present at (b) (4) mg/tablet (b) (4) mg total per day). According to the inactive ingredients database, the highest levels approved are at 42.5 mg/tablet. According to the National Institutes of Health, the upper tolerable intake limit of tocopherol is 1000 mg per day.[1] As such, the daily intake of tocopherol from Gle/Pib tablets will be below the upper tolerable intake limit at (b) (4) mg per day.

2.5 Comments on Impurities/Degradants of Concern

See APPENDIX 3

[1] https://ods.od.nih.gov/factsheets/VitaminE-HealthProfessional/#h7

2.6 Proposed Clinical Population and Dosing Regimen

Gle/Pib is indicated for the treatment of hepatitis C viral infection, including in patients with cirrhosis. The recommended adult oral dose is three 100 mg/40 mg tablets with food (total daily dose of 300 mg/120 mg). The duration of treatment with Gle/Pib is dependent on the patient population as described below:

Table 3 Recommended Gle/Pib Duration for Treatment Naïve Patients

2.7 Regulatory Background

These drugs were previously reviewed under INDs 116169 (Gle) and 116170 (Pib).

APPENDIX 1. ABT-493/A-1282596

Studies Reviewed

Secondary Pharmacology
Study RD 12700. A-1282576 Off-Target Binding Assays

Safety Pharmacology
Study RD 111267. A neurobehavioral safety evaluation of orally administered A-1282576 (ABT-493) in rats.

Study RD 11999. A-1282576.0 CNS/neurobehavioral safety pharmacology profile in the rat (p.o. administration).

Study RD 12698. A-1282576: in vitro effects on hERG current

Study RD 12643. Effects of A-1282576.0 on cardiovascular and hemodynamic function in the anesthetized dog.

Study RD 111269. A cardiovascular safety evaluation of orally administered A-1282576 (ABT-493) in beagle dogs.

Study RD 111268. A respiratory safety evaluation of orally administered A-1282576 (ABT-493) in rats.

Pharmacokinetics/ADME
Study RD 12621. Absorption, Distribution, Metabolism and Excretion of [14C]A-1282576 in Male Sprague Dawley Rats

Study A-1282576 Absorption Memo 2. A-1282576 Pharmacokinetics following Intravenous or Oral Dosing in Mouse

Study A-1282576 Absorption Memo 3. A-1282576 Pharmacokinetics following Intravenous or Oral Dosing in Rat

Study A-1282576 Absorption Memo 4. A-1282576 Pharmacokinetics following Intravenous or Oral Dosing in Monkey

Study A-1282576 Absorption Memo 5. A-1282576 Pharmacokinetics following Intravenous or Oral Dosing in Dog

Study RD 12622. Preclinical Pharmacokinetic Summary of A-1282576 Single Dose Studies in Mouse, Rat, Dog and Monkey

Study RD 140755. Quantitative Whole-Body Autoradiography of Pigmented Rats Following Oral Administration of 14C-ABT-493

Study RD 160244. Lacteal Excretion, Placental Transfer, and Tissue Distribution of Radioactivity in Pregnant Female Sprague Dawley Rats after Oral Administration of 14C-ABT-530 and 14C-ABT-493

Study RD 160374. Determination of the Unbound Fraction of A-1282576 in Plasma and Microsomal Protein and Blood-to-Plasma Concentration Ratios.

Study RD 12620. In Vitro Biotransformation of A-1282576

Study A-1282576 Drug Metabolism Memo 7. Determination of the Permeability and Transport of A-1282576 using the MDCK-MDR1 Model

Study RD 12619 Determination of the Binding of A-1282576 to Human and Animal Plasma Proteins

Study A-1282576 Drug Metabolism Memo 11. The Blood Cell Partitioning of A-1282576 in Mouse, Rat, Monkey, Dog and Human

Study A-1282576 Drug Metabolism Memo 6. Determination of the Metabolic Stability of A-1282576 in Liver Microsomes and Hepatocytes across Species

Study A-1282576 Drug Metabolism Memo 10. Assessment of the Enzymes Involved in the Metabolism of [14C]A-1282576 Using Recombinant Enzymes

Study RD 12617. Assessment of the Effect of A-1282576 on the Activity of Cytochrome P450 (CYP450) Isoforms in Human Liver Microsomes

Study RD 12618. Assessment of CYP Time Dependent Inhibition Potential by A-1282576 in Human Liver Microsomes

Study A-1282576 Drug Metabolism Memo 9. An In Vitro Investigation of Cytochrome P450 Induction by A-1282576 in Cultured Human Hepatocytes

Study A-1282576 Drug Metabolism Memo 13. Uptake of A-1282576 by Organic Anion Transporting Polypeptide (OATP) 1B1 and 1B3

Study A-1282576 Drug Metabolism Memo 8. Inhibitory Interaction of A-1282576 on Organic Anion Transporting Polypeptide (OATP) 1B1 and 1B3, Organic Cation Transporter (OCT) 2, and Organic Anion Transporter (OAT) 1 and 3

Study A-1282576 Drug Metabolism Memo 22. Preliminary A-1282576 (ABT-493) Metabolite Identification in Plasma after Multiple Oral Dosing in Dog

Study RD 151003. Study A-1282576 Drug Metabolism Memo 22. A-1282576: In Vitro Drug Transporter Assessment

Study RD 151004. Pharmacokinetic Drug-Drug Interactions: Metabolism and Transporters. 2016.

Study A1282576 Drug Metabolism Memo 20. Metabolism and Disposition of [14C]A-1282576 (ABT-493) after Single 60 mg/kg Oral Dose in Rat. 2015.

Study A-1282576 Metabolism Memo 22. Preliminary Metabolite Identification of A-1282576 (ABT-493) in Plasma after Multiple Oral Dosing in Sprague-Dawley Rats

Study A1282576 Drug Metabolism Memo 24. Metabolism and Disposition of [14C]A-1282576 (ABT-493) after a Single 30 mg/kg Oral Dose in Dog. 2016.

Study RD 151004Pharmacokinetic Drug-Drug Interactions: Metabolism and Transporters. 2016.

Study A-1282576. Drug Metabolism Memo 25. Plasma concentrations of A-1282576 and A-1325912 after Oral Co-Dosing in Sprague Dawley Rats.

Study RD 13482. Assessment of the Effect of A-1282576 on the Activity of UDP-glucuronosyltransferases (UGT) Isoforms in Human Liver Microsomes. 2014.

Study RD 16-0373. Assessment of Inhibitory Effects on Drug Metabolizing Enzyme Activity by A-1282576

Single and Repeat-Dose Toxicology

Study RD 11575. Two-Week Oral Dose Range-Finding Toxicity Study of A-1282576 Free Acid in Sprague-Dawley Rats (Non-GLP Study)

Study RD 11930. Thirteen-Week Oral Toxicity Study of A-1282576 Free Acid in Sprague-Dawley Rats with a Four-Week Recovery Period

Study RD 11535. Two-Week Oral (Capsule) Dose Range-Finding Toxicity Study of A-1282576 in Beagle Dogs (Non-GLP)

Study RD 11925. Thirteen-Week Oral (Capsule) Toxicity Study of A-1282576 in Beagle Dogs (With a Four-Week Recovery Period)

Study RD 140001. A-128276 Free Form: A 26-Week BID Oral Dose Toxicity Study in Sprague-Dawley Rats (CRL:SD) with a 4-Week Recovery Period

Study RD 140002. A-1282576 : A 39-Week BID Oral Dose Toxicity Study of in Beagle Dogs With an 8-Week Recovery Period

Genotoxicity

Study RD 12270. Bacterial Reverse Mutation Assay with A-1282576 Free Form

Study RD 12271. In Vitro Mammalian Chromosome Aberration Test with A-1282576 Free Form

Study RD 13002. In Vivo Micronucleus Assay in Rats with A-1282576 Free Form

Study RD 16-0433. Assessment of Cytochrome P450 mRNA Induction by A-1282576 in Cultured Human Hepatocytes

Reproductive and Developmental Toxicology

Study RD 140683. An Oral Fertility Study with A-1282576 in Rats

Study RD 13841. A-1282576: An Oral Developmental Toxicity Study in Rabbits, Including a Toxicokinetic Evaluation

Study RD 13679. A-1282576: An Oral Developmental Toxicity Study in Rabbits, Including a Toxicokinetic Evaluation

Study RD 13679. A-1282576: An Oral Developmental Toxicity Study in Rabbits, Including a Toxicokinetic Evaluation

Study RD 13913. A-1282576: An Oral Developmental Toxicity Study in Rats, Including a Toxicokinetic Evaluation

Study RD 160239. A Developmental and Perinatal/Postnatal Reproduction Study of A-1282576 by Oral (Gavage) in Rats, Including a Postnatal Behavioral/Functional Evaluation

Special Toxicology Studies

Study RD 13488. Neutral Red Uptake Phototoxicity Assay of A-1282576 Free Form in Balb/c 3T3 Mouse Fibroblasts

Study RD 13803. A Repeat Dose Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of A-1282576 Free Form on Eyes and Skin in Pigmented Rats

Study RD 160701. Capryol 90- Bacterial reverse mutation test (Plate incorporation)

Study RD 160094. A Study of the Effects of Capryol 90 on Embryo/Fetal Development in Rats

Study RD 160093. A 28-Day Oral (Gavage) Toxicity Study of Capryol 90 in Rats

Impurities

Study RD 140675. Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with (b) (4)

(b) (4) Study 8300181, AbbVie Study TX14-074. 2014.

Study RD 150776. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Component of the Synthetic Route of ABT-493. AbbVie Study TX15-228. 2015.

Study RD 150158. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Component of the Synthetic Route of ABT-493. AbbVie Study TX15-054. 2015.

Study RD 150881. 6-Well Bacterial Reverse Mutation Assay with (b) (4); Component of the Synthetic Route of ABT-493. AbbVie Study TX15-246. 2015.

Study RD 160245. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Components of the Synthetic Route of ABT-493. AbbVie Study TX16-072. 2016.

Study RD 160477. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Components of the Synthetic Route of ABT-493. AbbVie Study TX16-117. 2016.

Study RD 160552. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Component of the Synthetic Route of ABT-493. AbbVie Study TX16-120. 2016.

Study RD 160561. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Components of the Synthetic Route of ABT-493. AbbVie Study TX16-130. 2016.

Study RD 160634. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Components of the Synthetic Route of ABT-493. AbbVie Study TX16-140. 2016.

Study RD 160682. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Components of the Synthetic Route of ABT-493. AbbVie Study TX16-142. 2016.

Study RD 160721. 6-Well Bacterial Reverse Mutation Assay with (b) (4); Component of the Synthetic Route of ABT-493. AbbVie Study TX16-165. 2016.

Study RD 160822. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Component of the Synthetic Route of ABT-493. AbbVie Study TX16-177. 2016.

Study RD 160872. 6-Well Bacterial Reverse Mutation Assay with (b) (4); Components of the Synthetic Route of ABT-493. AbbVie Study TX16-194. 2016.

Study RD161006. 6-Well Bacterial Reverse Mutation Assay with (b) (4); Component of the Synthetic Route of ABT-493. AbbVie Study TX16-206. 2016.

Study RD 140063. 5-Strain MiniAmes Test with Components of the Synthetic Route of (b) (4). AbbVie Study TX14-041. Amended 2016.

Study RD 140416. 5-Strain MiniAmes Test with (b) (4) Components of the Synthetic Route of ABT-493. AbbVie Study TX14-141. 2014.

Study RD 11748. Miniscreen Ames Test with (b) (4) Impurity of A-1282576. AbbVie Study TX11-192. 2011.

Study RD 12942. MiniAmes Test with (b) (4) Starting Material of (b) (4) AbbVie Study TX12-138. Amended 2016.

Studies not reviewed

Studies considered irrelevant for the nonclinical safety assessment were not reviewed (e.g. analytical methodology, PK comparison for different formulations, etc.). Also, repeat-dose toxicity studies with CByB6F1-Tg(HRAS)2JIC (TgHras) wild-type mice were conducted with glecaprevir and pibrentasvir to prepare for carcinogenicity studies in case those were needed. However, carcinogenicity studies were not conducted since no causes for concern were identified in genotoxicity or general toxicity studies and since the optimal treatment duration for the proposed combination of glecaprevir and pibrentasvir is expected to be less than 6 months (anticipated duration ≤16 weeks). As such, studies with CByB6F1-Tg(HRAS)2JIC (TgHras) wild-type mice were not reviewed. Exploratory rat studies with pibrentasvir were also not reviewed.

All tables in this review are excerpted from the Sponsor's reports.

Pharmacology

Primary Pharmacology

Refer to Virology review for more details.

Secondary Pharmacology

In a panel of off-target binding assays, A-1282576 (10 μM) did not displace control specific binding by greater than 50% at any of 79 receptors, ion channels, or transporters. The GABA-gated Cl receptor was inhibited 45%.

Safety Pharmacology

CNS and neurobehavioral effects of A-1282576 were assessed in a modified Irwin Functional Observation Battery assay, as well as in assays for spontaneous locomotor activity, pro-convulsant activity, and ethanol interaction in rats.

In Irwin, spontaneous locomotor activity, pro-convulsant activity, and ethanol interaction assays in rats, oral administration of A-1282576 produced no effects through the highest oral dose of 100 mg/kg. A-1282576 was also tested in a GLP Functional Observational Battery assay in rats. A-1282576 was administered to female rats at oral doses of 5, 20 and 60 mg/kg (n = 8/group). A-1282576 did not produce any neurobehavioral effects through the highest oral dose of 60 mg/kg.

Potential effects of A-1282576 on the QT repolarization interval were assessed in the in vitro hERG assay. At the limit of solubility (24.70 μg/mL), hERG tail current was significantly reduced (28.8%).

In anesthetized dogs, A-1282576 had no effect on cardiovascular parameters through the highest plasma concentration of 110 μg/mL. In non-anesthetized, telemeterized dogs, oral doses of 10, 30, or 100 mg/kg A-1282576 had no effect on measured parameters. At 100 mg/kg the associated plasma concentration was 85.8 μg/mL.

The effects of A-1282576 on respiratory function in rats were assessed following oral administration of 10, 20, or 60 mg/kg. No effect on respiratory function was observed through 20 mg/kg (C_{max} = 31.1 μg/mL). A dose of 60 mg/kg (C_{max} = 56.7 μg/mL) slightly increased respiratory rate and decreased tidal volume; total ventilatory capacity (minute volume) was not affected at any dose.

Pharmacokinetics/ADME/Toxicokinetics

Absorption

The pharmacokinetic behavior of A-1282576 was evaluated in CD-1 mice, Sprague-Dawley rats, cynomolgus monkeys and beagle dogs. The pharmacokinetics were characterized by plasma elimination half-lives which ranged from 1.7 hours in monkey to 3.7 hours in dog. Volumes of distribution (Vss) were moderate in rat and dog (0.32-0.6 L/kg), with higher distribution volumes in mouse (3.3 L/kg) and monkey (1.2 L/kg). Plasma clearance values were high in monkey (1.7 L/hr●kg), dog (0.99 L/hr●kg) and mouse (1.6 L/hr●kg), but low in rat (0.21 L/hr●kg).

A-1282576 was rapidly absorbed from a solution formulation after oral dosing (T_{max} < 1 hr) in mouse, rat and dog, with slower absorption in monkey (T_{max} = 2.8 hr). A-1282576

bioavailability values from a solution formulation were >90% in mouse and rat, but were lower in dog (44%) and monkey (26%). Plasma concentrations obtained from the optimized lipid based solution formulation showed roughly linear increases in AUC between 10-100 mg/kg in rat, rabbit and dog, but no significant increase in AUC with further increases in dose. There were no apparent sex differences in mice, rats and dogs, nor any apparent accumulation with multiple daily dosing in all three species.

Table 5 Pharmacokinetics of A-1282576 after a Single Dose in Multiple Species

Intravenous Dose – Plasma

Species	Dose (mg/kg)	$t_{1/2}$ (hr)	V_c (L/kg)	V_{ss} (L/kg)	V_β (L/kg)	$AUC_{0-\infty}$ (µg•hr/ml)	CL_p (L/hr•kg)	n
CD1 Mouse	5	2.7	0.9	3.3	6.2	3.1	1.6	3
FVB Mouse	1	0.9	0.7	1.9	3.3	0.39	2.6	3
Mdr1a/b-Bcrp KO	1	3.5	1.2	5.1	6.3	0.79	1.3	3
Oatp1a/b KO	1	1.8	0.14	0.25	0.33	7.83	0.13	3
Rat	5	2.8	0.08	0.32	0.84	24.8 (4.5)	0.21 (0.04)	3
Monkey	5	1.7	0.3	1.2	4.0	3.18 (0.91)	1.7 (0.5)	3
Dog	3	3.7	0.2	0.6	5.3	3.40 (1.50)	0.99 (0.37)	3

Intravenous Dose – Liver

Species	Dose (mg/kg)	$t_{1/2}$ (hr)	C_{max} (µg/g)	$AUC_{0-\infty}$ (µg•hr/g)	L/P Ratio	n
CD1 Mouse	5	4.3	65.5	518.6	167	3
FVB Mouse	1	4.6	12.5	25.8	66	3
Mdr1a/b-Bcrp KO	1	4.6	3.3	34.0	43	3
Oatp1a/b KO	1	1.4	5.1	20.7	2.6	3
Rat	1	-	-	-	288[a]	3
Dog	3	1.4	28.4 (12.2)	56.1 (7.6)	16	3

Oral Dose – Plasma

Species	Dose (mg/kg)	$t_{1/2}$ (hr)	C_{max} (µg/ml)	T_{max} (hr)	$AUC_{0-\infty}$ (µg•hr/ml)	F (%)	n
CD-1 Mouse	5	4.3	0.92	1.0	3.8	123	3
Rat	5	2.0	6.3 (1.2)	0.5 (0.0)	22.4 (2.0)	90.1 (8.1)	3
Monkey	5	2.0	0.26 (0.13)	2.8 (1.3)	0.83 (0.35)	26.0 (11.0)	3
Dog	3	1.7	0.79 (0.19)	0.8 (0.3)	1.49 (0.47)	43.9 (14.0)	3
	10 [f]	0.9	12.9 (7.5)	1.3 (0.3)	26.9 (19.4)		3
	10 [nf]	1.3	6.5 (7.3)	1.3 (0.8)	12.4 (13.0)		3

Data provided as mean (standard deviation); L/P – liver to plasma AUC ratio
f - fasted dogs
nf - non-fasted (fed) dogs
a. liver to plasma ratio 24-hr after dosing (single time point)

Distribution

A-1282576 demonstrated extensive liver distribution in mouse, rat and dog, with the liver exposure 10-100 fold higher than the plasman exposure. In a quantitative whole-body autoradiography (QWBA) study, distribution of radioactivity to tissues was extensive 0.5 hours after a 5 mg/kg oral dose of [14C]glecaprevir ([14C] label inside the macrocyclic ring) to pigmented male Long-Evans rats. Maximum concentrations occurred 0.5-2 hours post-dose for most tissues. The liver contained the highest concentrations of radioactivity observed for any tissue from 0.5 through 96 hours post-dose. Radioactivity concentrations were below measurable levels in the arterial walls and lens of the eye at all collection times throughout this study. [14C]Glecaprevir-derived radioactivity showed no apparent affinity for tissues containing melanin. The elimination of radioactivity from all tissues was virtually complete by 24 hours post-dose, with only the liver, large intestine and small intestine having measurable levels of

radioactivity; radioactivity was not detected in any tissue, blood or urine at 168 and 192 hours post-dose. A-1282576 had average blood-to-plasma ratios (B/P) of 0.64, 0.60, 0.55, 0.75 and 0.57 in mouse, rat, dog, monkey and human, respectively.

Following a 5 mg/kg oral dose of [14C]glecaprevir ([14C] label inside the macrocyclic ring to pregnant Sprague-Dawley rats on Day 18 of gestation, radioactivity distributed into fetal tissues with the greatest concentrations observed in fetal liver and blood. Minimal amounts of radioactivity were detected in fetal brain, kidney and the residual fetus. Peak radioactivity for most maternal tissues occurred at 0.5 hours postdose, with liver the only observed exception when C_{max} occurred at 4 hours post-dose. Elimination of [14C]glecaprevir-derived radioactivity from maternal liver, placenta, plasma, uterus and amniotic fluid was not complete by 72 hours post-dose after a single oral gavage dose of [14C]glecaprevir.

Metabolism and Excretion

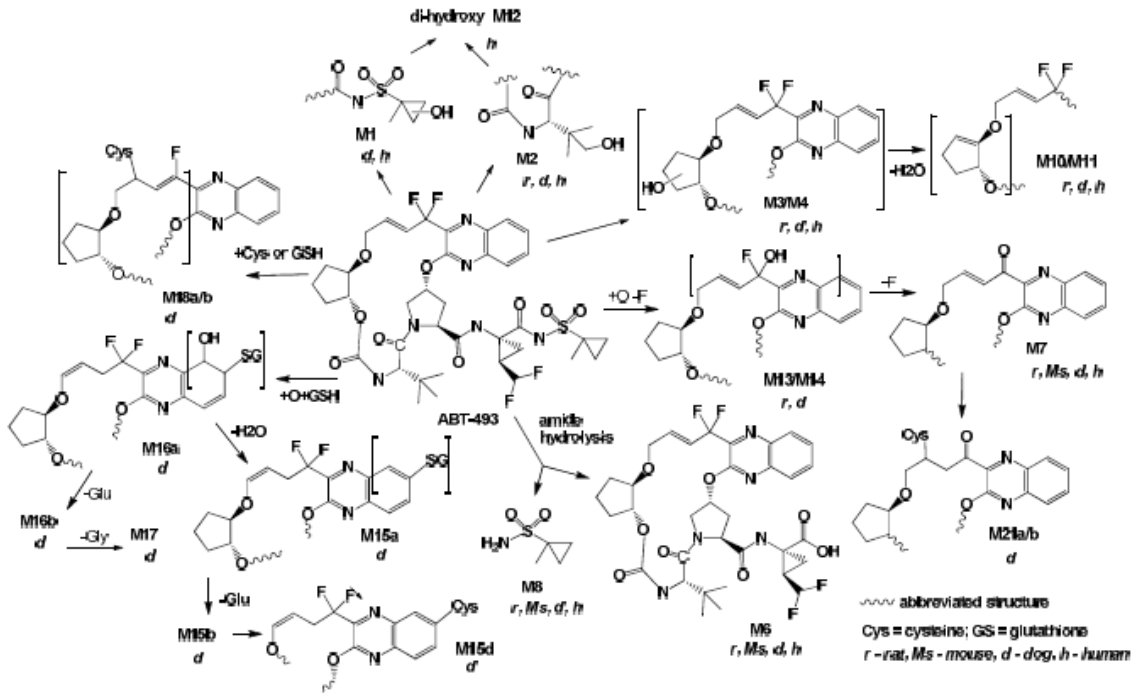
Metabolism of A-1282576, though limited, was primarily by CYP3A4/5. All the human metabolites formed in the *in vitro* hepatic system were present in one or more animal species. Excretion was mainly via the biliary/fecal route.

Table 6 Glecaprevir (A-1282576) Metabolite Profile in Plasma at Steady State

	Mouse (a)	Rat (b)	Dog (c)	Human (d)
Glecaprevir (parent drug)	**major**	**major**	**major**	**major**
M1 (monohydroxy)	-	-	minor	minor
M2 (monohydroxy)	-	minor	minor	minor
M3 (monohydroxy)	-	minor	minor	minor
M4 (monohydroxy)	-	minor	minor	minor
M6 (amide hydrolysis)	minor	minor	minor	minor
M7 (defluorination & oxidation)	minor	minor	minor	minor
M10 (dehydrogenation)	-	minor	minor	minor
M11 (dehydrogenation)	-	minor	minor	minor
M12 (dihydroxylation)	-	-	-	minor
M13 (defluorination & hydroxylation)	-	minor	minor	-
M14 (defluorination & hydroxylation)	-	minor	minor	-
M15a (GSH conjugate)	-	-	minor	-
M15b (GSH conjugate degradant)	-	-	minor	-
M15d (Cys conjugate)	-	-	minor	-
M16a (oxidation & GSH conjugate)	-	-	minor	-
M16b (oxidation & GSH conjugate degradant)	-	-	minor	-
M17 (hydroxylation & Cys conjugate)	-	-	minor	-
M18a (defluorination & Cys-conjugate)	-	-	minor	-
M18b (defluorination & Cys-conjugate)	-	-	minor	-
M21a (Cys-conjugate of M7)	-	-	minor	-
M21b (Cys-conjugate of M7)	-	-	minor	-

Designations of major and minor reflect the FDA MIST guidelines, where major is defined as >10% of total drug related materials in plasma and minor is defined as <10% of total drug related materials in plasma.
(**a**) 75 mg/kg BID x 7 days; (**b**) 60 mg/kg BID x 5 days; (**c**) 100 mg/kg BID x 273 days;
(**d**) M13-356: 800 mg QD x 10 days
M8 was not detected in the HPLC-MS analysis of plasma samples at steady state across species.

Table 7 Proposed Biotransformation Path of Glecaprevir (A-1282576) Based on Metabolites Observed in Plasma of Mouse, Rat, Dog and Human



Inhibition or induction of CYP enzymes is not expected to have a major impact on A-1282576 exposures. At the clinical dose, A-1282576 has the potential to inhibit intestinal CYP3A4. The minimal inhibition and induction of other major human CYPs at clinically relevant exposures indicates a low potential for significant drug-drug interactions with A-1282576 as a perpetrator drug.

The metabolite profiles were studied in milk and plasma, following a single 5 mg/kg oral dose of [14C]A-1282576 to lactating female rats. [14C]A-1282576 represented the majority of total radioactivity in both rat milk (96.5%) and plasma (100%). One minor uncharacterized radiochemical component U1 was also detected in rat milk.

Transporters

There is a potential for transporter mediated DDI given that A-1282576 is a substrate and inhibitor of hepatic uptake (OATP1B1 and 1B3) and efflux transporters (P-gp and BCRP). A-1282576 is an inhibitor of UGT1A1.

Co-Dosing

To support combination toxicology studies, the Gle and Pib pharmacokinetics following oral co-dosing in male Sprague-Dawley rats were evaluated. The formulations were prepared from tablets (Gle/Pib co-formulated tablets and Pib tablets) (b) (4) suspended in 0.5% HPMC for oral administration, with the intent to define

doses which would provide AUCs of both compounds within 2-4-fold of the target clinical exposure (target Gle exposure: ~11 µg•hr/mL; target Pib exposure: 1.2 µg•hr/mL). The 12.5 mg/kg dose of Gle in combination with the 20 mg/kg dose of Pib (5 mg/kg from the bilayer tablet + 15 mg/kg from the (b) (4) Pib tablets) was selected as the dose which provided AUCs for each compound ~2-4-fold above the clinical exposures.

General Toxicology

Single-Dose Toxicity

None conducted

Repeat-Dose Toxicity

Two-Week Oral Dosing Studies of A-1282576 Free Acid in Sprague Dawley Rats (RD 11575) Five Sprague Dawley rats per sex per group were administered 20, 60, or 300 mg/kg BID (total doses 40, 120, 600 mg/kg/day) A-1282576 via oral gavage for 14 or 15 days. Inflammatory changes such as neutrophilic infiltration and ulceration were noted in stomachs of animals at 600 mg/kg/day. The changes were considered non adverse, as they are due to the local irritation rather than systemic toxicity. However, such findings are potentially dose-limiting. The NOAEL was defined as the high dose, 600 mg/kg, corresponding to Day 14 systemic exposures of 543 µg*hr/mL in rats (males and females combined). However, higher systemic exposures were achieved at the middle dose (120 mg/kg; AUC = 739 µg*hr/mL).

Study title: Thirteen-Week Oral Toxicity Study of A-1282576 Free Acid in Sprague-Dawley Rats with a Four-Week Recovery Period (RD 11930)

Study no.:	TA11-141
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07 September 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576 free base (A-1282576, A-1282576.0, ABT-493), 07684ZW01, 93.7%

Key Study Findings

There were no toxicologically significant findings. The No-Observed-Adverse-Effect-Level (NOAEL) was the high dose (120 mg/kg/day), corresponding to day 91 systemic exposures of 580 µg•hr/mL (AUC_{0-24}; males and females combined).

Methods

Doses:	5, 20 and 60 mg/kg BID (10, 40, or 120 mg/kg/day)
Frequency of dosing:	BID for 91 days
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	70% Polyethylene glycol (PEG 400):20% Tween 20:10% Poloxamer 124
Species/Strain:	Rats/ Sprague Dawley
Number/Sex/Group:	15
Age:	9 weeks
Weight:	188-361 grams
Satellite groups:	5/sex/group for four week recovery
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: Rats were observed for survival and clinical signs twice a day during dosing. Detailed observations were made six to eight hours after dosing at least twice a week. There were no unscheduled deaths.

Clinical Signs: Detailed clinical observations were recorded twice per week during dosing. There were no remarkable findings.

Body Weights: Body weights were recorded twice weekly during dosing. There were no remarkable findings.

Feed Consumption: Food consumption was recorded once weekly during dosing. There were no remarkable findings.

Ophthalmologic Examination: Ophthalmic exams were performed on all rats pretreatment and at the end of dosing on group 1 and 4 rats. There were no remarkable findings.

Hematology/Coagulation: Blood samples were collected at necropsy. There were no remarkable findings.

Clinical Chemistry: There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Adrenal glands, brain, heart, kidney, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and parathyroid were weighed. There were no remarkable findings.

Histopathology

Adequate Battery- Yes.

Peer Review- Yes.

Histological Findings

Histological findings listed below are considered to be non-adverse due to minimal severity and/or low incidence relative to controls.

Heart: Minimal infiltration of mononuclear cells was noted in 5/10 high dose males and 2/10 high dose females. The finding was noted in 3/10 control males and 3/10 control females.

Kidney: Mild cysts were noted in 3/10 high dose males (0/10 control males)

Liver: Minimal necrosis in 2/10 high dose males (0/10 control males)

Pancreas: Minimal mononuclear cell infiltration in 4/10 high dose males (2/10 control males)

Skeletal muscle: Minimal myofiber degeneration/necrosis 2/10 females (0/10 control females but Mild myofiber degeneration/necrosis seen in 1/10 control females)

Tongue: Minimal fibrosis in 4/10 high dose females (1/4 control females). Minimal mixed cell infiltration in 2/10 high dose females (1/10 control females)

Toxicokinetics: Blood samples were collected at 0.5, 1.5, 6, 6.5, 7.5, 12 and 24 hours after the first daily dosing on Day 1, and again towards the end of the dosing period (Day 91) via sparse sampling. Toxicokinetic parameters for A-1282576 are presented below.

Toxicokinetic Parameters for A-1282576, Mean

Collection Interval	Sex	A-1282576 Dosage (mg/kg/day)[a,b]		
		10	40	120
		Mean Plasma $AUC_{0\text{-}24hr}$ (µg•hr/mL)		
Day 1	Overall	37.5	301	597
Day 91	Overall	45.8	253	580
		Mean Plasma C_{max}^{1} (µg/mL)[c]		
Day 1	Overall	4.22	23.6	52.4
Day 91	Overall	5.93	30.6	68.2
		Mean Plasma C_{max}^{2} (µg/mL)[c]		
Day 1	Overall	4.98	28.2	65.6
Day 91	Overall	5.74	30.3	61.0

a. Sparse sampling, toxicokinetic parameters calculated on the mean composite profile, therefore no standard deviation determined.

b. Dose levels expressed in total daily dose. Daily dose via BID (six hour) administration.

c. BID dosing: C_{max}^{1} refers to the period following the first of the two daily doses and C_{max}^{2} refers to the period following the second of the two daily doses.

Dosing Solution Analysis: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Study title: A-1282576 Free Form: A 26-Week BID Oral Dose Toxicity Study in Sprague-Dawley Rats (CRL:SD) with a 4-Week Recovery Period (RD 140001)

Study no.:	TA13-005
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07 May 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576 free base (A-1282576, A-1282576.0, ABT-493), 25986ZW01, 94.3%

Key Study Findings

There were no toxicologically significant findings. The No-Observed-Adverse-Effect-Level (NOAEL) was defined as the high dose (120 mg/kg/day), corresponding with Day 181 systemic exposures of 626 µg·hr/mL in males and 843 µg·hr/mL in females. This provides an exposure margin of 70X (avg. male and female combined), based on observed human exposure to 300 mg A-1282576.

Methods

Doses:	5, 20 and 60 mg/kg BID (10, 40, or 120 mg/kg/day)
Frequency of dosing:	BID for 26 weeks
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	70% Polyethylene glycol (PEG 400):20% Tween 20:10% Poloxamer 124
Species/Strain:	Rats/ Sprague Dawley
Number/Sex/Group:	20
Age:	9 weeks
Weight:	Males:154-175 g Females: 135-162 g
Satellite groups:	7/sex/group for four week recovery
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect results of study.

Observations and Results

Mortality: Rats were observed for survival and clinical signs twice a day during dosing. Detailed observations were made 1 to 2 hours after the first daily dosing at least twice a week. Six of eight early deaths were attributed to dosing injuries. The cause of death of the remaining two animals (both from control group) could not be determined.

Clinical Signs: Detailed clinical observations were recorded twice weekly during dosing. The incidence of sparse hair was increased in a dose dependent manner in females from the high dose group. This finding was also present in 3/7 males from the high dose group following the 4 week recovery period.

Body Weights: Body weights were recorded twice weekly during dosing. There were no remarkable findings.

Feed Consumption: Food consumption was recorded once weekly during dosing. There were no remarkable findings.

Ophthalmologic Examination: Ophthalmic exams were performed on all rats pretreatment and at the end of dosing on all rats. There were no remarkable findings.

Hematology/Coagulation: Blood samples were collected at necropsy. There were no remarkable findings.

Clinical Chemistry: There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Adrenal glands, brain, heart, kidney, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and parathyroid were weighed. There were no remarkable findings.

Histopathology

Adequate Battery- Yes.

Peer Review- Yes.

Histological Findings

There were no remarkable findings.

Toxicokinetics: Blood samples were collected at 0.5, 1.5, 6, 6.5, 7.5, 12 and 24 hours after the first daily dosing on Day 1 and Day 181 via sparse sampling. Toxicokinetic parameters for A-1282576 are presented below.

Mean Toxicokinetic Parameters for A-1282576

			Day 1			
Group	Dose Level (mg/kg BID)	Sex	C_{max}[1] (µg/mL)	C_{max}[2] (µg/mL)	T_{max} (hr)	AUC (µg·hr/mL)
2	5	Male	1.32	4.59	0.5	25.3
		Female	3.77	5.02	0.5	29.4
		Overall	2.54	4.46	0.5	27.3
3	20	Male	11.3	39.5	0.5	190
		Female	17.4	33.9	0.5	250
		Overall	14.4	36.7	0.5	220
4	60	Male	88.5	118	1.5	1030
		Female	71.8	141	6	934
		Overall	70.8	130	1.5	983
			Day 181			
Group	Dose Level (mg/kg BID)	Sex	C_{max}[1] (µg/mL)	C_{max}[2] (µg/mL)	T_{max} (hr)	AUC (µg·hr/mL)
2	5	Male	4.40	4.62	0.5	33.5
		Female	5.07	6.74	0.5	42.3
		Overall	4.73	5.25	0.5	37.9
3	20	Male	21.7	53.8	1.5	241
		Female	31.5	47	0.5	308
		Overall	26.3	42.7	0.5	274
4	60	Male	98.5	63.6	1.5	626
		Female	101	135	1.5	843
		Overall	99.8	99.2	1.5	735

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

2-week Oral Range-finding Toxicity Study with A-1282576 in Beagle Dogs (RD 11535) Two Beagle dogs per sex per group were dosed with 40, 100, 200 mg/kg/day A-1282576 via oral gavage daily for two weeks. There were no adverse findings in this pilot study. The NOAEL was defined as the high dose (200 mg/kg/day). The corresponding day 14 systemic exposures (AUC) was 961 µg·hr/mL (males/females combined).

Study title: Thirteen-Week Oral (Capsule) Toxicity Study of A-1282576 in Beagle Dogs (With a Four-Week Recovery Period) (RD 11925)

Study no.:	TB11-142
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	07 September 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576 free base (A-1282576.0, ABT-493) Lots: 07684ZW01 (93.7%), 09691ZW01 (95.8%), and 10693ZW00 (97.3%)

Key Study Findings

There were no toxicologically significant findings. The NOAEL is defined as the high dose, 200 mg/kg/day, corresponding to systemic exposure values of 765 µg•hr/mL (AUC_{0-24}) (males and females combined).

Methods

Doses:	10, 30, or 100 mg/kg BID (20, 60, and 200 mg/kg/day)
Frequency of dosing:	BID for 3 months
Route of administration:	Oral (gelatin capsules)
Dose volume:	1 mL/capsule
Formulation/Vehicle:	Gelatin capsule/70% Polyethylene glycol (PEG 400), 20% Tween® 20, 10% Poloxamer 124
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4
Age:	9 months
Weight:	6.712 to 10.287 kg

Satellite groups: See table below
Unique study design: None
Deviation from study protocol: Reported deviations did not affect study results.

Test Group	Dosage (A-1282576.0)[a,b]		Concentration	Number of Dogs[d]	
	(mg/kg/day)	(mg/kg/dose)	(mg/mL of A-1282576.0)[c]	Males	Females
1 (control)	0[e]	0[e]	0	4 (2)	4 (2)
2 (low)	20	10	10	4	4
3 (mid)	60	30	30	4 (2)	4 (2)
4 (high)	200	100	100	4 (2)	4 (2)

a. A-1282576 (A-1282576.0, Lot: 07684ZW01, 09691ZW01, and 10693ZW00; Expressed as free active moiety. Assigned chemical potency: 945 mg/g of test item (Lot 07684ZW01), 951 mg/g of test item (Lot 09691ZW01), and 967 mg/g of test item (Lot 10693ZW00).

b. Dosing was administered BID for Groups 1-4, with a 6-hour ± 15-minute interval between two dosings; Administered as a liquid formulation placed in gelatin capsules immediately prior to dosing.

c. Dose volume: 1 mL/kg.

d. Two males and two females within parentheses were designated recovery dogs.

e. Capsules containing vehicle.

Observations and Results

Mortality: Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. There were no early deaths.

Clinical Signs: Clinical observations were conducted twice weekly at 2 hours to 3 hours postdose. There were no remarkable findings. Fecal changes (unformed, mucoid, or watery) were noted in middle and high dose group dogs to a greater extent than in control group animals. In the absence of diet or body weight changes the effects were not considered to be significant.

Body Weights: Body weights were recorded twice weekly. There were no remarkable findings.

Feed Consumption: Food consumption was measured and recorded twice weekly during the study. There were no remarkable findings.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted and prior to the terminal necropsy and on recovery Day 23. There were no remarkable findings.

Hematology: Blood samples for clinical pathology were collected at terminal necropsy. There were mild decreases in red cell mass (red blood cells, hemoglobin, and/or hematocrit) at the end of dosing in animals administered 60 mg/kg/day (one male, two females) and 200 mg/kg/day (one male, one female) as compared to baseline average (range -12 to -23%). The baseline average was calculated from data collected on Baseline Days 27 and 34. The red cell mass parameter decreases had resolved by the

end of the recovery period. The red cell mass decreases were considered test item-related but not adverse due to mild magnitude and low incidence.

Electrocardiogram: Electrocardiograms were recorded once during the baseline period, on Days 7-8 and on Days 86-87 of the dosing period and on Day 24 of the recovery period for all surviving animals. Electrocardiograms were recorded two-three hours after the first BID dose. There were no remarkable findings.

Clinical Chemistry: Blood samples for clinical pathology evaluations were collected from all animals once on Dosing Day 88 and on Recovery Day 28. Eight of twelve males and four of twelve females at ≥60 mg/kg/day had minimally to mildly increased alkaline phosphatase (ALKP) at the end of the dosing phase (approximately 2- to 3.3-fold increase with the exception of one female with a 5.5-fold increase compared to baseline average). The baseline average was calculated from data collected on Baseline Days 27 and 34. There was no histologic correlate.

ALKP was increased in a control male 1.6-fold and in one control female 1.7-fold over baseline average at the end of the dosing period.

At the end of the recovery period the ALKP values were within the range of controls.

Alanine aminotransferase (ALT) was mildly increased in one male (3.8-fold, compared to baseline average) and two females (2.2- and 3-fold, compared to baseline average) at 200 mg/kg/day at the end of the dosing phase with no histologic correlate and no other transaminase changes. The ALT values were within the range of controls by the end of the recovery period. The changes in ALKP and ALT were considered test item-related but nonadverse due to low magnitude, low incidence, and lack of histologic correlate.

Urinalysis: Urine samples were collected once pretest (Day -8), once during Week 4 (Day 25), and prior to the terminal necropsy (Day 88). There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Adrenals, brain, epididymis, heart, kidney, liver, pituitary, prostate, spleen, ovaries, testes, thyroid/parathyroid, and thymus were weighed. There were no remarkable findings.

Histopathology

Adequate Battery- Yes.

Note, only gallbladder (males and females), kidney (males) and peripheral nerve (males) were processed and examined in recovery groups due to potential test item-related findings in main study animals.

Peer Review- Yes.

Histological Findings

Heart: mild, focal, mesothelial hyperplasia in one middle dose male; minimal, focal mesothelial hyperplasia in one high dose male and one middle dose female

Skeletal muscle: minimal, multifocal myofiber degeneration/necrosis in one middle dose female

Peripheral nerve: minimal multifocal degeneration in one high dose male and one middle dose female

Gall bladder: Minimal gallbladder edema was observed in one male at 20 mg/kg/day, one male and two females at 60 mg/kg/day, and one female at 200 mg/kg/day at the end of the dosing phase without a similar finding in females at the end of the recovery period. This finding was considered related to test item administration but nonadverse due to minimal severity, absence of cellular response, and maintenance of gallbladder epithelial integrity.

Testes: minimal unilateral or bilateral multifocal atrophy of tubules in one low dose and one middle dose dog. Minimal unilateral multifocal degeneration of tubules in one low dose dog.

Toxicokinetics: Blood samples were collected predose and at 1, 3, 6, 9, 12, and 24 hours postdose on Days 1, 28 and 91. Toxicokinetic parameters in dogs following 91 days of oral administration of A-1282576 are presented below. Exposures were similar in males and females.

Pharmacokinetic Parameters for A-1282576, Mean ± Standard Deviation (SD)

Collection Interval	Sex	A-1282576 Dosage (mg/kg/day)		
		20	60	200
		Mean Plasma $AUC_{0\text{-}24}$ (µg•hr/mL) ± SD		
Day 1	Overall	23.5 ± 9.95	441 ± 331	1180 ± 527
Day 91	Overall	16.1 ± 8.31	368 ± 217	765 ± 281
		Mean Plasma C_{max}[1] (µg/mL) ± SD		
Day 1	Overall	8.05 ± 3.06	62.0 ± 30.5	87.4 ± 40.3
Day 91	Overall	4.34 ± 3.22	60.6 ± 32.2	83.9 ± 33.2
		Mean Plasma C_{max}[2] (µg/mL) ± SD		
Day 1	Overall	1.97 ± 1.75	42.9 ± 31.1	92.0 ± 37.8
Day 91	Overall	1.73 ± 1.21	40.5 ± 22.6	66.5 ± 19.7

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Study title: A-1282576 : A 39-Week BID Oral Dose Toxicity Study of in Beagle Dogs With an 8-Week Recovery Period (RD140002)

Study no.:	TB13-006
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 18, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576 free base (A-1282576.0, ABT-493) Lots: 25986ZW01 (94.3%)

Key Study Findings

There were no toxicologically significant findings. The NOAEL is defined as the high dose, 200 mg/kg/day, corresponding to systemic exposure values of 1440 µg•hr/mL (AUC_{0-24}) (Day 273; males and females combined). This provides an exposure margin of 137X based on observed human exposure to 300 mg A-1282576.

Methods

Doses:	10, 25, or 100 mg/kg BID (20, 50, or 200 mg/kg/day)
Frequency of dosing:	BID for 9 months
Route of administration:	Oral (gelatin capsules)
Dose volume:	1 mL/capsule
Formulation/Vehicle:	Gelatin capsule/70% Polyethylene glycol (PEG 400), 20% Tween® 20, 10% Poloxamer 124
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4
Age:	7-8 months
Weight:	Males: 7.30 to 9.20 kg Females: 5.05 to 7.15 kg
Satellite groups:	2 in control and high dose groups (recovery)
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. There were no unscheduled deaths.

Clinical Signs: Clinical observations were conducted twice weekly at 2 hours to 3 hours postdose. There were no remarkable findings.

Body Weights: Body weights were recorded weekly. There were no remarkable findings.

Feed Consumption: Food consumption was measured and recorded weekly during the study. There were no remarkable findings.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted and prior to the terminal and recovery necropsies. There were no remarkable findings.

Hematology: Blood samples for clinical pathology evaluations were collected from all animals once pretest (Day -8 and -5), Week 4, and prior to the terminal and recovery necropsies. There were no remarkable findings.

Electrocardiogram: All animals received an electrocardiographic examination prior to initiation of dosing and again approximately 2 to 3 hours following the first daily dose on Day 1, approximately 2 to 3 hours post the first daily dose on the day prior to the terminal necropsy, and once prior to the recovery necropsy. There were no remarkable findings.

Clinical Chemistry: Blood samples for clinical pathology evaluations were collected from all animals once pretest (Day -8 and -5), Week 4, and prior to the terminal and recovery necropsies.

Mild increases in alanine aminotransferase (ALT) and/or gamma glutamyltransferase (GGT) were noted sporadically in females administered ≥25 mg/kg BID. The finding is not considered adverse due to the sporadic nature, lack of dose-response, and a lack of histopathologic correlate. However, it should be noted that similar effects on liver enzymes (without microscopic correlate) were noted in both males and females in the 13-week study.

Urinalysis: Urine samples were collected once pretest (Day -8), once during Week 4 (Day 25), and prior to the terminal necropsy. There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Adrenals, brain, epididymis, heart, kidney, liver, pituitary, prostate, spleen, ovaries, testes, thyroid/parathyroid, and thymus were weighed. There were no remarkable findings.

Histopathology

Adequate Battery- Yes.

Peer Review- Yes.

Histological Findings

There were no remarkable findings.

Toxicokinetics: Samples were collected from treated animals at 0.5, 1.5, 4, 6 (within 5 minutes prior to the second daily dose), 6.5, 7.5, 9, 12, and 24 hours post the first daily dose on Days 1 and 273. Exposures were similar in males and females.

Mean Toxicokinetic Parameters for A-1282576

			Day 1			
Group	Dose Level (mg/kg BID)	Sex	C_{max}[1] (µg/mL)	C_{max}[2] (µg/mL)	T_{max}[1] (hr)	AUC (µg·hr/mL)
2	10	Male	5.95	2.17	1.5	23.2
		Female	5.62	1.02	1.5	18.5
		Overall	5.78	1.60	1.5	20.9
3	25	Male	16.1	23.2	1.5	112
		Female	19.8	23.7	2.1	114
		Overall	17.9	23.5	1.8	113
4	100	Male	68.1	162	2.6	1750
		Female	30.1	110	2.3	873
		Overall	49.1	136	2.5	1310
			Day 273			
Group	Dose Level (mg/kg BID)	Sex	C_{max}[1] (µg/mL)	C_{max}[2] (µg/mL)	T_{max}[1] (hr)	AUC (µg·hr/mL)
2	10	Male	15.5	5.05	1.5	60.2
		Female	9.92	5.31	1.5	40.8
		Overall	12.7	5.18	1.5	50.5
3	25	Male	54.7	30.9	1.5	252
		Female	60.7	18.8	2.1	221
		Overall	57.7	24.9	1.8	236
4	100	Male	110	118	2.8	1500
		Female	124	121	2.8	1380
		Overall	117	119	2.8	1440

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Genetic Toxicology

Study title: Bacterial Reverse Mutation Assay with A-1282576 Free Form (RD 12270)

Study no.:	AD44TC.504002.BTL
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	21 February 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576, 10693ZW00, 97.3%

Key Study Findings

A-1282576 was negative for mutations under the conditions of this *Salmonella–E. coli* Reverse Mutation Assay.

Methods

Strains:	TA98, TA100, TA1535, TA1537, WP2*uvr*A
Concentrations in definitive study:	15.0, 50.0, 150, 500, 1500, and 5000 µg/plate with and without S9
Basis of concentration selection:	Dose range-finding assay
Negative control:	dimethylsulfoxide (DMSO)
Positive control:	See table below
Formulation/Vehicle:	dimethylsulfoxide (DMSO),
Incubation & sampling time:	48 to 72 hours

Strain	S9	Positive Control	Concentration (µg/plate)
Salmonella Strains	Rat	2-aminoanthracene	1.0-2.0
WP2 *uvr*A			10-20
TA98	None	2-nitrofluorene	1.0
TA100, TA1535		sodium azide	1.0
TA1537		9-aminoacridine	75
WP2 *uvr*A		methyl methanesulfonate	1,000

Study Validity

Study validity criteria were met.

Results

A-1282576 was negative for mutations under the conditions of this *Salmonella–E. coli*/Mammalian-Microsome Reverse Mutation Assay

Study title: *In Vitro* Mammalian Chromosome Aberration Test with A-1282576 Free Form (RD12271)

Study no.:	AD44TC.341.BTL
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	22 February 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576, 10693ZW00, 97.3%

Key Study Findings

No statistically significant increase in the number of cells with chromosomal aberrations, polyploidy, or endoreduplication was observed in the cultures analyzed. A-1282576

was considered to be negative for causing chromosomal aberrations under the conditions of this assay.

Methods

Cell line:	Cultured human lymphocytes
Concentrations in definitive study:	See table below
Basis of concentration selection:	Dose range-finding study
Negative control:	dimethylsulfoxide (DMSO)
Positive control:	mitomycin C (MMC): without metabolic activation cyclophosphamide (CP): with metabolic activation
Formulation/Vehicle:	dimethylsulfoxide (DMSO)
Incubation & sampling time:	See table below

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	50, 100, 150, 170, 200, 256, 320, 400
	20 hr	0 hr	20, 40, 50, 60, 70, 85, 100
S9-activated	4 hr	16 hr	20, 50, 100, 135, 150, 170, 185, 200

Study Validity

All validity criteria were met.

Results

A-1282576 was considered to be negative for causing chromosomal aberrations under the conditions of this assay.

Study title: *In Vivo* Micronucleus Assay in Rats with A-1282576 Free Form (RD 13002)

Study no.:	TA12-188
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 20, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576; 10693ZW00; 94.5%

Key Study Findings: No A-1282576 related effects were noted following oral administration at up to 2000 mg/kg to male rats. A-1282576 did not induce statistically significant increases in micronucleated PCEs.

Methods

Doses in definitive study:	0, 500, 1000 and 2000 mg/kg/day
Frequency of dosing:	Single dose
Route of administration:	Oral Gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	PEG400:Tween20:Poloxamer-124 (70:20:10 by weight)
Species/Strain:	SD Rats
Number/Sex/Group:	5
Satellite groups:	None
Basis of dose selection:	Dose range study
Negative control:	Vehicle
Positive control:	Cyclophosphamide

Results: There were no A-1282576-related effects. A-1282576 showed no signs of bone marrow cytotoxicity (no decreases in the PCE:NCE ratios). A-1282576 did not induce biologically relevant increases in micronucleated PCEs at any dose level.

The vehicle control micronucleated PCE value was within the vehicle historical control range showing a normal background level in this test. The positive control, cyclophosphamide, induced a statistically significant increase in micronucleated PCEs, confirming the validity of the assay.

Therefore, A-1282576 was negative in the rat bone marrow micronucleus assay when tested up to 2000 mg/kg/day and under the conditions of this assay.

Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Study title: An Oral Fertility Study with A-1282576 in Rats (RD 140683)

Study no.:	TA14-066
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 11, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576; 27013ZW01; 94.0%

Key Study Findings

Based on the lack of findings, the NOAEL for general toxicity and reproductive and fertility parameters in males and females was 120 mg/kg/day, the highest dose level evaluated. No toxicokinetics assessment was performed. Based on exposure levels from 13 and 26-week studies, the 120 mg/kg/day NOAEL provides an exposure margin of 63X based the human dose of 300 mg A-1282576.

Methods

Doses:	0, 10, 40, or 120 mg/kg/day
Frequency of dosing:	BID
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	PEG 400:Tween 20:Poloxamer 124 (70:20:10, %w/w)
Species/Strain:	CD® [Crl:CD® (SD)] rats
Number/Sex/Group:	25
Study design:	25 rats/group/sex were dosed daily via gavage (F: 14 days prior to pairing to GD 7; M: 14 days prior to pairing to study termination)
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily.

One male at 120 mg/kg/day was found dead on Study Day 34 (mating period). Clinical observations for this animal were normal with the exception of Days 18-20 and 33 where the animal was observed with red material around the nose. At necropsy the only

finding was thymus discolored red. No other deaths were observed in males or females at 120 mg/kg/day, no test article-related clinical or macroscopic findings were observed at this dose level, therefore this one death was not considered test article-related.

One female at 40 mg/kg/day was found dead on Day 19 (mating period). Clinical observations for this animal were normal with the exception of Days 12-14 and 18 where the animal was observed with red material around the nose. No macroscopic findings were observed at necropsy. No additional deaths occurred at 40 mg/kg/day or at 120 mg/kg/day in the females and without a dose-response and no test article-related clinical or macroscopic findings at this dose level, therefore this one death at 40 mg/kg/day was considered unrelated to the test article.

Clinical Signs: Detailed clinical examinations were conducted daily during treatment (2 hours post the second dose on dosing days). Mated females were also given a detailed clinical examination on GD 13.

No test article-related clinical findings were observed in the treated males and females. Red material around the nose (chromodacryorrhea related to porphyrin, common finding in rats, especially when there is some degree of stress), hypersensitivity to touch, and/or vocalization were observed in males and females (predominately males) in all treated and control groups throughout the study period. The number of animals affected/occurrences in the treated males and females was similar to concurrent controls and was not considered test article-related.

Body Weight: Body weights of males were recorded at initiation of dosing and twice weekly at 3- and 4-day intervals during treatment until euthanasia. Females were weighed at initiation of dosing, twice weekly at 3- and 4-day intervals prior to and during cohabitation, and on GD 0, 3, 7, 10, and 13.

No test article-related differences were observed in mean body weights and body weight change in males and females treated with A-1282576.

Feed Consumption: Food consumption was recorded twice weekly at 3- and 4-day intervals prior to pairing for mating. No test article-related differences were observed in mean food consumption in males and females treated with A-1282576.

Toxicokinetics: Toxicokinetics were not determined.

Necropsy: On GD 13, each female was euthanized, and the uterus and ovaries were exposed using a mid-abdominal incision. Beginning at the distal end of the left uterine horn, the number of normally developing embryos, resorptions, and the total number of implantations were recorded. The number of corpora lutea on each ovary was also recorded.

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

Estrous cycle length and mean number of cycles in the treated groups during the premating period were comparable to controls and unaffected by treatment with A-1282576. Estrous cycles in individual animals from treated groups appeared normal relative to controls.

These mating and fertility indices were all comparable to controls and within recent historical control data for this laboratory

Ovarian and uterine parameters (mean number of corpora lutea, implantation sites, viable fetuses, resorptions, pre- and post-implantation loss) in all treated groups were comparable to controls and unaffected by treatment with A-1282576.

Embryonic Fetal Development

A-1282576: An Oral Developmental Toxicity Study in Rabbits, Including a Toxicokinetic Evaluation (RD 13841) Due to significant clinical findings (inappetence, soft stool, diarrhea, respiratory abnormalities) at animal receipt and continuing following placement of animals on study, all animals were terminated on GD 12-14, due to the possibility that the illness observed in these animals was present at receipt and is not vehicle or test article related. The Study Director noted that the animals were received during a period of unusual heat (>90°) and may have been stressed by the environmental conditions. No fetal assessments were conducted in the current study. The segment II developmental toxicity study in rabbits was repeated (see review below).

Study title: A-1282576: An Oral Developmental Toxicity Study in Rabbits, Including a Toxicokinetic Evaluation (RD 13679)

Study no.:	TE13-250
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 12, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576.0, ABT-493, 25986ZW01, 943 µg/mg

Key Study Findings

No effects of treatment were noted on pregnancy or fetal development parameters. The NOAEL is defined as the high dose (60 mg/kg/day) corresponding to maternal exposures (AUC) of 0.728 µg.hr/mL. This low exposure is inadequate to make any safety conclusions based on observed human exposure to A-1282576 (10.5 µg.hr/mL) .

Methods

Doses:	0, 20, 60 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	EtOH:PEG400:Phosal 53MCT (10:30:60, %w/w)
Species/Strain:	New Zealand White Hra:(NZW)SPF
Number/Sex/Group:	20
Satellite groups:	Toxicokinetics (5/group)
Study design:	Test article was administered from GD 7 to 19. Dose levels were selected based on findings of maternal toxicity (lower maternal body weights, body weight gain, and food consumption at 100 and 300 mg/kg in dose range-finding studies. Also, early deliveries and lower fetal body weights were observed at 100 and 300 mg/kg.

Observations and Results

Mortality: All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily. There were no unscheduled deaths.

Clinical Signs: Clinical signs were recorded daily from GD 7 through 29 (60 to 90 minutes postdose on dosing days). Soft/mucoid/few and/or absent feces was observed in 1/20 control animals, 6/20 animals at the low dose (20 mg/kg) and 3/20 animals in the high dose (60 mg/kg). Discolored hair around the anogenital region or hind limbs was consistent with the fecal findings.

Body Weight: Body weights for all animals were measured and recorded on GD 0, 7, and daily thereafter. Mean body weights and body weight changes were comparable among control and treatment groups.

Feed Consumption: Food consumption for main study animals was recorded daily and reported on the corresponding body weight days. No effects of treatment on feed consumption were noted.

Toxicokinetics: Samples were collected prior to dosing and at 0.25, 0.5, 1, 3, 6, 12, and 24 hours postdose on GD 18. The estimated mean AUCs on GD 18 were 0.130 and 0.728 µg*hr/mL in the low and high dose groups, respectively.

Mean (±SD) Toxicokinetic Parameters for A1282576 in Rabbit Plasma – GD 18

Dose (mg/kg/day)	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)
20	0.0125 ±0.00401	5.4 ±1.3	0.130 ±0.0498
60	0.0986 ±0.0617	1.8 ±1.1	0.728 ±0.272

Necropsy: Gross necropsy of does did not reveal any treatment-related effects.

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

The pregnancy index was 85%(17/20) in the low dose group and 80% (16/20) in the high dose group, comparable to 85% (17/20) in the control group. Uterine and ovarian parameters (corpora lutea count, number of implantation sites, viable fetuses, litter size, pre- and post-implantation loss, and number of resorptions) in the treatment groups were comparable to controls and unaffected by the test article.

Offspring (Malformations, Variations, etc.)

No visceral malformations or variations were noted in low dose (20 mg/kg) animals. In high dose (60 mg/kg) animals, visceral malformations and variations were within historical control data.

No external malformations or variations were observed in control and treated groups.

Skeletal malformations and variations were observed in fetuses from treated animals. Total malformations (% litter incidence) were 23.5 % and 25% in the 20 and 60 mg/kg/day groups, respectively, and were comparable to controls at 23.5%. The low incidence (comparable to controls), lack of dose response, and correlation with historical control ranges suggest that the malformations and variations observed were not related to treatment.

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Study title: A-1282576: An Oral Developmental Toxicity Study in Rats, Including a Toxicokinetic Evaluation (RD 13913)

Study no.:	TA13-168
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 24, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576.0, ABT-493, 10693ZW00, 970 µg/mg

Key Study Findings

A-1282576 was not teratogenic at the dose levels evaluated. Based on the lack of dose-related findings, the NOAEL for maternal and developmental/fetal toxicity was the high dose of 120 mg/kg/day (AUC: 559 µg*hr/mL). This provides an exposure margin of 53X based on observed human exposure to 300 mg A-1282576.

Methods

Doses:	0, 10, 40, 120 mg/kg/day
Frequency of dosing:	BID
Dose volume:	2 mL/kg/dose
Route of administration:	Oral gavage
Formulation/Vehicle:	PEG 400:Tween 20:Poloxamer 124 (70:20:10, %w/w; with 1 molar equivalent of NaOH)
Species/Strain:	Rat/ CD® [Crl:CD®(SD)]
Study design:	Animals were dosed twice per day from GD 6 to 18 at a dose volume of 2 mL/kg/day.
Deviation from study protocol:	Reported deviations did not affect study results.

GROUP	DOSE LEVEL mg/kg/day	DOSE LEVEL mg/kg/dose (BID)	DOSE CONCENTRATION mg/mL	NUMBER OF ANIMALS INITIAL F	CESAREAN SECTION/ NECROPSY F	TOXICOKINETIC F
1	0	0	0	25	25	-
2	10	5	2.5	25	25	-
3	40	20	10	25	25	-
4	120	60	30	25	25	-
5	10	5	2.5	5	-	5
6	40	20	10	5	-	5
7	120	60	30	5	-	5

Observations and Results

Mortality: Animals were observed for morbidity, mortality and injury twice daily. More detailed observations of clinical signs were recorded daily from GD 6 through 20. All animals survived to scheduled necropsy.

Clinical Signs: Clinical signs were recorded daily from GD 6 through 20. Clinical findings of hair discolored (yellow) in the anogenital region and sparse hair (fore feet and abdominal region) were seen sporadically in ≤ 5 animals per treatment group.

Body Weight: Body weights were recorded on GD 0, 6, then daily. There were no remarkable findings.

Feed Consumption: Feed consumption was recorded on GD 0, 6, then daily. There were no remarkable findings.

Toxicokinetics: Samples were collected prior to dosing and at 0.5, 1.5, 6 (prior to second dose), 6.5, 7.5, 12, and 24 hours post the first dose on GD 6 and 17.

The average mean AUCs on GD 6 were 25.3, 261, and 1030 µg*hr/mL in the low, mid and high dose groups, respectively. The mean exposure (AUC) appeared to be more than proportional to dose from the low to the high dose groups on GD 6. The average mean AUCs on GD 17 were 55.9, 318, and 559 µg*hr/mL in the low, mid, and high dose groups, respectively.

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

The pregnancy index was 100% in the control and all treatment groups. Uterine and ovarian parameters (corpora lutea count, number of implantation sites, viable fetuses, litter size, pre and post-implantation loss, and number of resorptions [early, late, and combined]) in the treated groups were comparable to controls and unaffected by the test article.

Offspring (Malformations, Variations, etc.)

No fetal external malformations or variations were observed in the treated groups.

No fetal visceral malformations were observed in the control or treated groups. A few visceral variations (increased renal pelvic cavitation, dilated ureter, smaller than normal thyroid) were observed in the treated groups, but were seen at a low incidence (one or two fetuses), were not dose dependent, were within the range seen in recent historical control data, or were comparable to controls and were therefore not considered test article-related.

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Peri/Postnatal Studies

Study title: A Developmental and Perinatal/Postnatal Reproduction Study of A-1282576 by Oral (Gavage) in Rats, Including a Postnatal Behavioral/Functional Evaluation (RD 160239)

Study no.:	TA15-093
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 2, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576; 42338ZW00; 99.8%

Key Study Findings

The maternal and reproductive no-observable-adverse effect level (NOAEL) for A-1282576 was 120 mg/kg/day (AUC of 492 µg*hr/mL), the highest dose tested.
The NOAEL for viability and growth in the offspring was 120 mg/kg/day. This provides an exposure margin of 47X based on observed human exposure to 300 mg A-1282576.

Methods

Doses:	0, 10, 40, 120 mg/kg/day total (administered as 5, 20 and 60 mg/kg twice daily)
Frequency of dosing:	F0 female rats on Gestation Day (GD) 6 through Lactation Day (LD) 20 daily (GD24 for rats that did not deliver)

Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	PEG 400:Tween 20:Poloxamer 124 (70:20:10, %w/w)
Species/Strain:	Rat/ Sprague Dawley
Number/Sex/Group:	F0:22 females per group (and additional 6 TK females per group); F1: 22 per sex per group
Age:	75 to 81 days
Weight:	180 to 225 grams
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Maternal Mortality: All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily. One female (#3334) dosed with 10 mg/kg was euthanized due to adverse clinical signs on LD 13. Adverse clinical signs included rales and dyspnea and decreased motor activity, as well as gasping. There was also minor weight loss (8.9%) between LD 11 and LD 13. There were no abnormalities detected at necropsy. The Sponsor suspected a possible misintubation or aspiration of the test article on or about LD 12. One female (#3384) dosed with 120 mg/kg was found dead on LD 10. There were no clinical observations observed and no abnormalities detected at necropsy. These deaths were not considered test article related in the low dose due to possible handling error and in the high dose due to lack of associated signs of toxicity.

Maternal Clinical Signs: Detailed clinical examinations were conducted daily during treatment. Adverse clinical signs were observed in female 3334 as described above. No other treatment-related findings were observed.

Maternal Body Weights: Body weights were recorded daily GD6 to LD21 and day of euthanasia. There were no remarkable findings.

Maternal Feed Consumption: Food consumption was recorded GD6 to GD21 or 25 and LD 1,4,7,10,12 and 14. There were no remarkable findings.

Maternal Gross Examination: Examinations were conducted on LD 21 or GD 25.There were no remarkable findings.

Natural Delivery Observations: Pregnancy was confirmed in 22, 22, 21, and 22 rats in the 0, 10, 40 and 120 mg/kg/day dose groups, respectively. All pregnant rats delivered their litters. There were no A-1282576-related effects on any natural delivery parameter at any dose. The values for the number of dams delivering litters, the duration of

gestation, implantation sites per delivered litter, the gestation index, the number of dams with stillborn pups, and the number of dams with all pups dying was similar among the four dose groups.

Litter Observations: There were no A-1282576-related effects on any litter parameter at any dose. The values for the lactation index (percentage of pups born that survived to weaning), surviving pups per litter, the sex ratio, live litter size at weighing, and pup weights per litter were similar among the four dose groups.

F1 Generation - Preweaning

Clinical signs: Clinical signs were recorded daily. No treatment-related findings were observed.

Pup gross pathology: Prior to weaning on Day 21 postpartum, there were necropsy examinations performed on 1, 2, 4, and 4 F1 pups that were found dead, stillborn, or euthanized in the 0, 10, 40, and 120 mg/kg/day dose groups, respectively. Of the pups that were found dead or euthanized and necropsied, 1, 2, 2, and 0 pups in the 0, 10, 40, and 120 mg/kg/day dose groups, respectively, did not have milk present in the stomach at necropsy examination.

F1 Generation -Postweaning

Unscheduled deaths: Animals were checked for morbidity twice daily. All F1 generation rats survived to scheduled euthanasia.

Clinical signs: Clinical signs were recorded daily. There were no remarkable findings.

Body weight: Body weights were recoded weekly and GD 0, 7, 10 and 13 for females. There were no remarkable findings.

Food consumption: Food consumption was measured weekly and GD 0, 7, 10 and 13 for females. There were no remarkable findings.

Developmental Signs:

Vaginal opening: Measurements were conducted starting PND 28. There were no remarkable findings.

Prepupial separation: Measurements were conducted starting PND 39. There were no remarkable findings.

Behavioral assessments:

Acoustic Startle Habituation: Measurements were conducted on Day 61± 2 days postpartum. There were no remarkable findings.

Motor Activity: Measurements were conducted on Day 61± 2 days postpartum. There were no remarkable findings.

M-Shaped Water Maze: Measurements were conducted on Day 70± 2 days postpartum. There were no remarkable findings.

F1 Rats from Mating until Sacrifice:

Mating performance and fertility: PND 89 ± 2 days. There were no remarkable findings.

Necropsy: There were no remarkable findings.

Organ Weights, Ratio of Organ Weights to Terminal Body Weights, and Ratio of Organ Weights to Brain Weights: There were no remarkable findings.

Ovarian and Uterine Examinations: There were no A-1282576-related effects on any ovarian or uterine parameter at any dose. Pregnancy was confirmed in 21 (95.4%), 21 (95.4%), 20 (90.9%) and 22 (100.0%) F1 generation females in the 0, 10, 40, and 120 mg/kg/day dose groups, respectively.
The litter averages for corpora lutea, implantations, the percentage of preimplantation loss, viable and nonviable embryos and the percentage of postimplantation loss were similar among the four dose groups. No dam had a litter consisting of only nonviable embryos. No placentae examined had any detectable abnormalities.

Toxicokinetics: Blood samples were collected 0. 0.5, 1.5, 6.5, 7.5, 12 and 24 hours postdose. C_{max} and AUC levels were more than dose proportional on LD14 between 10 and 40 mg/kg/day. However values were less than dose proportional at the next dose level of 120 mg/kg/day.

Table 8 Mean Toxicokinetic Parameters for A1282576 in Rat Plasma on LD 14

Dose (mg/kg/day)	C_{max} (µg/mL)[a]		C_{max}/D (µg/mL/ mg/kg/day)[a]		T_{max} (hr)[a]		AUC (µg*hr/mL)	AUC/D (µg*hr/mL/ mg/kg/day)	SE of Composite AUC (NJH)
	1	2	1	2	1	2			
10	1.90	4.89	0.190	0.489	0.5	6.5	22.0	2.20	3.15
40	18.6	31.5	0.465	0.788	1.5	6.5	208	5.20	49.8
120	36.4	76.4	0.303	0.637	1.5	7.5	492	4.10	88.4

[a] - $Cmax^1$ and $Tmax^1$ refer to the period following the first of the two daily doses and $Cmax^2$ and $Tmax^2$ refer to the period following the second of the two daily doses

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Special Toxicology Studies

Neutral Red Uptake Phototoxicity Assay of A-1282576 Free Form in Balb/c 3T3 Mouse Fibroblasts (RD 13488; GLP)

The phototoxicity potential of A-1282576 free form was evaluated by measurement of the relative reduction in viability of Balb/c 3T3 mouse fibroblasts exposed to A-1282576 free form and ultraviolet radiation (+UVR; 5 J/cm2 of UVA and 21 mJ/cm2 of UVB from a xenon arc solar simulator equipped with a Schott WG 320 filter), as compared with the viability of fibroblasts exposed to A-1282576 free form in the absence of ultraviolet radiation (-UVR), at concentrations up to the solubility limit of 100 mg/L. Promethazine was used as the positive control.

A-1282576 demonstrated potential phototoxicity as measured by the Photoirritancy Factor (PIF; >10.263) and Mean Photo Effect (MPE; 0.240). A-1282576 was not cytotoxic. Promethazine cytotoxicity and phototoxicity criteria were met indicating that the assay was valid (sponsor's table below). An *in vivo* study was conducted to follow up on this result (Study RD 13803).

Table 9 Phototoxicity Potential in Mouse Fibroblasts

Assay Results					
	IC_{50} (mg/L) –UVR (cytotoxicity)	IC_{50} (mg/L) +UVR (phototoxicity)	Photoirritancy Factor (PIF)	Mean Photo Effect (MPE)	Phototoxic Potential
Promethazine	45.21	2.074	21.814	0.364	Phototoxic
A-1282576 free form	–	9.747	> 10.263	0.240	Phototoxic

IC_{50}: 50% Inhibitory Concentration
+UVR: with exposure to 5 J/cm^2 of UVA and 21 mJ/cm^2 of UVB in the assay
– UVR: without UVR exposure
>PIF : If a test article is phototoxic (+UVR) but not cytotoxic (-UVR): the > PIF = Cmax (-UVR) / EC50 (+UVR), any value > 1 predicts phototoxicity.

A Repeat Dose Phototoxicity Study to Determine the Effects of Oral (Gavage) Administration of A-1282576 Free Form on Eyes and Skin in Pigmented Rats (RD 13803; GLP)

Phototoxicity on the eyes and skin of female Crl:LE (Long-Evans) pigmented rats was evaluated following A-1282576 administration by oral gavage twice daily for 3 consecutive days at 0, 10, 40, and 600 mg/kg total per day, followed by exposure to radiation from a xenon lamp (to simulate sunlight; 10.3 J/cm2 of UVA and approximately 145 mJ/cm2 of UVB) 60 min after the last A-1282576 administration. An additional group of male rats were dosed with 600 mg/kg A-1282576 and no UV radiation. Also, 8-Methoxypsoralen (8-MOP) at 50 mg/kg was used as the control (applied once on the day of UVR exposure).

The toxicokinetic results demonstrated dose-dependent exposure to A-1282576 over the time of UVR exposure. The estimated mean AUCs on Day 3 were 28.7, 223 and 1070 µg*hr/mL in the low, mid and high dose groups, respectively. The increase of the

mean exposure (AUC) from the low to the high dose groups appeared to be approximately proportional to dose on Day 3.
The average T_{max1} occurred at 0.5 hours post dosing on Day 3 and the average T_{max2} occurred between 13.5 and 18 hours post dosing on Day 3.
A single administration of the comparator article 8-MOP followed by a single UVR exposure approximately one hour later resulted in skin (erythema grade 1, edema grade 1 and/or flaking grade 1) and eye reactions (diffuse corneal edema) in female rats consistent with phototoxicity.
A-1282576 elicited no evidence of cutaneous or ocular phototoxicity.

Excipient Studies

Capryol 90

The Gle/Pib film-coated bilayer tablets contain the excipient propylene glycol monocaprylate II (Capryol 90) at a level of (b) (4) mg per tablet. To achieve the recommended human doses, 3 tablets are required, therefore, the maximum daily dose of propylene glycol monocaprylate II that will be administered is (b) (4) mg/day. The sponsor conducted genotoxicity, EFD and 28 day repeat dose studies with this excipient to qualify the proposed clinical amounts.

Capryol 90- Bacterial reverse mutation test (Plate incorporation) (RD 160701)
The genotoxicity of Capryol-90 was carried out in a bacterial mutagenicity test by assessing the effects on 5 histidine-dependent strains of Salmonella typhimurium (TA98, TA100, TA1535, TA1537 and TA102) in the presence and absence of metabolic activation, at up to 5000 µg/plate. Two independent experiments were carried out using the plate incorporation method. Each experiment was performed in triplicate with concurrent negative and positive controls. All the criteria for a valid study were met. Capryol-90 did not induce any mutagenic effects in the Ames test either with or without metabolic activation.

A 28-Day Oral (Gavage) Toxicity Study of Capryol 90 in Rats (RD 160093) In a 28-day repeat dose toxicology study, male and female Crl:CD(SD) rats were dosed with 0, 500, 1500 or 2500 mg/kg Capryol 90 via oral gavage. The following parameters were recorded: mortality, clinical observations, body weights, food consumption, ophthalmic exams, clinical pathology. Complete necropsies were conducted, and selected organs were weighed from all animals at the scheduled necropsy. Gross lesions were examined microscopically from all animals and selected tissues were examined microscopically from all animals in the control and 2500 mg/kg/day groups. Although blood was collected for toxicokinetic analysis, the samples were not analyzed.
Excessive salivation including clear material noted around the mouth and yellow or red material around the mouth and nose and on the forelimbs, hindlimbs, ventral neck and ventral trunk were noted at all doses, with the highest incidence at the highest dose of 2500 mg/kg/day. However these effects were not considered adverse in absence of any other parameter changes. The NOAEL for Capyrol 90 in this study was the highest dose of 2500 mg/kg/day.

A Study of the Effects of Capryol 90 on Embryo/Fetal Development in Rats (RD 160094) In an EFD study, three groups of 25 female Crl:CD(SD) rats were dosed with 0, 500, 1500 or 2500 mg/kg/day Capryol 90 from gestational days (GD) 6 to 17. All animals were observed twice daily for mortality. Clinical observations, body weights and food consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed on each female. The uteri, placentae and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations. Clinical findings that were attributed to the test article in the 1500 and 2500 mg/kg/day groups consisted of salivation, noted immediately prior to dose administration, and clear material on various body surfaces, noted approximately 1 hour following dose administration; these salivation-related findings were not considered adverse. Similar findings were noted in the 28-day repeat dose toxicology study with Capryol 90. The NOAEL for maternal and embryo/fetal developmental toxicity was 2500 mg/kg/day Capryol 90.

Integrated Summary and Safety Evaluation

This marketing application was submitted in support of a for Gle/Pib (Glecaprevir/Pibrentasvir; ABT-493/ABT-530;A-1282576/A-1325912) indicated for the treatment of chronic HCV genotype 1-6 infection in adults, including those with cirrhosis, who are either treatment-naïve or previously treated with interferon, pegylated interferon (pegIFN) and/or ribavirin. Gle (3/4A protease inhibitor) and Pib (an inhibitor of the NS5A protein) are both new molecular entities. The proposed clinical dosing regimen of Gle/Pib is 300 mg/120 mg (Pib/Gle) for 8 to 16 weeks, depending on patient population.

The following sections summarize nonclinical results for Glecaprevir (Gle).

PK/ADME: The oral bioavailability of Gle was 90% in rat and 44% in dog. In vitro protein binding was >97% in all species, including human. Following an oral dose of ^{14}C-Gle in rat, radioactivity was widely distributed with the highest concentrations found in liver. Gle crossed the blood:brain barrier, blood:eye barrier, blood:testis barrier however binding to melanin was not observed. The metabolite profile of the pooled plasma samples from multiple oral dosing in human showed that Gle was the major drug-related component, with nine minor metabolites, including products of hydroxylation, acylsulfonamide hydrolysis and defluorination with subsequent oxidation to the α,β unsaturated ketone. All the human metabolites formed in the in vitro hepatic system were present in one or more animal species. There were no significant circulating clinical metabolites (e.g., >10% of total drug-related exposure) requiring nonclinical safety assessment. Elimination primarily occurred through the biliary route in rats and humans. Gle was transferred to rat pups during lactation. Inhibition or induction of CYP enzymes is not expected to have a major impact on Gle exposures. At the clinical dose,

Gle has the potential to inhibit intestinal CYP3A4. The minimal inhibition and induction of other major human CYPs at clinically relevant exposures indicates a low potential for significant drug-drug interactions with Gle as a perpetrator drug. There is a potential for transporter mediated DDI as a victim and perpetrator given that Gle is a substrate and inhibitor of hepatic uptake (OATP1B1 and 1B3) and efflux transporters (P-glycoprotein [P-gp] and Breast Cancer Resistance Protein [BCRP]).

Safety Pharmacology: Gle was not associated with clinically relevant adverse effects on safety pharmacology parameters. These studies included neurological and respiratory assessments in rats. Cardiovascular evaluations included both in vitro (i.e., hERG inhibition) and in vivo study in telemetered dogs.

Repeat-Dose Toxicology: General toxicology evaluations included GLP repeat-dose studies of ≤ 26 weeks in mice and ≤ 39 weeks in dogs. Overall, Gle was not associated with clinically relevant adverse effects in pivotal studies. However, non-adverse findings or findings of limited clinical relevance were noted in clinical chemistry, hematology, heart, peripheral nerve, gall bladder and testes in dogs as well as liver and kidneys in rats. All effects listed below were recoverable with the exception of necrosis in the liver (rat). Absence of effects in the long-term studies may be indicative of an adaptive effect or incidental findings.

- Clinical Chemistry: Alkaline phosphatase (ALP) levels were increased up to 5.5X in dogs dosed with ≥60 mg/kg/day for 13 weeks. Alanine transferase (ALT) levels were elevated up to 3.8X in dogs dosed with 200 mg/kg/day for 13 weeks and mild increases were observed in dogs dosed with ≥60 mg/kg/day in the 39-week study. Gamma glutamyltransferase (GGT) levels were also mildly elevated in dogs dosed with ≥60 mg/kg/day in the 39-week study.

  Increased ALT and GGT levels were also observed in clinical trials.

- Hematology: Red blood cell mass was decreased up to 23% in dogs dosed with ≥60 mg/kg/day in the 13 week study. This was not observed in the 39-week dog study.
- Kidney: Cysts were noted in the kidneys of rats dosed with 200 mg/kg/day in the 13-week study but not the 26-week study.
- Heart: mesothelial hyperplasia was observed in dogs dosed with ≥60 mg/kg/day in the 13-week study but not the 39-week study.
- Liver: Necrosis was noted in rats dosed with 120 mg/kg/day in the 13-week study but not the 26-week study. The necrosis was also noted in one recovery animal (13-week study; low dose).
- Peripheral nerve: Minimal multifocal degeneration was observed in dogs dosed with 60 mg/kg/day in the 13-week study but not the 39-week study.
- Gall bladder: Edema was observed in dogs dosed with ≥20 mg/kg/day in the 13-week study but not the 39-week study.

- Testes: Atrophy of the tubules was observed in dogs dosed with ≥20 mg/kg/day in the 13-week study but not the 39-week study.

Genetic Toxicology and Carcinogenicity: Gle was not considered genotoxic based on negative results in the *in vitro* bacterial mutation assay, *in vitro* mammalian chromosome aberration assay in human peripheral blood lymphocytes, and *in vivo* rat micronucleus assay. Because Gle was not genotoxic and clinical administration is limited to 16 weeks (maximum), carcinogenicity studies were not required.

Developmental and Reproductive Toxicology: There were no clinically relevant adverse effects on male or female fertility in rats, embryofetal development in rats or rabbits, or pre/post-natal development in rats. An initial dose range finding rabbit embryo fetal study was repeated due to excessive maternal toxicity associated with the vehicle (PEG-400:Tween 20:Poloxamer 124). A new vehicle (Ethanol:PEG-400:Phosal 53 MCT) was utilized in a subsequent study. Neither maternal toxicity nor teratogenic effects were observed in the subsequent embryo fetal development rabbit studies. However the low exposure in rabbits was inadequate for any safety conclusions.

Other Studies: Gle tested positive in the in vitro neutral red uptake phototoxicity assay. An *in vivo* repeat dose phototoxicity study was conducted to determine the effects of oral administration of Gle on the eyes and skin in pigmented rats. Gle elicited no evidence of cutaneous or ocular phototoxicity in this study. The weight of evidence suggests that Gle is not phototoxic.

Excipients, Metabolites, and Impurities: Excipient exposures fell below maximum potencies listed for approved products in the FDA Inactive Ingredient Database, except tocopherol and were, therefore, considered acceptable. Tocopherol is present at levels below the tolerable upper intake level recommended by the NIH and Institute of Medicine.

Table 10 Gle exposure margins

Study	NOAEL	Nonclinical AUC[a]	Exposure margin[b]
General Toxicology			
6-month rat	120 mg/kg/day	734 µg·hr/mL[c]	70x
9-month dog	200 mg/kg/day	1440 µg·hr/mL[d]	137x
Developmental and Reproductive Toxicology			
Segment 1			
fertility and early embryonic development in rat	120 mg/kg/day	657[e]	63x[e]
Segment 2			
embryofetal development in rat	120 mg/kg/day	559 µg·hr/mL[f]	53x
embryofetal development in rabbit	60 mg/kg/day	0.728 µg·hr/mL[g]	0.07x[i]
Segment 3			
pre-/post-natal development in rat	120 mg/kg/day	492 µg·hr/mL[h]	47x

[a] based on A- A-1282576
[b] compared to clinical $AUC_{24,ss}$ = 10.5 µg·hr/mL
[c] Day 181 data
[d] Day 273 data
[e] Based on average exposure from 13- and 26-week studies (male)
[f] GD 17 data
[g] GD 18 data
[h] LD 14 data
[i] Exposure too low to conclude safety

APPENDIX 2 Pibrentasvir/ABT-530/A-1325912

Studies Reviewed

Secondary Pharmacology
Study RD 12750. In vitro pharmacology: (b) (4) high-throughput profile study of A-1325912.0.

Safety Pharmacology
Study RD 111000. A-1325912.0: effects on spontaneous locomotor activity in the mouse (p.o. administration).

Study RD 12371. A neurobehavioral safety evaluation of orally administered A-1325912 (ABT-530) in mice.

Study RD 12699. A-1325912: in vitro effects on hERG current.

Study RD 12664. Effects of A-1325912.0 on cardiovascular and hemodynamic function in the anesthetized dog.

Study RD 12373. A cardiovascular safety evaluation of orally administered A-1325912 (ABT-530) in beagle dogs.

Study RD 12372. A respiratory safety evaluation of orally administered A-1325912 (ABT-530) in mice.

ADME
Study A-1325912 Absorption Memo 02. A-1325912 Pharmacokinetics following Intravenous or Oral Dosing in Mouse

Study A-1325912 Absorption Memo 03. A-1325912 Pharmacokinetics following Intravenous or Oral Dosing in Rat

Study A-1325912 Absorption Memo 04. A-1325912 Pharmacokinetics following Intravenous or Oral Dosing in Monkey

Study A-1325912 Absorption Memo 05. A-1325912 Pharmacokinetics following Intravenous or Oral Dosing in Dog

Study RD 12-631. Preclinical Pharmacokinetic Summary of A-1325912 Single Dose Studies in Mouse, Rat, Rabbit, Dog and Monkey

Study RD 12-634. Absorption, Distribution, Metabolism and Excretion of [3H]A-1325912 (ABT-530) in Male Sprague-Dawley Rats

Study RD 14-0262. Quantitative Whole-Body Autoradiography of Pigmented Rats Following Oral Administration of 14C-ABT-530

Study RD 16-0244. Lacteal Excretion, Placental Transfer, and Tissue Distribution of Radioactivity in Pregnant Female Sprague Dawley Rats After Oral Administration of 14C-ABT-530 and 14C-ABT-493

Study RD 16-0372. Determination of the Unbound Fraction of A-1325912 in Plasma and Microsomal Protein and Blood-to-Plasma Concentration Ratios

Study RD 12-629. In Vitro Biotransformation of A-1325912

Study A-1325912 Drug Metabolism Memo 7. Determination of the Permeability and Transport of A-1325912 using the MDCK-MDR1 Model

Study RD 16-0368. Metabolite Profiles of [14C]A-1282576 (ABT-493) &[14C]A-1325912 (ABT-530) in Milk and Plasma after a 5 mg/kg Oral Dose in Female Sprague-Dawley Rats

Study RD 16-0370. Metabolism and Disposition of [14C]A-1325912 (ABT-530) after a Single 50 mg/kg Oral Dose in CD-1 Mice

Study RD 16-0371. Assessment of Inhibitory Effects on Drug Metabolizing Enzyme Activity by A-1325912

Study RD 16-0434. Assessment of Cytochrome P450 mRNA Induction by A-1325912 in Cultured Human Hepatocytes

Study A-1325912 Drug Metabolism Memo 06. Determination of the Metabolic Stability of A-1325912 in Liver Microsomes and Hepatocytes Across Species

Study A-1325912 Metabolism Memo 16. Metabolism and Disposition of [14C]A-1325912 (ABT-530) after Single 5 mg/kg Oral Dose in Rat

Study A-1325912 Metabolism Memo 17. Preliminary Metabolite Identification of A-1325912 (ABT-530) in Plasma after Multiple Oral Dosing in Sprague-Dawley Rats

Study A-1325912 Metabolism Memo 20. Preliminary Metabolite Identification of A-1325912 in Dog Plasma.

Study A-1325912 Metabolism Memo 18. Metabolism and Disposition of [14C]A-1325912 (ABT-530) after Single 10 mg/kg Oral Dose in Beagle Dogs

Study A-1325912 Metabolism Memo 19. Preliminary Metabolite Identification of A-1325912 (ABT-530) in Human Plasma Samples

Study RD 140243. Assessment of the Effect of A-1325912 on the Activity of UDP-glucuronosyltransferases (UGT) Isoforms in Human Liver Microsomes. 2014.

Study RD 151005. A-1325912. In Vitro Drug Transporter Assessment

Study RD 160368. Metabolite Profiles of [14C]A-1282576 (ABT-493) &[14C]A-1325912 (ABT-530) in Milk and Plasma after a 5 mg/kg Oral Dose in Female Sprague-Dawley Rats

Study A-1325912 Drug Metabolism Memo 21. A-1325912 Pharmacokinetics following Intravenous or Oral Dosing in Transporter Knockout Mouse. 2016.

Repeat-dose Toxicity

Study RD 13309. 10 Day Vehicle Tolerability Study of PEG-300: D5W: Tween80 (89.5:10:0.5, v/v/v) in Rabbits. Study TE13-075. Amended 2016.

Study RD 11840. Two-week oral dose range-finding toxicity study of A-1325912 free base in mice.

Study RD 12138. Thirteen-week oral toxicity study of A-1325912 in CD-1 mice with a four-week recovery period.

Study RD 11928. Two-week oral (capsule) dose range-finding toxicity study of A-1325912 free form in beagle dogs.

Study RD 12345. Thirteen-week oral (capsule) toxicity study of A-1325912 in beagle dogs with a four-week recovery period.

Study RD 13434. A-1325912 Free Form: A 26-Week Oral Toxicity Study with an 8-Week Recovery Period in Mice

Study RD 13172. Thirty-Nine Week Oral Capsule Toxicity Study of A-1325912 Free Form in Beagle Dogs with an Eight-Week Recovery Period

Reproductive Toxicity

Study RD 13958. A Maternal Tolerability Study with A-1325912 Free Form by Oral Gavage in Rabbits. (b) (4) Study 20043617, AbbVie Study TE13-167. 2013.

Study RD 13248. A Dose Range-Finding Intravenous Infusion Study with A-1325912 in Non-Pregnant Rabbits. (b) (4) Study 20034546, AbbVie Study TE12-186. 2013.

Study RD 13321. A Dose Range-Finding Intravenous Infusion Embryo-fetal Development Study with A-1325912 Free Form in Rabbits. (b) (4) Study 20034547, AbbVie Study TE12-187. Amended 2014.

Study RD 13638. An Embryo-Fetal Development Study of A-1325912 Free Form by Oral Gavage in Rabbits

Study RD 13950. An Embryo-Fetal Development Study of A-1325912 Free Form by Oral Gavage in Mice

Study RD 140682. Study of Fertility and Early Embryonic Development to Implantation of A-1325912 Free Form Administered by Oral Gavage in Mice

Study RD 150822. A Developmental and Perinatal/Postnatal Reproduction Study of A-1325912 Free Form by Oral (Gavage) in Mice, Including a Postnatal Behavioral/Functional Evaluation

Genotoxicity

Study RD 12269. A-1325912 free form:Salmonella – E. coli/mammalian microsome reverse mutation assay.

Study RD 12265. In vitro chromosome aberration test in cultured human peripheral blood lymphocytes.

Study RD 13014. In Vivo Micronucleus Assay in Rats with A-1325912 Free Form

Impurity Study

Study RD 160640. Four-Week Oral Dose Toxicity Study of A-1325912 Free Form (with Impurity) in CD-1 Mice

Special Toxicology Studies

Study RD 13656. Neutral Red Uptake Phototoxicity Assay of A-1325912 Free Form in Balb/c 3T3 Mouse Fibroblasts

Study RD 160097. Four-Week Oral Gavage Toxicity Study of A-1282576 and A-1325912 (Free Form) in Sprague-Dawley Rats

Impurities

Study RD 140229. Bacterial Reverse Mutation Assay with (b) (4) (b) (4) Study AD89HI.502ICH.BTL, AbbVie Study TX13-331. 2014.

Study RD 11492. MicroAmes Test with (b) (4) of A-1325912. Study TX11-117. 2011.

Study RD 140045. 5-Strain MiniAmes Test with Components of the Synthetic Route of ABT-530. Study TX13-332. 2015.

Study RD 140675. Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay with (b) (4)

(b) (4) Study 8300181, AbbVie Study TX14-074. 2014.

Study RD 141294. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Components of the Synthetic Route of ABT-530. Study TX14-284. 2015.

Study RD 150081. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Component of the Synthetic Route of ABT-530. Study TX15-025. 2015.

Study RD 150423. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Component of the Synthetic Route of ABT-530. Study TX15-137. 2015.

Study RD 50497. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Components of the Synthetic Route of ABT-530. Study TX15-168. 2015.

Study RD 150602. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Component of the Synthetic Route of ABT-530. Study TX15-195. 2015.

Study RD 150879. 6-Well Bacterial Reverse Mutation Assay with (b) (4); Component of the Synthetic Route of ABT-530. Study TX15-247. 2015.

Study RD 151234. 6-Well Bacterial Reverse Mutation Assay with (b) (4); Component of the Synthetic Route of ABT-530. Study TX15-273. 2016.

Study RD 160165. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Component of the Synthetic Route of ABT-530. Study TX16-056. 2016.

Study RD 160688. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Component of the Synthetic Route of ABT-530. Study TX16-146. 2016.

Study RD 160736. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Components of the Synthetic Route of ABT-530. Study TX16-162. 2016.

Study RD 161095. 6-Well Bacterial Reverse Mutation Assay with (b) (4) Components of the Synthetic Route of ABT-530. Study TX16-234. 2016.

Studies not reviewed

Studies considered irrelevant for the nonclinical safety assessment were not reviewed (e.g. analytical methodology, PK comparison for different formulations, etc.). Also, repeat-dose toxicity studies with CByB6F1-Tg(HRAS)2JIC (TgHras) wild-type mice were conducted with glecaprevir and pibrentasvir to prepare for carcinogenicity studies in case those were needed. However, carcinogenicity studies were not conducted since no causes for concern were identified in genotoxicity or general toxicity studies and since the optimal treatment duration for the proposed combination of glecaprevir and pibrentasvir is expected to be less than 6 months (anticipated duration ≤16 weeks). As such, studies with CByB6F1-Tg(HRAS)2JIC (TgHras) wild-type mice were not reviewed.

Exploratory rat studies with pibrentasvir were also not reviewed.

Pharmacology

Primary Pharmacology

Refer to Virology review.

Secondary Pharmacology

There was no significant (i.e., >50%) binding in a screening assay for off-target receptor binding (10 uM A-1325912).

Safety Pharmacology

CNS and neurobehavioral effects of A-1325912 were assessed in a Functional Observation Battery assay, as well as in assays for spontaneous locomotor activity in rats. There were no apparent central nervous system/neurobehavioral effects in mice at doses up to 60 mg/kg (locomotor) and 100 mg/kg C_{max} 9.8 µg/mL (FOB test).

In non-anesthesized dogs, A-1325912 at oral doses of 3, 10 and 100 mg/kg had no effect on cardiovascular parameters. The resulting exposure at the high dose was 2.2 µg/mL. In anesthetized dogs, IV infusion of A-1325912 had no effect up to systemic exposures of 1.5 µg/mL.

Potential effects of A-1325912 on the QT repolarization interval were assessed in the in vitro hERG assay. A-1325912 reduced hERG channel tail current by 8.4% at 0.5 µg/mL (limit of solubility).

The effects of A-1325912 on respiratory function in mice were assessed following oral administration of 3, 10 or 100 mg/kg. A-1325912 had no effect on respiratory function in mice through the high dose of 100 mg/kg (C_{max} = 6.3 µg/mL).

Table 11 Summary of Safety Pharmacology Studies for A-1325912

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses or Concentrations	Gender and No. Per Group	Noteworthy Findings	GLP	Study Number
Receptor Binding	Receptors, ion channels, and transporters	In vitro	10 µM	n = 2	No displacement of control specific binding > 80% at any of 79 receptors, ion channels, and transporters.	No	R&D/12/750
CNS/Neurobehavior							
Locomotor Activity	Mouse Rj:NMRI	PO	0.6, 2, 6, 20, and 60 mg/kg	Male n = 10	No consistent effects.	No	R&D/11/1000
Functional Observational Battery	Mouse Crl:CD1(ICR)	PO	3, 10, and 100 mg/kg	Female n = 8	No neurobehavioral effects through 100 mg/kg (C_{max} = 9.8 µg/mL).	Yes	R&D/12/371 R&D/12/138
Cardiovascular							
hERG Current	Stably transfected HEK 293 cells	In vitro	0.5 µg/mL	n = 5	8.4% inhibition of hERG tail current.	No	R&D/12/699
Cardiovascular	Dog Beagle	IV	56, 187, and 562 µg/kg/30 min	Male n = 6	No cardiovascular effects through the highest plasma concentration of 1.5 µg/mL.	No	R&D/12/664
Cardiovascular	Dog Beagle	PO	3, 10, and 100 mg/kg	Male n = 6	No effects on MAP, HR, or QTc through 100 mg/kg (C_{max} = 2.2 µg/mL).	Yes	R&D/12/373 R&D/12/345
Respiratory							
Respiratory	Mouse Crl:CD1(ICR)	PO	3, 10, and 100 mg/kg	Male n = 8	No effect on respiratory rate, tidal volume or minute volume through 100 mg/kg (C_{max} = 6.3 µg/mL).	Yes	R&D/12/372 R&D/12/138

GLP = good laboratory practice; PO = oral; hERG = human ether-a go-go-related gene; IV = intravenous; MAP = mean arterial pressure; HR = heart rate; QTc = QT interval corrected for heart rate; C_{max} = maximum observed concentration

Pharmacokinetics/ADME/Toxicokinetics

Absorption

The pharmacokinetic behavior of A-1325912 was evaluated in mice, rats, cynomolgus monkeys and beagle dogs. The pharmacokinetics were characterized by plasma elimination half-lives which ranged from 5.7 hours in monkey to 8.3 hours in dog. Volumes of distribution (Vss) were between 0.5 and 0.9 L/kg. Plasma clearance values were lowest in rat (0.07 L/hr●kg), and highest in monkey (0.15 L/hr●kg).

A-1325912 exhibited relatively slow absorption after oral dosing in mouse, rat and dog, with slowest absorption in the monkey (T_{max} = 11 hr). Bioavailability values were highest in dog and lowest in mouse. AUC values were highest in the dog and mouse.

Table 12 Pharmacokinetics of A-1325912 after a Single Dose in Multiple Species

Intravenous Dose

Species	Dose (mg/kg)	$t_{1/2}$ (hr)	V_c (L/kg)	V_{ss} (L/kg)	V_β (L/kg)	$AUC_{0-\infty}$ (µg•hr/ml)	CL_p (L/hr•kg)	n
Mouse	3	n.f.	0.1			>252.1[a]	<0.003	3
Rat	3	6.2	0.1	0.5	0.7	41.0 (7.3)	0.07 (0.01)	3
Monkey	1	8.3	0.1	0.8	1.8	6.85 (1.45)	0.15 (0.03)	3
Dog	1	7.1	0.1	0.9	1.0	10.6 (2.0)	0.10 (0.02)	3

Oral Dose

Species	Dose (mg/kg)	$t_{1/2}$ (hr)	C_{max} (µg/ml)	T_{max} (hr)	$AUC_{0-\infty}$ (µg•hr/ml)	F (%)	n
Mouse	3	n.f.	1.51 (0.01)	11.0 (1.7)	20.0[a]	7.9	3
Rat	3	7.0	0.28 (0.05)	5.3 (1.2)	4.07 (0.59)	9.9 (1.4)	3
Monkey	2.5	5.7	0.29 (0.09)	4.0 (0.0)	2.42 (0.70)	14.1 (4.1)	3
Dog	2.5	8.3	0.63 (0.16)	3.7 (0.6)	7.86 (2.11)	29.8 (8.0)	3
	10 [f]	n.f.	1.32 (0.12)	1.8 (0.3)	11.1 (0.96)[a]		3
	10 [nf]	n.f.	1.10 (0.69)	2.5 (1.3)	10.7 (7.64)[a]		3

Data provided as mean (standard deviation); n.f. – unable to estimate plasma elimination half-life

a. AUC 0-24 hr
f - fasted dogs
nf - non-fasted (fed) dogs

Distribution

In a quantitative whole-body autoradiography (QWBA) study in pigmented Long-Evans rats treated with a 5 mg/kg single oral dose of [14C]pibrentasvir, drug-derived radioactivity was slowly absorbed and distributed into tissues, with peak concentrations generally noted 4-8 hours after dosing. Most radioactivity was eliminated within 48-hours post-dose and radioactivity was below measurable levels in the brain and eye all times; binding to melanin was not observed. Radioactivity concentrations were below measurable levels in the non-circumventricular central nervous system (CNS) tissues (cerebellum, cerebrum, medulla, olfactory lobe, and spinal cord), the lens of the eye, abdominal fat, arterial wall, bone, eyes, intra-orbital lacrimal gland, meninges, seminal vesicles, and urine at all collection times throughout the time course of this study. Bile had higher amounts of radioactivity than those measured for tissues and blood from 1 through 8 hours postdose. The adrenal gland and liver contained the highest amounts of radioactivity observed for any tissue from 1 through 4 hours postdose. Excluding bile, tissues with the highest Cmax values were adrenal gland, liver, small intestines, lymph nodes, and myocardium. Tissues with the lowest Cmax values were epididymis, testis, uveal tract, exorbital lacrimal gland, and nonpigmented skin. The pituitary gland, a CNS tissue not protected by the blood:brain barrier, had measurable concentrations of radioactivity from 2 to 8 hours postdose. The data illustrated that [14C]pibrentasvir did not cross the blood:brain barrier at levels above the QWBA lower quantitation limit of 48.5 ng eq/g.

Based on radioactivity studies, A-1325912 was excreted in milk of lactating rats and crossed the placenta of pregnant rats (minimal distribution into blood and tissues of developing fetus) after oral administration of 5 mg/kg [14C]A-1325912.

Metabolism and Excretion

A-1325912 did not inhibit nor induce any major human CYPs at clinically relevant concentrations, suggesting a low potential for CYP mediated drug-drug interactions for pibrentasvir as a perpetrator drug. CYP inhibitors or inducers are not expected to have an impact on pibrentasvir clinical exposures. In general, all the human metabolites formed in the in vitro hepatic system were present in one animal species. Excretion was via the biliary/fecal route.

Table 13 Proposed Biotransformation Path for Pibrentasvir (A-1325912) in Mouse, Rat, Dog and Human

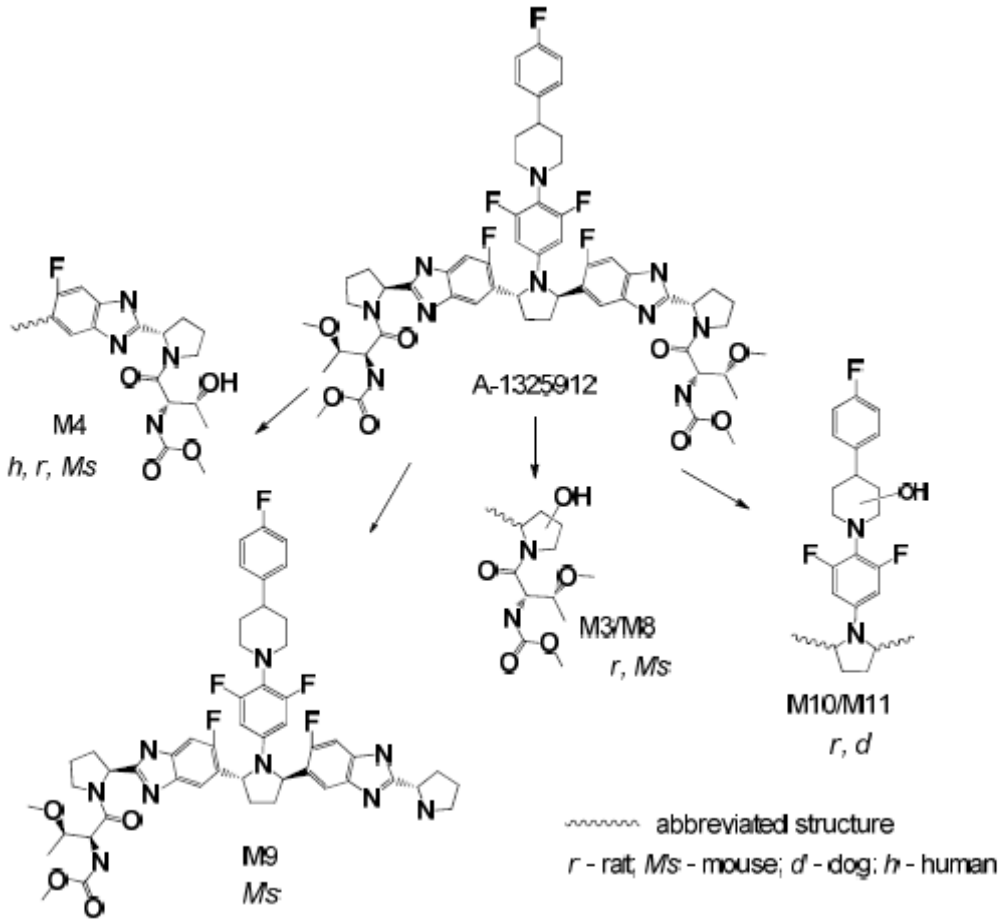


Table 14 Pibrentasvir (A-1325912) Metabolite Profile in Plasma at Steady State

Plasma Metabolites	Mouse (a)	Rat (b)	Dog (c)	Human (d)
Pibrentasvir (parent drug)	major	major	major	major
A-1325912 isomer	minor	-		minor
M4 (O-demethylation)	minor	-	-	minor
M8 (mono-oxidation)	minor	-	-	-
M9 (amide hydrolysis)	minor	-		-
M10 (mono-oxidation)	-	-	minor	-
M11 (mono-oxidation)	-	-	minor	-

Designations of major and minor reflect the FDA MIST guidelines, where major is defined as >10% of total drug related materials in plasma and minor is defined as <10% of total drug related materials in plasma.

(a) 100 mg/kg/day x 8 days, (b) 30 mg/kg/day x 85 days; (c) 100 mg/kg/day x 280 days;

(d) Study M13-355, 600 mg daily dose x 10 days.

The metabolite profiles were studied in milk and plasma, following a single 5 mg/kg oral dose of [14C]A-1325912 to lactating female rats. [14C]A-1325912 represented the majority of total radioactivity in both rat milk (100%) and plasma (97.8%). One minor uncharacterized radiochemical component U1 was also detected in rat plasma.

Transporters

A-1325912 exposure may be impacted by inhibitors or inducers of P-gp and/or BCRP. Inhibition of P-gp, BCRP or OATP1B1 by A-1325912 may increase the plasman exposure of sensitive substrates. A-1325912 is an inhibitor of UGT1A1 and UGT1A4. A weak but not clinically relevant interaction with raltegravir (UGT1A1 substrate) was observed and no clinically significant interaction was observed with lamotrigine (UGT1A4 substrate).

General Toxicology

Single-Dose Toxicity

No single dose studies were conducted.

Repeat-Dose Toxicity

Two-Week Oral Dose Range-Finding Toxicity Study of A-1325912 Free Base in Mice (RD 11840) A-1325912 was administered to five CD-1 mice per sex per group by oral gavage at 10, 30 or 300 mg/kg/day for 14 or 15 days. There were no dose limiting toxicities or adverse effects. Consequently, the no observed adverse effect level (NOAEL) in this study was the high dose of 300 mg/kg/day, which was associated with an AUC of 197.5 µg●hr/mL and a Cmax of 10.16 µg/mL (Day 14, mean of combined male and female values).

Study title: Thirteen-week oral toxicity study of A-1325912 in CD-1 mice with a four-week recovery period (RD 12138)

Study no.:	TA12-007
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	27 January 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912 free form, A-1325912.0, Lot number 11722ZW01), 98.7%

Key Study Findings

There were no toxicologically significant findings. The No-Observed-Adverse-Effect Level (NOAEL) in this study was 100 mg/kg/day. This resulted in a mean steady state (Day 91, combined male and female exposure values) C_{max} of 6.14 µg/mL and AUC of 127 µg●hr/mL.

Methods

Doses:	3, 10 and 100 mg/kg
Frequency of dosing:	Once daily for 91 days
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	Phosal 53 MCT:Peg-400:Poloxamer 124:Cremophor RH40 (40:20:20:20)
Species/Strain:	Mice/CD-1
Number/Sex/Group:	15 (includes 5/sex/group for four week recovery)
Age:	9 weeks
Weight:	188-361 grams
Satellite groups:	Toxicokinetics (38/sex/group)
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: Observations for morbidity, mortality, and injury were conducted twice daily for all animals. One animal administered 100 mg/kg/day was euthanized on Day 17 due to an eye injury determined to be unrelated to test item administration. Additionally, three TK satellite animals were euthanized in poor clinical condition. The condition of the TK animals necessitating euthanasia was considered not to be test item-related.

Clinical Signs: Clinical signs were recorded twice weekly during dosing. There were no remarkable findings.

Body Weights: Body weights were recorded twice weekly during dosing. There were no remarkable findings.

Feed Consumption: Food consumption was recorded once weekly during dosing. There were no remarkable findings.

Hematology/Coagulation: Blood samples were collected at necropsy. There were no remarkable findings.

Clinical Chemistry: There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Adrenal glands, brain, heart, kidney, liver, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid and parathyroid were weighed. There were no significant findings.

Non dose-dependent and non-adverse changes in absolute and relative spleen weights (up to -30% absolute and -20% relative as compared to controls) were observed; there

was no microscopic correlate, and spleen weight changes were not present at end of recovery period.

Histopathology

Adequate Battery- Yes.

Peer Review- Yes.

Histological Findings

There were no remarkable findings.

Toxicokinetics: Blood samples for determination of plasma test item concentration were collected from three satellite mice/sex/group/time point in Groups 2 through 4 at 1, 3, 6, 9, 12 and 24 hours after dosing on Day 1, and again near the end of the dosing period.

Pharmacokinetic Parameters for A-1325912, Mean[a]

Collection Interval	Sex	A-1325912 Dosage (mg/kg/day)		
		3	10	100
		Mean Plasma $AUC_{0\text{-}24\ h}$ (µg•hr/mL)		
Day 1	Males	11.6	30.9	107
	Females	21.2	55.2	175
	Overall	16.4	43.0	141
Day 91	Males	15.7	52.9	114
	Females	21,9	43.0	141
	Overall	18.8	48.0	127
		Mean Plasma C_{max} (µg/mL)		
Day 1	Males	0.576	1.61	6.30
	Females	1.09	3.47	9.78
	Overall	0.820	2.50	8.04
Day 91	Males	0.781	2.78	5.05
	Females	1.11	2.48	7.46
	Overall	0.917	2.29	6.14

a Standard deviations could not be computed due to the use of composite data.

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Study title: A-1325912 Free Form: A 26-Week Oral Toxicity Study with an 8-Week Recovery Period in Mice (RD 13434)

Study no.:	TD13-007
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 20, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912.0 (A-1325912); Lot 11722ZW01; 98.7%

Key Study Findings

Based on the lack of findings, the NOAEL is the high dose of 100 mg/kg/day, corresponding to an overall mean AUC of 123 µg·hr/mL on Day 182. This provides an exposure margin of 88X based on observed human exposure to 120 mg A-1325912.

Methods

Doses:	0, 3, 10, 100
Frequency of dosing:	Once per day
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	Phosal 53 MCT:PEG-400:Poloxamer 124:Cremophor RH40 (40:20:20:20 w/w)
Species/Strain:	Crl:CD1® (ICR) mice
Number/Sex/Group:	20
Age:	7 weeks
Weight:	Male: 27.1 to 35.0 g Female: 21.4 to 27.6 g,
Satellite groups:	10 (control and high dose) for recovery
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: Animals were checked twice daily for mortality. Eight mice did not survive to the scheduled necropsy or termination. Two control males were found dead on Days 117 and 149, and one male at 100 mg/kg/day died after dosing on Day 147. One control

male, two males at 10 mg/kg/day (one of which was in the TK group), and two females at 100 mg/kg/day were euthanized in extremis on Days 152, 131, 97, 19, and 134 respectively. None of these found dead or early terminations were considered test article-related due to occurrence in control group and/or proximity to dose administration (e.g. gavage and/or handling error).

Clinical Signs: Detailed clinical signs were recorded weekly. There were no remarkable findings.

Body Weights: Body weights were recoded twice weekly. There were no remarkable findings.

Food Consumption: Food consumption was recoded twice weekly There were no remarkable findings.

Ophthalmoscopy: Ophthalmoscopy was conducted before necropsy in main and recovery animals. There were no remarkable findings.

Hematology: Blood samples for clinical pathology were collected from main study animals at the terminal and recovery necropsies. There were no remarkable findings.

Clinical Chemistry: Blood samples for clinical pathology were collected from main study animals at the terminal and recovery necropsies. There were no remarkable findings.

Urinalysis: Urine samples were collected from main study animals at the terminal and recovery necropsies. There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: There were no remarkable findings.

Histopathology

Adequate Battery: The battery was adequate. Tissues were microscopically examined at terminal necropsy for all control and high dose (100 mg/kg/day) mice. Mice from low and middle dose (3 and 10 mg/kg) with gross lesions or unscheduled deaths were examined microscopically.

Peer Review- Yes

Histological Findings

There were no remarkable findings.

Toxicokinetics: Samples were collected at 1, 3, 6, 9, 12, and 24 hours postdose on Days 1 and 182. Toxicokinetic parameters are below (table excerpted from sponsor).

Mean Toxicokinetic Parameters for A-1325912

Dose Level (mg/kg/day)	Sex	Day 1 C_{max} (µg/mL)	Day 1 AUC (µg·hr/mL)	Day 182 C_{max} (µg/mL)	Day 182 AUC (µg·hr/mL)
3	Male	0.581	10.8	0.908	18.4
	Female	0.543	10.7	0.642	13.6
	Overall	0.553	10.7	0.774	16.0
10	Male	2.22	32.9	2.39	44.1
	Female	1.84	32.0	2.82	53.9
	Overall	2.03	32.4	2.61	49.1
100	Male	5.58	91.8	5.72	104
	Female	6.50	96.3	6.87	141
	Overall	5.43	94.0	6.08	123

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Two-week oral (capsule) dose range-finding toxicity study of A-1325912 free form in beagle dogs (RD 11928) Administration of A-1325912 at dosages of 10, 30 and 300 mg/kg/day to two Beagle dogs per sex per group via oral capsules, did not result in any adverse or test item-related effects. Therefore, the No-Observed-Adverse-Effect-Level (NOAEL) in this study was 300 mg base/kg/day, resulting in a Cmax of 2.39 µg/mL and 3.81 µg/mL and an AUC_{0-24} of 35.6 µg●hr/mL and 54.8 µg●hr/mL for males and females, respectively.

Study title: Thirteen-week oral (capsule) toxicity study of A-1325912 in beagle dogs with a four-week recovery period (RD 12345)

Study no.:	TB12-008
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	31 January 2012
GLP compliance:	Yes
QA statement:	Yes

Drug, lot #, and % purity:	A-1325912 (A-1325912.0, A-1325912 free form, A-1325912, Lot 11722ZW01), (93.7%),

Key Study Findings

Administration of A-1325912 at dosages of 10, 30 and 100 mg/kg/day did not result in any adverse or test item-related effects. The NOAEL was 100 mg base/kg/day, corresponding to an AUC (males and females combined) of 32 µg●hr/mL.

Methods

Doses:	10, 30, or 100 mg/kg/day
Frequency of dosing:	Once daily for 3 months
Route of administration:	Oral (gelatin capsules)
Dose volume:	1 mL/capsule
Formulation/Vehicle:	Gelatin capsule/ Phosal 53 MCT:Polyethylene Glycol 400:Poloxamer 124:Cremophor RH40 (40%:20%:20%:20%, by weight)
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4
Age:	7 months
Weight:	6 to 9 kg
Satellite groups:	See table below
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. There were no unscheduled deaths.

Clinical Signs: Clinical observations were conducted twice weekly at 2 hours to 3 hours postdose. There were no remarkable findings. Fecal changes (unformed, mucoid, or watery) were noted in middle and high dose group dogs to a greater extent than in control group animals. In the absence of diet or body weight changes the effects were not considered to be significant.

Body Weights: Body weights were recorded twice weekly. There were no remarkable findings**.**

Feed Consumption: Food consumption was measured and recorded twice weekly during the study. There were no remarkable findings.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted and prior to the terminal necropsy (Day 88) and on recovery Day 25. There were no remarkable findings.

Hematology: Blood samples for clinical pathology evaluations were collected from all animals once on Dosing Day 88 and on Recovery Day 28. There were no remarkable findings.

Electrocardiogram: Electrocardiograms were recorded once during the baseline period, on Days 7-8 and on Days 86-87 of the dosing period and on Day 24 of the recovery period for all surviving animals. Electrocardiograms were recorded two-three hours after the first BID dose. There were no remarkable findings.

Clinical Chemistry: Blood samples for clinical pathology evaluations were collected from all animals once on Dosing Day 88 and on Recovery Day 28. There were no remarkable findings.

Urinalysis: Urine samples were collected prior to the terminal necropsy and end of recovery. There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Adrenals, brain, heart, kidney, liver, pituitary, prostate, spleen, ovaries, testes, thyroid/parathyroid, and thymus were weighed. There were no remarkable findings.

Histopathology

Adequate Battery- Yes.

Peer Review- Yes.

Histological Findings

There were no remarkable findings.

Toxicokinetics: Blood samples were collected predose and at 1, 3, 6, 9, 12, and 24 hours postdose on Days 1 and 91.

Pharmacokinetic Parameters for A-1325912, Mean ± Standard Deviation (SD)

Collection Interval	Sex	A-1325912.0 Dosage (mg/kg/day)		
		3	10	100
		Mean Plasma AUC_{24} (µg•hr/mL) ± SD		
Day 1	Males	3.80±0.162	11.6±3.5	29.8±7.41
	Females	3.65±0.682	10.4±2.09	25.5±7.52
	Overall	3.72±0.466	11.0±2.75	27.7±7.46
Day 91	Males	3.45±1.41	11.4±4.07	31.5±6.91
	Females	5.7±2.08	14.2±7.48	33.4±17.9
	Overall	4.57±2.04	12.8±5.78	32.4±13.0
		Mean Plasma C_{max} (µg/mL) ± SD		
Day 1	Males	0.365±0.0146	1.30±0.351	2.22±0.496
	Females	0.407±0.101	0.913±0.182	1.92±0.328
	Overall	0.386±0.0703	1.11±0.333	2.07±0.429
Day 91	Males	0.264±0.0915	1.00±0.240	2.11±0.514
	Females	0.578±0.342	1.12±0.427	2.75±1.26
	Overall	0.421±0.286	1.06±0.327	2.43±0.975

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Study title: Thirty-Nine Week Oral Capsule Toxicity Study of A-1325912 Free Form in Beagle Dogs with an Eight-Week Recovery Period (RD 13172)

Study no.:	TB13-008
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	20 March 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912 free form (A-1325912.0, A-1325912); Lot: 11722ZW01 (equivalent to 11722ZW00) and 30083ZW00.

Lot 117222W00 was used during (b) (4) (02 APR to 08 SEP 2013). Lot 30083ZW00 was used during (b) (4) (09 SEP 2013) to (b) (4) (08 JAN 2014).
Assigned Chemical Potency:
30083ZW00, 992 µg/mg substance.
11722ZW00, 987 µg/mg substance.

Key Study Findings

There were no toxicologically significant findings. The NOAEL is defined as the high dose, 100 mg/kg/day, corresponding to systemic exposure values of 25 µg•hr/mL (AUC_{0-24}) (Day 280; males and females combined). This provides an exposure margin of 17X based on observed human exposure to 120 mg A-1325912.

Methods

Doses:	3, 10, or 100 mg/kg
Frequency of dosing:	QD for 9 months
Route of administration:	Oral (gelatin capsules)
Dose volume:	1 mL/kg in capsule
Formulation/Vehicle:	Gelatin capsule/ Phosal 53 MCT: PEG-400: Poloxamer 124: Kolliphor RH40 (40:20:20:20 w/w).
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4
Age:	10-11 months
Weight:	6.2 to 9.8 kg
Satellite groups:	2 in control and high dose groups (recovery)
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. There were no early deaths.

Clinical Signs: Clinical observations were conducted once weekly at 2 hours to 4 hours postdose. There were no remarkable findings.

Body Weights: Body weights were recorded weekly. There were no remarkable findings.

Feed Consumption: Food consumption was measured and recorded weekly during the study. There were no remarkable findings.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted and prior to the terminal and recovery necropsies. There were no remarkable findings.

Hematology: Venous blood samples were withdrawn from all surviving dogs on Baseline Days 2 and 9, Dosing Days 92, 184, 277 and Recovery Day 56. Absolute reticulocytes were decreased up to 46% at the high dose of 100 mg/kg/day on Day 277. This effect was not observed in recovery animals.

Electrocardiogram: Electrocardiograms were recorded near the end of the first week of dosing (Days 7, 8) and again near the end of the dosing (Days 269, 270, 273) and recovery (Recovery Day 52) periods for all surviving animals. There were no remarkable findings.

Clinical Chemistry: Venous blood samples were withdrawn from all surviving dogs on Baseline Days 2 and 9, Dosing Days 92, 184, 277 and Recovery Day 56.

Urinalysis: A urine sample from the bladder of each dog was collected at scheduled necropsies. There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Terminal body weights were obtained and the organs indicated in the table below were weighed at scheduled necropsy. There were no remarkable findings.

Histopathology

Adequate Battery- Yes.

Peer Review- Yes (sponsor).

Histological Findings

There were no remarkable findings.

Toxicokinetics: Venous samples of blood were collected from each dog at approximately 1, 3, 6, 9, 12 and 24 hours after dosing on Dosing Days 1, 98 and 280.

Toxicokinetic parameters in dogs following 280 days of oral administration of A-1325912 are presented below. Exposures were similar in males and females.

Table 15 Toxicokinetic parameters in dogs following 280 days of oral administration of A-1325912

	Day 1		Day 98		Day 280	
Dose Level (mg/kg/day)	C_{max} (µg/mL)	AUC (µg·hr/mL)	C_{max} (µg/mL)	AUC (µg·hr/mL)	C_{max} (µg/mL)	AUC (µg·hr/mL)
3	0.244	1.54	0.377	3.66	0.439	4.70
10	0.905	5.96	1.13	13.5	1.10	13.7
100	1.42	9.72	2.20	27.5	1.92	25.2

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Genetic Toxicology

Study title: Salmonella-E.Coli/Mammalian Microsome Reverse Mutation Assay (RD 12269)

Study no.:	TX12-012
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	16 February 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912 (lot no. 11722ZW01), 98.7%

Key Study Findings

A-1325912 was negative for mutations under the conditions of this *Salmonella–E. coli* Reverse Mutation Assay.

Methods

Strains:	TA98, TA100, TA1535, TA1537, WP2*uvr*A
Concentrations in definitive study:	25, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate with and without S9
Basis of concentration selection:	Dose range-finding assay
Negative control:	dimethylsulfoxide (DMSO)
Positive control:	Without metabolic activation, ICR-191 acridine (0.5 µg/plate) with strain TA1537; 2-nitrofluorene (2.5 µg/plate) with strain TA98; sodium azide (1.0 µg/plate) with strains TA100 and TA1535; and 4-nitroquinoline-N-oxide (2.0 µg/plate) for WP2 *uvrA*. With metabolic activation, 2-aminoanthracene was used as the positive control for all strains.
Formulation/Vehicle:	dimethylsulfoxide (DMSO),
Incubation & sampling time:	2 days

Study Validity

Study validity criteria were met.

Results

A-1325912 (A-1325912) was negative for mutations under the conditions of this *Salmonella–E. coli*/Mammalian-Microsome Reverse Mutation Assay

Study title: In vitro Chromosome Aberration Test in Cultured Human Peripheral Blood Lymphocytes (RD 12265)

Study no.:	MBR12-101
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	16 February 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912 (lot no. 11722ZW01), 98.7%

Key Study Findings

A-1325912 (A-1325912) was considered negative for inducing structural and numerical chromosome aberrations in human peripheral blood lymphocytes with or without metabolic activation under the conditions of this test system.

Methods

Cell line:	Cultured human lymphocytes
Concentrations in definitive study:	See table below
Basis of concentration selection:	Dose range-finding study
Negative control:	dimethylsulfoxide (DMSO)
Positive control:	mitomycin C (MMC): without metabolic activation cyclophosphamide (CP): with metabolic activation
Formulation/Vehicle:	dimethylsulfoxide (DMSO)
Incubation & sampling time:	See table below

Treatment	Treatment Conditions and Concentrations		
	3 h without S9	3 h with S9	22 h without S9
DMSO (%)	1.0	1.0	1.0
Mitomycin C (µg/mL)	0.60	--	0.30
Cyclophosphamide (µg/mL)	--	20	--
A-1325912 (µg/mL)	15.6 125 250	15.6 125 250	7.81 125 250

Study Validity

All validity criteria were met.

Results

A-1325912 was considered to be negative for causing chromosomal aberrations under the conditions of this assay.

Study title: In Vivo Micronucleus Assay in Rats with A-1325912 Free Form (RD 13014)

Study no.:	TA12-189
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 29, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912; 11722ZW01; 98.7%

Key Study Findings: No A-1282576 related effects were noted following oral administration at up to 2000 mg/kg to male rats. A-1325912 induced a statistically significant increase in the mean percent micronucleated PCEs at the highest dose tested (2000 mg/kg) in the 48-hour time point animals. This was not considered to be biologically significant since the mean percent micronucleated PCE in the high dose animals was within the historical vehicle control range.

Methods

Doses in definitive study:	0, 500, 1000 and 2000 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral Gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	Phosal® 53 MCT:Polyethylene glycol 400 (PEG-400):Poloxamer 124: Cremophor® RH40 (40:20:20:20)
Species/Strain:	SD Rats
Number/Sex/Group:	5
Satellite groups:	TK animals
Basis of dose selection:	Dose range study
Negative control:	Vehicle
Positive control:	Cyclophosphamide

Results: There were no A-1325912-related effects. A-1325912 induced a statistically significant increase in the mean percent micronucleated PCEs at the highest dose tested (2000mg/kg) in the 48-hour time point animals. This was not considered to be biologically significant since the mean percent micronucleated PCE in the high dose

animals (0.11 ± 0.05%) was within the historical vehicle control range (0.00-0.20%). Otherwise, A-1325912 was not cytotoxic to the bone marrow (i.e., no statistically significant decreases in the PCE:NCE ratios) at any dose of the test article.

The vehicle control micronucleated PCE value was within the vehicle historical control range showing a normal background level in this test. The positive control, cyclophosphamide, induced a statistically significant increase in micronucleated PCEs, confirming the validity of the assay.

Therefore, A-1325912 was negative in the rat bone marrow micronucleus assay when tested up to 2000 mg/kg/day and under the conditions of this assay.

Reproductive and Developmental Toxicology

10 Day Vehicle Tolerability Study of PEG-300: D5W: Tween80 (89.5:10:0.5, v/v/v) in Rabbits (RD 13309). To evaluate the tolerability of PEG-300:D5W:Tween80 (89.5:10:0.5, v/v/v), female rabbits (three/group) were administered a dosage of 1 mL/kg/day via IV infusion over 15 minutes once daily for ten consecutive days. Parameters that were evaluated in the study consisted of survival, clinical signs, body weight and food consumption. Although administration of PEG-300:D5W:Tween80 (89.5:10:0.5, v/v/v) for up to 10 days resulted in bruising, swelling and scabbing of the ears, the test item was deemed tolerable for a subsequent intravenous embryo-fetal developmental toxicity study in rabbits. The vehicle was next tested in a dose range finding IV study in non-pregnant rabbits (RD 13248). In the EFD dose range finding study (RD 13321), the vehicle-mediated toxicity resulted in early termination of the study.

A Dose Range-Finding Intravenous Infusion Study with A-1325912 in Non-Pregnant Rabbits. (RD 13248) Twelve non-pregnant New Zealand White [Hra:(NZW)SPF] female rabbits were randomly assigned to four dose groups, three rabbits per group. Formulations of the test item, pibrentasvir, or the control item, PEG-300: D5W: Tween 80 (89.5/10/0.5 v/v), were administered once daily to these rabbits on Days 1 through 10 of study (DS 1 through 10) via 15 ± 1 minute IV infusion (marginal ear vein) at doses of 0 (control), 2.5, 5 or 10 mg/kg/day. Checks for viability were made twice daily. Clinical observations were recorded daily before dose administration. Postdose observations were recorded at hourly intervals (± 10 minutes) for the first four hours and at the end of the normal working day. Special attention was paid to the site of injection (ear) for any signs of irritation. Body weights and feed consumption values were recorded daily during the dose period. Rabbits were euthanized on Day 11 and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The administration site(s) (both ears) were collected from all rabbits and preserved in 10% neutral buffered formalin. Discoloration at the site of injection was observed in all groups. Rabbits in the 10 mg/kg group also exhibited injection site swelling. The pibrentasvir dosages used in this study were considered sufficiently well tolerated to be evaluated in a range-finding IV infusion embryo-fetal development (EFD) study in

rabbits (RD 13321). However the EFD study was ultimately terminated early due to excessive deaths.

A Dose Range-Finding Intravenous Infusion Embryo-fetal Development Study with A-1325912 Free Form in Rabbits (RD 13321) Forty timed-mated New Zealand White [Hra:(NZW)SPF] rabbits were randomly assigned to four dose groups, 10 rabbits per group. The control item (PEG-300: D5W: Tween 80 [89.5/10/0.5 w/w/w]) or the test item (A-1325912) were administered via 15 ± 1 minute IV infusion once daily on GD 7 through 19 for rabbits assigned to the main study and on GD 7 through 20 for rabbits assigned to toxicokinetic (TK) sample collection at doses of 0, 2, 5 and 10 mg/kg/day. Excessive maternal toxicity resulting in mortality (death or unscheduled euthanasia) occurred in all study groups over GD 17 to 24 (three, five, four and four rabbits in the 0, 2, 5 and 10 mg/kg/day dose groups, respectively). These rabbits exhibited adverse clinical signs (including ear swelling/discharge, ungroomed coat, dehydration and/or evidence of abortion), body weight loss/decreased body weight gain and a concomitant decrease in food consumption. Since this increased incidence of mortality was considered vehicle-related, all surviving rabbits were euthanized on GD 23 or 24. As such, no valid fetal examinations were performed.

A Maternal Tolerability Study with A-1325912 Free Form by Oral Gavage in Rabbits (RD 13958) Nine timed-mated New Zealand White [Hra:(NZW)SPF] female rabbits were randomly assigned to three dose groups, three rabbits per group. Dose formulations of the control item (Phosal 53MCT:PEG 400:Poloxamer 124: Cremophor RH40 [40:20:20:20 w/w]) or A-1325912 were administered via oral gavage once daily to these rabbits on Gestation Days (GD) 7 through 19 at doses of 0, 10 and 100 mg/kg/day. On GD 7 and GD 19, blood samples were collected from each rabbit at approximately 1.5, 3, 4, 6, 9, 12 and 24 hours postdose for bioanalysis and toxicokinetic evaluation. The following parameters were evaluated in this study: maternal viability, maternal clinical signs, maternal body weight and maternal body weight gain, maternal food consumption, toxicokinetic parameters, pregnancy status and maternal gross necropsy findings. One rabbit in the 100 mg/kg group died due to gavage error on GD 11. One out of two rabbits in the 100 mg/kg dose group exhibited scant feces, body weight loss and a concomitant decrease in food consumption over GD 7 to 20. Based on the limited number of animals evaluated, the relationship of these effects to A-1325912 was uncertain. The vehicle in this study was later tested in the definitive rabbit embryofetal development study.

Fertility and Early Embryonic Development

Study title: Study of Fertility and Early Embryonic Development to Implantation of A-1325912 Free Form Administered by Oral Gavage in Mice (RD 140682)

Study no.:	TD14-107
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 3, 2014
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912; 37209ZW10; 98.4%

Key Study Findings

Based on the lack of findings, the NOAEL for general toxicity and reproductive and fertility parameters in males and females was 100 mg/kg/day, the highest dose level evaluated (C_{max} 6.95 µg/mL; AUC_{0-24}: 153.0 µg*hr/mL, in males). This provides an exposure margin of 106X based on observed human exposure to 120 mg A-1325912.

Methods

Doses:	0, 3, 10, or 100 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Phosal 53MCT:PEG 400:Poloxamer 124: Cremophor RH40 [40:20:20:20 w/w]
Species/Strain:	Crl:CD1 (ICR) mice
Number/Sex/Group:	30
Study design:	Male and female mice were given the test article or the control article formulations once daily beginning 14 days before and during cohabitation. Dose administration for males continued through the day before euthanasia (for a total of 34 to 36 doses each) and for females through Gestation Day (GD) 6 (for a total of 22 to 30 doses each). Females were euthanized on GD 13.
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily. One female in the control group was euthanized on day of study 2 (DS 2) due to a corneal abrasion on the left eye and one female in the 10 mg/kg/day group was euthanized on DS 8 due to misintubation as confirmed in necropsy (esophageal perforation). There were no other unscheduled deaths.

Clinical Signs: Detailed clinical examinations were conducted daily during treatment. There were no remarkable findings.

Body Weight: Body weights were recorded twice weekly and on GD 0, 3, 7, 10, and 13 for females. There were no remarkable findings.

Feed Consumption: Food consumption was recoded twice weekly and on GD 0, 3, 7, 10, and 13 for females. There were no remarkable findings.

Toxicokinetics: Blood was collected 1, 3, 6, 9, 12 and 24 hours post dose on day of euthanasia in males only. C_{max} and AUC_{0-24} values were less than dose proportional with increasing dose between 10 and 100 mg/kg/day. The 100 mg/kg dose was selected as the high dose, since this was the maximal feasible exposure as determined in previously conducted pharmacokinetic studies.

Table 16 Summary of Steady State Toxicokinetic Parameters of A-1325912 in Male Mice on Day 35/36 of the Study

Dose level (mg/kg)	C_{max} (µg/mL)	C_{max}/D [(µg/mL)/(mg/kg)]	T_{max} (hr)	AUC_{0-24} (µg*hr/mL)	AUC/D [(µg*hr/mL)/(mg/kg)]
3	1.01	0.3370	12	21.2	7.06
10	3.54	0.3540	12	69.1	6.91
100	6.95	0.0695	12	153.0	1.53

Sex Organ Weights (Males only): There were no remarkable findings.

Necropsy
On GD 13, female mice were euthanized, and a gross necropsy of the thoracic, abdominal and pelvic viscera was performed. The reproductive tract was dissected from the abdominal cavity. The uterus was opened and the contents were examined. The embryos were removed from the uterus.

The ovaries and uterus were examined for number and distribution of:

- Corpora Lutea
- Implantation Sites
- Placentae (size, color or shape)
- Resorptions
- Live and Dead Embryos

Uteri of apparently nonpregnant female mice were examined while being pressed between glass plates to confirm the absence of implantation sites.

Male mice were euthanized following completion of at least 80% of the female ovarian/uterine examinations. Male mice were examined for gross lesions and subjected to a gross necropsy of the thoracic, abdominal and pelvic viscera.

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

There were no A-1325912 -related effects on estrous cycling in the females at any dose level. All values for estrous cycling were similar across the groups.

There were no A-1325912-related effects on days needed for mating (2.5 to 2.9 days), mating index (100%) or fertility index (93.3% to 100.0%) in the males at any dose.

Pregnancy was confirmed in 29/29 (100.0%), 28/30 (93.3%), 28/29(96.6%), and 30/30 (100.0%) females in the 0, 3, 10, and 100 mg/kg dose groups, respectively.

The litter averages for corpora lutea, implantations, and viable and nonviable were similar among the four dose groups.

A statistically significant increase in pre-implantation loss (2.6 vs. 0.4 in the control group) and post implantation loss (8 vs. 2.6 in control) was observed in the 3 mg/kg/day dose group. The increases were considered unrelated to the test article, because the percent pre- and post-implantation values observed in all study groups were within the Testing Facility's Historical Control Data maximum ranges (4.6% and 12%, respectively), and there was no evidence of a dose response at 10 or 100 mg/kg/day dose groups and high doses. The post implantation loss in the low dose was driven by a single animal (#8549) that lost 6 embryos post implantation. No dam had a litter consisting of only nonviable embryos. No placentae examined had any detectable abnormalities.

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Embryonic Fetal Development

Study title: An Embryo-Fetal Development Study of A-1325912 Free Form by Oral Gavage in Rabbits (RD 13638)

Study no.:	TE13-163
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	28JUN2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912 (A-1325912.0, A-1325912); 11722ZW00; 98.1%

Key Study Findings

No effects of treatment were noted on pregnancy or fetal development parameters. Based on the results of this study, the maternal no-observed-adverse-effect level (NOAEL) could not be established due to confounding effects of the vehicle formulation. The NOAEL for embryo-fetal development was 100 mg/kg (AUC of 2.11 µg*hr/mL), the highest dose tested. This provides an exposure margin of 1.5X based on observed human exposure to 120 mg A-1325912.

Methods

Doses:	0, 10, 100 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Phosal 53MCT:PEG 400:Poloxamer 124: Cremophor RH40, 40:20:20:20 w/w
Species/Strain:	New Zealand White Hra:(NZW)SPF rabbits
Number/Sex/Group:	20
Satellite groups:	Toxicokinetics (5/group)
Study design:	Test article was administered from GD 7 to 19. Dose levels were selected based on no findings of maternal toxicity at oral doses of up to 100 mg/kg in a dose range finding study

Observations and Results

Mortality: The rabbits were observed for clinical observations and/or general appearance once during the acclimation period, and once daily during the dose and postdose periods. Excessive maternal toxicity resulting in mortality (found dead or unscheduled euthanasia) or abortions occurred in all study groups over GD 20 to GD 29 (5, 4, and 3 rabbits in the 0, 10, and 100 mg/kg dose groups, respectively). These rabbits exhibited adverse clinical signs, which included dehydration and/or thin body condition, body weight loss/suppressed body weight gain and a concomitant decrease in food consumption. It is likely that the vehicle formulation contributed to the declining clinical condition of the rabbits across the dose groups.

Clinical Signs: Clinical signs were recorded daily. The surviving rabbits in all dose groups exhibited clinical signs that included ungroomed coat, soft/liquid/reduced feces, apparent decreases in body weight/body weight loss and concomitant decreases in food consumption. Since these findings occurred in all dose groups, including controls and there was no dose-response relationship to A-1325912, they were considered vehicle-related rather than A-1325912-related.

Body Weight: Body weights were recorded once during the acclimation period, and daily during the dose and postdose periods. The surviving rabbits in all dose groups, including controls, exhibited suppressed maternal body weight gain or maternal body weight losses. Changes were attributed to vehicle effects, rather than test article effects.

Feed Consumption: Food consumption values were recorded daily during acclimation (values not tabulated), and daily during the dose and postdose periods. The surviving rabbits in all dose groups exhibited decreases in food consumption that corresponded with suppressed maternal body weight gain or maternal body weight losses. Changes were attributed to vehicle effects, rather than test article effects.

Toxicokinetics: Samples were collected on GD 7 and GD 19, at approximately 1.5, 3, 4, 6, 9, 12 and 24 hours postdose; the 24 hour postdose time point occurred prior to the last dose administered on GD 20.

The estimated mean AUCs on GD 19 were 0.449 and 1.26 µg*hr/mL in the low and high dose groups, respectively.

Mean GD 7 and 19 Maternal Toxicokinetic Parameters for A-1325912

Dose (mg/kg)	Gestation Day 7			Gestation Day 19		
	Cmax (µg/mL)	Tmax (hr)	AUC (µg*hr/mL)	Cmax (µg/mL)	Tmax (hr)	AUC (µg*hr/mL)
10	0.0789	3.3	0.449	0.0728	3.3	0.404
100	0.210	3.4	1.26	0.259	3.8	2.11

Text Table 12
Mean GD 20 Fetal Toxicokinetic Parameters for A-1325912

Dose (mg/kg)	Fetal Concentration – 2 h (ng/mL)	Maternal Concentration – 2 h (ng/mL)	Ratio (Fetal:Maternal)
0	0.00	0.00	N/A
10	0.165	5.31	0.0237
100	1.27	58.4	0.0251

Necropsy: Gross necropsy of does did not reveal any treatment-related effects.

Pregnancy was confirmed in 20, 18, and 20 rabbits in the 0, 10, and 100 mg/kg dose groups, respectively.

There were no A-1325912-related ovarian, uterine or placental findings in the rabbits that survived to scheduled euthanasia (15, 14 and 17 rabbits at 0, 10, and 100 mg/kg, respectively). In addition, there were no apparent effects on embryo-fetal survival in these rabbits.

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

In the surviving rabbits, administration of A-1325912 did not affect any ovarian or uterine parameter, embryo-fetal survival or fetal body weight.

Although abortions occurred in all dose groups including the control group, these were considered to be secondary to maternal toxicity that was vehicle related. In the surviving rabbits, there were no A-1329512-related effects on post-implantation loss, or fetal body weight.

Offspring (Malformations, Variations, etc.): There were no A-1325912-related fetal external, soft tissue or skeletal malformations or variations at any dose.

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Study title: An Embryo-Fetal Development Study of A-1325912 Free Form by Oral Gavage in Mice (RD 13950)

Study no.:	TE13-162
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	04JUN2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912 (A-1325912.0, A-1325912); 11722ZW00; 98.1%

Key Study Findings

No effects of treatment were noted on pregnancy or fetal development parameters. Based on the results of this study, the maternal no-observed-adverse-effect level (NOAEL) and the NOAEL for embryo-fetal development was 100 mg/kg (AUC of 73.1 µg*hr/mL), the highest dose tested. This provides an exposure margin of 52X based on observed human exposure to 120 mg A-1325912.

Methods

Doses:	0, 3, 10, 100 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	2 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Phosal 53MCT:PEG 400:Poloxamer 124: Cremophor RH40, 40:20:20:20 w/w
Species/Strain:	Crl:CD1(ICR) female mice
Number/Sex/Group:	25
Satellite groups:	Toxicokinetics (18/group [an additional 6 mice were used for the vehicle control group])
Study design:	Test article was administered from GD 6 to 15.

Observations and Results

Mortality: Animals were checked for morbidity twice daily. There were no deaths during the study. All mice survived to scheduled euthanasia.

Clinical Signs: Mice were observed daily for clinical signs. There were no remarkable findings.

Body Weight: Body weights were recorded once during the acclimation period, and daily during the dose and postdose periods. There were no remarkable findings.

Toxicokinetics: Samples were collected on GD 15 or GD16 at approximately 1, 3, 6, 9, 12 or 24 hours postdose. The estimated mean AUCs on GD 15 were 9.63 and 73.1 µg*hr/mL in the low and high dose groups, respectively.

Summary of Mean Maternal Toxicokinetic Parameters for A-1329512

Dose (mg/kg)	Gestation Day 15		
	C_{max} (µg/mL)	T_{max} (hr)	AUC (µg*hr/mL)
3	0.534	9	9.63
10	1.29	6	23.6
100	3.48	3	73.1

Text Table 3
Summary of Mean Fetal Toxicokinetic Parameters for A-1329512

Dose (mg/kg)	Gestation Day 15		
	Fetal (µg/mL)	Maternal (µg/mL)	AUC Ratio (Fetal/Maternal)
3	0.0101	0.404	0.0239
10	0.0691	0.967	0.0725
100	0.228	3.04	0.0729

Necropsy: Gross necropsy of does did not reveal any treatment-related effects.

Pregnancy was confirmed in 24, 22, 21, and 23 mice in the 0, 3, 10, and 100 mg/kg dose groups, respectively. There were no A-1325912-related ovarian, uterine or placental findings in the mice In addition, there were no apparent effects on embryo-fetal survival.

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

Administration of A-1325912 did not affect any ovarian or uterine parameter, embryo-fetal survival or fetal body weight.

Offspring (Malformations, Variations, etc.): There were no A-1325912-related fetal external, soft tissue or skeletal malformations or variations at any dose, except for cleft palate. Cleft palate was observed in three fetuses (out of 289; 1%; p≤0.05) in three litters (out of 22; 13%; p≤0.05) at the low dose only (3 mg/kg A-1325912). These numbers were **within** the observed historical data. Historical control data (provided by the Sponsor) indicated a litter incidence of cleft palate at a range of 0-13.6% and fetal incidence at a range of 0-1.7%.

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Pre and Postnatal Development

Study title: A Developmental and Perinatal/Postnatal Reproduction Study of A-1325912 Free Form by Oral (Gavage) in Mice, Including a Postnatal Behavioral/Functional Evaluation (RD 150822)

Study no.:	TD15-099
Study report location:	Electronic
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 25, 2015
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912; 37209ZW10; 98.4%

Key Study Findings

The maternal and reproductive no-observable-adverse effect level (NOAEL) for A-1325912 is 100 mg/kg/day. There were no test article-related effects on postnatal development including sexual maturation, neurobehavioral endpoints or reproductive function in the F1 generation mice. On the basis of these data, the NOAEL for viability and growth in the offspring (F1) is 100 mg/kg/day, which corresponded to an AUC of 107 µg*hr/mL in the F0 animals on LD14. This provides an exposure margin of 74X based on observed human exposure to 120 mg A-1325912.

Methods

Doses:	0, 3, 10, 100 mg/kg/day
Frequency of dosing:	F0 female rats on Gestation Day (GD) 6 through Lactation Day (LD) 20 daily (GD 22 for rats that did not deliver)
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	Phosal 53MCT:PEG 400:Poloxamer 124:Cremophor RH40 (40:20:20:20 w/w)
Species/Strain:	Mouse/ CD1
Number/Sex/Group:	F0:25 females per group (and additional 6-18 TK females per group); F1: 25 per sex per group
Age:	62 days
Weight:	23.9 to 29.5 grams
Satellite groups:	None
Unique study design:	None

Deviation from study protocol: Reported deviations did not affect study results.

Observations and Results

Maternal Mortality: Animals were checked daily for mortality. Five mice were either found dead or euthanized early due to dosing errors or unknown causes (2 control mice, one 3 mg/kg mouse and two 10 mg/kg mice). The deaths were not considered test-article related.

Maternal Clinical Signs Clinical signs were recorded daily. There were no remarkable findings.

Maternal Body Weights: Body weights were recorded daily during dosing period and on day of euthanasia. Overall, mean maternal body weight gains were reduced at all dose levels for the interval of LD 1 to 21 compared to controls (71%, 74%, and 79% of controls in the 3, 10, and 100 mg/kg/day dose groups, respectively). However, the mean maternal body weights on LD 21 were comparable across the four dose groups. As such, the effect was not considered adverse.

Maternal Feed Consumption: Food consumption was recorded on GD 0, 6, 9, 12, 15, 18 (21 and 23 for females that did not deliver a litter; and on LD 1, 2, 4, 7, 10, 12 and 14): There were no remarkable findings.

Natural Delivery Observations: There were no A-1325912-related effects on any natural delivery parameter at any dose. The values for the number of dams delivering litters, the duration of gestation, implantation sites per delivered litter, the gestation index, the number of dams with stillborn pups, and the number of dams with all pups dying was similar among the four dose groups.

Maternal Necropsy: No treatment-related findings were observed.

Litter Observations: There was a statistically significant increase in found dead pups on PND 2-4 in the 10 mg/kg/day dose group (n=22 vs. 7 in controls; $p \leq 0.01$), as well as PND 5-7 in the 100 mg/kg/day dose group (n=7 vs 0 in controls; $p \leq 0.01$). However survival was similar to control over intervals prior and post PND 2-4.
Viability index was statistically lower in dams dosed with 10 mg/kg/day than control dams (92.4% vs. 97.6%; $p \leq 0.01$). Although viability index was also lower at the highest dose (100 mg/kg/day), it was not statistically significant (94 vs. 97.6). These effects were not considered adverse because a dose response was not observed and the changes were minimal.

F1 Generation - Preweaning

Clinical signs: Clinical signs were recorded daily. No treatment-related findings were observed.

Pup Gross Pathology: No treatment-related findings were observed.

F1 Generation - Postweaning

Mortality: Animals were check for mortality twice daily. Male 6462 (10 mg/kg/day) was found dead on PND 59. No clinical signs were observed in this animal prior to death. Body weights and food consumption were unremarkable. No abnormalities were detected at necropsy. The cause of death of this male was undetermined.
Male 6475 (10 mg/kg/day) was euthanized on PND 34 due to adverse clinical observations. Clinical signs observed in this animal prior to death included decreased motor activity, ptosis, all paws and both ear pale, whole body cold to touch, and moderate dehydration (based on skin turgor) on PND 34. No abnormalities were detected at necropsy. This death was considered to be due to a failure to thrive (the animal was 5.9 g at weaning compared to a range of 7.6 g to 15.8 g in all other F1 generation mice) and unrelated to A-1325912. Given the isolated incidence of these deaths, low number and no dose response, they were not considered test article related.

Clinical signs: Clinical signs were recoded daily. No treatment-related findings were observed with the exception of those described above.

Body weight: Body weights were recorded weekly for males and GD 0, 6, 9, 12 and 13 for females. There were no remarkable findings.

Food consumption: Food consumption was recorded weekly and GD 0, 6, 9, 12 and 13. There were no remarkable findings.

Developmental Signs:

Vaginal opening: Measurements started on PND 21. There were no remarkable findings.

Prepupial separation: Measurements started on PND 22. There were no remarkable findings.

Behavioral assessments:

Acoustic Startle Habituation: Measurements were conducted on Day 60± 2 days postpartum. There were no remarkable findings.

Motor Activity: Measurements were conducted on Day 60± 2 days postpartum). There were no remarkable findings.

M-Shaped Water Maze: Measurements were conducted between days 66 and 90 days postpartum. There were no remarkable findings.

F1 Mice from Mating until Sacrifice:

Mating performance and fertility: PND 90. There were no A-1325912-related effects on days needed for mating (1.6 to 2.3 days), mating index (100%) or fertility index (87.5% to 100.0%) in the F1 generation mice at any dose. All values were similar among the four dose groups.

Necropsy: The only observation noted at macroscopic necropsy (an enlarged spleen in 2 and 1 females in the 3 and 100 mg/kg/day dose groups, respectively) was considered unrelated to A-1325912 because it was not dose dependent and occurred in a limited number of animals.

Terminal Body Weights, Testes and Epididymides Weights and Ratios (%) of Testes and Epididymides Weights to Terminal Body Weight: There were no remarkable findings.

Ovarian and Uterine Examinations: Pregnancy was confirmed in 23 (92.0%), 22 (88.0%), 23 (92.0%), and 25 (100.0%) F1 generation females in the 0, 3, 10, and 100 mg/kg/day dose groups, respectively. There was an apparent increase in the number of nonviable embryos in the 100 mg/kg/day dose group, compared to controls (1.7 nonviable embryos/litter versus 0.5 in controls). As a result, there was a corresponding increase in the percentage of postimplantation loss at 100 mg/kg/day compared to controls (11.4% versus 3.5% in controls). The increases in the number of nonviable embryos and postimplantation loss slightly exceeded the historical range of the Testing Facility (Historical Control Data nonviable embryo range was 0.3 to 1.3 and the postimplantation loss range was 2.0 to 10.9). These effects were not considered adverse because a dose response was not observed, the changes were minimal and the changes were within or very close to historical controls.
The litter means for corpora lutea, implantations, the percentage of preimplantation loss, and viable embryos were similar among the four dose groups and did not significantly differ. No dam had a litter consisting of only nonviable embryos. No placentae examined had any detectable abnormalities.

Toxicokinetics Blood was collected on LD or PND 14; 1, 3, 6, 9, 12 and 24 hours postdose. The F0 animal mean A-1325912 Cmax and AUC exposure appeared to be approximately proportional to dose from the low to the mid dose, but were less than proportional from the mid to the high dose on LD14. The mean plasma concentrations of the F1 animals at 0 hr on PND 14 were approximately one quarter to one third of those to the mean concentrations at 1 hr of the F0 animals on LD 14 in each dose group.

Table 17 Mean Toxicokinetic Parameters for A1325912 in F0 Mouse Plasma on LD 14 and F1 Pups on PND 14

	F0 Generation: Lactation Day 14	
Dose (mg/kg)	Cmax (µg/mL)	AUC (µg*hr/mL)
3	1.19	14.5
10	2.99	32.4
100	7.58	107

Dose (mg/kg)	F1 Generation: Postnatal Day 14
3	0.0837 µg/mL
10	0.210 µg/mL
100	1.22 µg/mL

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Other Toxicology Studies

Study title: Four-Week Oral Dose Toxicity Study of A-1325912 Free Form (with Impurity) in CD-1 Mice (RD160640)

Study no.:	TA16-109
Study report location:	Electronic
Conducting laboratory and location:	AbbVie Inc. Research and Development Preclinical Safety Toxicology 1 North Waukegan Road North Chicago, IL 60064, USA
Date of study initiation:	May 06, 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1325912 free form (A-1325912; undetectable impurity); Lot No: 2288349 repackaged from Lot FT00065105- purity (b) (4)%; and A-1325912 free form (A-1325912 (b) (4) Lot No: 2288348-purity (b) (4)%

Key Study Findings

To investigate potential toxicologic and pathologic effects of a chemical species identified as a degradant impurity during the manufacture of the drug product, mice (10/sex/group) were administered vehicle alone (control group) or dosages of 100 mg/kg/day A-1325912 with or without the (b) (4) impurity by oral gavage once daily for 28 to 29 consecutive days. Mice were administered either A-1325912.0 a comparator lot with undetectable levels of (b) (4) impurity or A-1325912 (b) (4) There were no adverse effects in this study and the NOAEL was (b) (4) mg/kg A-1325912.0 (with impurity).

Methods

Doses:	0, 100 mg/kg A-1325912.0 (impurity undetectable) or 100 mg/kg A-1325912.0 (b) (4)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	2 mL/kg
Formulation/Vehicle:	Phosal 53 MCT: PEG 400: Poloxamer 124: Kolliphor RH40 (40:20:20:20, by weight)
Species/Strain:	Mouse/ CD-1[CD® (SD)]
Number/Sex/Group:	10 mice/sex/group analysis
Age:	9 weeks old
Weight:	25.7 to 37.2 grams
Satellite groups:	Additional 4-5 mice/sex/group for TK analysis
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. There were no unscheduled deaths.

Clinical Signs: Clinical observations were conducted twice weekly. There were no remarkable findings.

Body Weights: Body weights were recorded twice weekly. There were no remarkable findings.

Food Consumption Food consumption was measured and recorded weekly during the study. There were no remarkable findings.

Hematology: Blood samples for clinical pathology were collected at terminal necropsy. Mean platelet levels were 20% higher in males dosed with A-1325912.0 (impurity undetectable) as compared to controls. Considering the minimal change and lack of

effect in females as well as in males dosed with A-1325912.0 (spiked with impurity), this effect was not considered adverse.

Clinical Chemistry: Blood samples for clinical pathology were collected at terminal necropsy. There were no remarkable findings.

Gross Pathology: Blood samples for clinical pathology were collected at terminal necropsy. There were no remarkable findings.

Organ Weights: Blood samples for clinical pathology were collected at terminal necropsy. There were no remarkable findings.

Histopathology

Adequate Battery: yes

Peer Review: yes

Histopathology Findings:
Minimal dilation of the stomach glands were noted in one out of 10 females dosed with the test article and three out of 10 females dosed with spiked test article, in females. Due to the minimal severity of the finding and absence of the finding in males, this effect was not considered adverse.

Toxicokinetics: Blood samples for determination of plasma test item concentration were collected from all satellite mice in Groups 1-3 at six hours after dosing on Dosing Day 28. There were no gender differences in exposure nor was exposure affected by presence of impurity.

Table 18 Plasma levels of A-1325912 on Day 28

Collection Interval	Sex	A-1325912 Dosage (mg/kg/day) 0	100[b]	100[c]
		Mean Plasma A-1325912 Concentration (µg/mL) ± SEM		
Day 28	Males	BQL[a]	6.03 ± 0.574	6.36 ± 0.969
	Females	BQL[a]	8.23 ± 0.937	6.59 ± 0.228
	Overall	BQL[a]	7.13 ± 0.635	6.48 ± 0.471

a. Below the lower quantitation limit.
b. A-1325912 comparator lot, Group 2 with undetectable levels of (b) (4)
c. A-1325912 spiked with (b) (4)

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Neutral Red Uptake Phototoxicity Assay of A-1325912 Free Form in Balb/c 3T3 Mouse Fibroblasts (Study RD 13656)

The phototoxicity potential of A-1325912 free form was evaluated by measurement of the relative reduction in viability of Balb/c 3T3 mouse fibroblasts exposed to A-1325912 free form and ultraviolet radiation (+UVR; 5 J/cm2 of UVA and 21-22 mJ/cm^2 of UVB from a xenon arc solar simulator equipped with a Schott WG 320 filter), as compared with the viability of fibroblasts exposed to A-1325912 free form in the absence of ultraviolet radiation (-UVR), at concentrations up to the solubility limit of 3 mg/L. Promethazine was used as the positive control.

A-1325912 did not demonstrate any potential phototoxicity by the Photoirritancy Factor (PIF; 1) and Mean Photo Effect (MPE; 0.00). A-1325912 was not cytotoxic. Promethazine cytotoxicity and phototoxicity criteria were met indicating that the assay was valid (sponsor's table below).

Table 19 Phototoxicity Potential of A-1325912 in Mouse Fibroblasts

Assay Results					
	IC_{50} (mg/L) –UVR (cytotoxicity)	IC_{50} (mg/L) +UVR (phototoxicity)	Photoirritancy Factor (PIF)	Mean Photo Effect (MPE)	Phototoxic Potential
Promethazine	56.15	1.302	43.383	0.430	Phototoxic
A-1325912 free form	-	-	*1	0.000	Non Phototoxic

IC_{50}: 50% Inhibitory Concentration
+UVR: with exposure to 5 J/cm^2 of UVA and 21-22 mJ/cm^2 of UVB in the assay
– UVR: without UVR exposure
*1: Both IC_{50}(-UVR) and IC_{50}(+UVR) could not be calculated, this indicated a lack of phototoxic potential

Study title: Four-Week Oral Gavage Toxicity Study of A-1282576 and A-1325912 (Free Form) in Sprague-Dawley Rats

Study no.:	TA16-040
Study report location:	Electronic
Conducting laboratory and location:	AbbVie Inc. Research and Development Preclinical Safety Toxicology 1 North Waukegan Road North Chicago, IL 60064, USA
Date of study initiation:	January 19, 2016
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	A-1282576 and A-1325912 Lot Nos: 14-002580 and 14-002133; 95.7% and 102.2%

Key Study Findings

Oral administration of a combination of A-1282576 and A-1325912 ((b) (4) suspended in 0.5% HPMC) at a dosage of 12.5/20 mg/kg/day was well tolerated and did not result in any signs of toxicity or changes in anatomic or clinical pathology. This

dosage resulted in an average Cmax of 5.37/0.282 µg/mL (A-1282576/A-1325912) and an AUC(0-24) of 10.1/2.11 µg•hr/mL (A-1282576/A-1325912) on Day 24. The daily dosage of ABT-493/ABT-530 selected for the study was 12.5/20 mg/kg/day. This dosage was selected to produce AUC exposures approximately 2- to 4-fold above the efficacious AUC for this drug combination, and is supported by pharmacokinetic data in rats (non-fasted). In the current study, this dosage was not expected to produce toxicity to the study animals.

Methods

Doses:	0, or 12.5/20 mg/kg/day A-1282576/A-1325912
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% hydroxypropylmethylcellulose (HPMC) in sterile water
Species/Strain:	Rat/ Sprague Dawley
Number/Sex/Group:	10 rats/sex/group
Age:	7 weeks old
Weight:	198.7 to 347.8 grams
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Reported deviations did not affect study results.

Observations and Results

Mortality: Observations for morbidity, mortality, injury, and the availability of food and water were conducted daily for all animals. One female animal, Animal 2008, was euthanized on Dosing Day 7, based on observations consistent with a dosing error. Perforation of the esophagus was confirmed during necropsy. All other animals survived to scheduled necropsy.

Clinical Signs: Clinical observations were conducted twice weekly. There were no remarkable findings.

Body Weights: Body weights were recorded twice weekly. There were no remarkable findings.

Food Consumption: Food consumption was measured and recorded weekly during the study. There were no remarkable findings.

Ophthalmologic Examination: Ophthalmoscopic examinations were conducted on Day 22. There were no remarkable findings.

Hematology: Blood samples for clinical pathology were collected at terminal necropsy. On Dosing Day 29, males administered 12.5/20 mg/kg/day A-1282576/A-1325912

exhibited a minimally decreased reticulocyte count (-23%; not statistically significant) relative to the mean for control males; no other changes were noted, nor was this observed in females, and as such, this effect was not considered adverse.

Coagulation: Blood samples for clinical pathology were collected at terminal necropsy. There were no remarkable findings.

Clinical Chemistry: Blood samples for clinical pathology were collected at terminal necropsy. On Dosing Day 29, males administered 12.5/20 mg/kg/day A-1282576/A-1325912 exhibited a minimally decreased potassium concentration (-19%) relative to the mean for control males. No other changes were observed, nor was this observed in females, and as such this effect was not considered adverse.

Urinalysis: Urine samples were collected at terminal necropsy. There were no remarkable findings.

Gross Pathology: There were no remarkable findings.

Organ Weights: Female thyroid weight was 18% higher than controls in treated animals. This was not observed in males and the effect was not considered adverse.

Histopathology

Adequate Battery: yes

Peer Review: yes

Histopathology Findings:
No treatment-related findings were observed.

Toxicokinetics: Blood samples were collected 0.5, 1.5, 3, 7, 12, and 24 hours after dosing on Day 1 and Day 24. AUC values were higher in males than females for A-1325912 on Days 1 and 24. T_{max} and C_{max} was also higher on Day 24 in males. C_{max} of A-1282576 was higher in females on Day 24 but other parameters were similar across both sexes.

Mean Toxicokinetic Parameters for A1325912 in Rat Plasma

			Day 1						Day 24					
Treatment Group	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)	C_{max}/D (µg/mL/mg/kg/day)	T_{max} (hr)	AUC (µg*hr/mL)	AUC/D (µg*hr/mL/mg/kg/day)	SE of Composite AUC (µg*hr/mL)	C_{max} (µg/mL)	C_{max}/D (µg/mL/mg/kg/day)	T_{max} (hr)	AUC (µg*hr/mL)	AUC/D (µg*hr/mL/mg/kg/day)	SE of Composite AUC (µg*hr/mL)
2	20	Male	0.302	0.0151	1.5	2.30	0.115	0.260	0.437	0.0219	3.0	2.97	0.148	0.140
		Female	0.296	0.0148	1.5	1.25	0.0623	0.144	0.220	0.0110	1.5	1.19	0.0593	0.144
		Overall	0.299	0.0150	1.5	1.77	0.0886	0.194	0.282	0.0141	3.0	2.11	0.105	0.270

Mean Toxicokinetic Parameters for A1282576 in Rat Plasma

			Day 1						Day 24					
Treatment Group	Dose (mg/kg/day)	Sex	C_{max} (µg/mL)	C_{max}/D (µg/mL/mg/kg/day)	T_{max} (hr)	AUC (µg*hr/mL)	AUC/D (µg*hr/mL/mg/kg/day)	SE of Composite AUC (µg*hr/mL)	C_{max} (µg/mL)	C_{max}/D (µg/mL/mg/kg/day)	T_{max} (hr)	AUC (µg*hr/mL)	AUC/D (µg*hr/mL/mg/kg/day)	SE of Composite AUC (µg*hr/mL)
2	12.5	Male	4.75	0.380	0.5	13.1	1.05	2.36	4.06	0.325	0.5	9.97	0.798	1.73
		Female	7.93	0.634	0.5	13.6	1.09	2.25	6.68	0.534	0.5	10.0	0.803	0.926
		Overall	6.34	0.507	0.5	13.3	1.06	1.71	5.37	0.430	0.5	10.1	0.808	1.11

Stability and Homogeneity: Acceptance criteria were met for both homogeneity and concentration. Test article was not detected in control formulations.

Integrated Summary and Safety Evaluation

This marketing application was submitted in support of a for Gle/Pib (Glecaprevir/Pibrentasvir; ABT-493/ABT-530) indicated for the treatment of chronic HCV genotype 1-6 infection in adults, including those with cirrhosis, who are either treatment-naïve or previously treated with interferon, pegylated interferon (pegIFN) and/or ribavirin. Gle (3/4A protease inhibitor) and Pib (an inhibitor of the NS5A protein) are both new molecular entities. The proposed clinical dosing regimen of Gle/Pib is 300 mg/120 mg (Gle/Pib) for 8 to 16 weeks (depending on patient population; see prescribing information for more details).

The following sections summarize nonclinical results for Pibrentasvir (Pib).

PK/ADME: The oral bioavailability of Pib was 7.9% in mouse and 30% in dog. *In vitro* protein binding was >99% in all species, including human. Following an oral dose of ^{14}C-Pib in rat, radioactivity was widely distributed with the highest concentrations found in liver. The drug did not cross the blood:brain barrier; minimal radioactivity was detected in testis and uveal tract (but not the lens or eye). Binding to melanin was not observed. In human, Pib was the only component in plasma and the administered dose was virtually all eliminated in feces as unchanged parent drug. There were no significant circulating clinical metabolites (e.g., >10% of total drug-related exposure) requiring nonclinical safety assessment. Elimination primarily occurred through the biliary route in rats and humans, with minimal renal excretion. Pib was transferred to rat pups during lactation and crossed the placenta of pregnant rats. The lack of inhibition and induction of major human CYPs at clinically relevant concentrations suggest a low potential for CYP mediated drug-drug interactions for Pib as a perpetrator drug. CYP inhibitors or inducers are not expected to have an impact on Pib clinical exposures. Pib exposure may be impacted by inhibitors or inducers of P-gp and/or BCRP. Inhibition of P-gp, BCRP or OATP1B1 by Pib may increase the plasman exposure of sensitive substrates. Selection of the mouse as the rodent toxicology species was based on superior systemic exposures when compared to other species (rat).

Safety Pharmacology: Pib was not associated with clinically relevant adverse effects on safety pharmacology parameters. These studies included neurological and respiratory assessments in rats. Cardiovascular evaluations included both in vitro (i.e., hERG inhibition) and *in vivo* study in telemetered dogs.

Repeat-Dose Toxicology: General toxicology evaluations included GLP repeat-dose studies of ≤ 26 weeks in mice and ≤ 39 weeks in dogs. Overall, Pib was not associated with clinically relevant adverse effects in pivotal studies. However, non-adverse findings and/or findings of limited clinical significance were noted in spleen weight in mice and clinical signs and hematology in dogs; all were recoverable except the clinical signs in the dog. Absence of effects in the long-term studies may be indicative of an adaptive effect or incidental findings.

- Spleen weight: Spleen weight was decreased up to 30% at all doses in the 13-week mouse study. This was not observed in the 26 week study.

- Hematology: Absolute reticulocytes were decreased up to 46% at the high dose of 100 mg/kg/day in the 39-week study in dogs.

- Clinical signs: Unformed, watery or mucoid feces were observed at a higher incidence in dogs dosed with 10 or 100 mg/kg/day versus controls in the 13-week dog study.

  Diarrhea was reported in clinical trials.

Genetic Toxicology and Carcinogenicity: Pib was not considered genotoxic based on negative results in the in vitro bacterial mutation assay, in vitro mammalian chromosome aberration assay in human peripheral blood lymphocytes, and in vivo rat micronucleus assay. Because Gle was not genotoxic and clinical administration is limited to 16 weeks (maximum), carcinogenicity studies were not required.

Developmental and Reproductive Toxicology: There were no relevant adverse effects on male or female fertility in mice or embryo-fetal development in mice or rabbits. There was a slight increase in found dead pups in all groups dosed with the test article and an increase in nonviable embryos (F2) as well as associated increase in post-implantation loss (F1 females) at the highest dose tested (100 mg/kg/day) in the pre/post-natal study in mice. However due to the small magnitude in change and lack of dose response, these effects were not considered adverse.

In rabbits, the intravenous route (IV) was initially evaluated in an attempt to improve overall systemic Pib exposure in the embryo fetal development study. Due to tolerability issues associated with IV dosing of the vehicle (PEG-300:D5W:Tween-80), oral gavage administration of Pib in Phosal 53MCT: PEG-400: Poloxamer 124: Cremophor RH40 vehicle was selected for the definitive EFD study in rabbits. Although some rabbits in

the EFD study exhibited maternal toxicity in the control and all dose groups, there were no adverse effects on embryo-fetal development at Pib doses up to 100 mg/kg/day.

Other Studies: The weight of evidence suggests that Pib is not phototoxic. Although there were no concerning safety signals or overlapping toxicity with Gle and Pib, a combination study was not conducted. (b) (4) a four-week safety study at low doses of Gle and Pib was conducted in Sprague-Dawley rats. Oral administration of a combination of A-1282576 (Gle) and A-1325912 (Pib; (b) (4) tablets) at a dosage of 12.5/20 mg/kg/day was well tolerated and did not result in any signs of toxicity or changes in anatomic or clinical pathology. This dosage was selected to produce AUC exposures approximately 2- to 4-fold above the efficacious AUC for this drug combination.

Excipients, Metabolites, and Impurities: Excipient exposures fell below maximum potencies listed for approved products in the FDA Inactive Ingredient Database and were, therefore, considered acceptable (with the exception of Capryol 90).
All impurities were qualified per appropriate ICH guidelines. There were no significant circulating clinical metabolites (e.g., >10% of total drug-related exposure) requiring nonclinical safety assessment.
To investigate potential toxicologic and pathologic effects of a chemical species identified as a degradant impurity (b) (4) during the manufacture of the drug product, mice were administered vehicle alone (control group) or dosages of 100 mg/kg/day A-1325912 with or without the (b) (4) impurity by oral gavage once daily for 28 to 29 consecutive days. There were no adverse effects in this study and the NOAEL was (b) (4) mg/kg A-1325912.0 (with impurity).

The Gle/Pib film-coated bilayer tablets contain the excipient propylene glycol monocaprylate II (Capryol 90) at a level of (b) (4) mg per tablet. To achieve the recommended human doses, 3 tablets are required. Therefore, the maximum daily dose of propylene glycol monocaprylate II that will be administered is (b) (4) mg/day. The sponsor conducted genotoxicity, EFD and 28 day repeat dose studies with this excipient. No adverse effects were noted in any of the studies.

Table 20 Pib exposure margins

Study	NOAEL	Nonclinical AUC[a]	Exposure margin[b]
General Toxicology			
6-month mouse	(b) (4) mg/kg/day	123 µg·hr/mL[c]	88x
9-month dog	mg/kg/day	25 µg·hr/mL[d]	17x
Developmental and Reproductive Toxicology			
Segment 1			
fertility and early embryonic development in mouse	mg/kg/day	153[e]	106x
Segment 2			
embryofetal development in mouse	mg/kg/day	73.1 µg·hr/mL[f]	52x
embryofetal development in rabbit	mg/kg/day	2.11 µg·hr/mL[g]	1.5x
Segment 3			
pre-/post-natal development in mouse	mg/kg/day	107 µg·hr/mL[h]	74x

[a] based on A-1325912
[b] compared to clinical $AUC_{24,ss}$ = 1.4 µg·hr/mL
[c] Day 182 data
[d] Day 280 data
[e] Day 35/36 data
[f] GD 15 data
[g] GD 19 data
[h] LD 14 data

APPENDIX 3: Comments on Impurities/Degradants of Concern

Glecaprevir

There are three Class 1 impurities in the drug substance of Gle; [redacted] The levels of each will be below the specific permissible daily exposure limits for all three impurities.

Based on available rodent carcinogenicity data, the compound specific limit for [redacted] is [redacted] µg/day. This converts to [redacted] µg/day for a drug given from >1 to 12 months. The value is less than the proposed controlled value of [redacted] ppm in the DS [redacted] µg/day).

The draft ICH M7 addendum lists a lifetime acceptable intake of [redacted] as [redacted] µg/day. This converts to [redacted] µg/day for a drug given from >1 to 12 months and is below the proposed controlled value of [redacted] ppm in the DS [redacted] µg/day).

Based on available rodent carcinogenicity data, the compound specific limit for [redacted] µg/day . This converts to [redacted] µg/day for a drug given from >1 to 12 months. This is below the proposed controlled value of [redacted] ppm in the DS [redacted] µg/day).

The potential manufacturing impurities for glecaprevir drug substance, including the [redacted] were subjected to mutagenicity assessment (i.e., DEREK analysis, version Nexus 4.0.05 or 4.1.0); CASE Ultra analysis, version 1.5.0.0, 1.5.1.8 or 1.5.2.0); literature review). The impurities that showed structural alerts for mutagenicity and were subsequently shown to be negative in a bacterial mutation (Ames) testing were not considered impurities of genotoxic concern. Mutagenic and potentially mutagenic impurities are controlled to a daily intake of ≤ 20 µg/day based on a >1 to 12 month treatment duration as per ICH M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. The six positive impurities were classified as Class 2 M7 control option impurities by the Sponsor (impurities under characterization; table below).

RD 11748 evaluated the effect of the process impurity on bacterial strains of TA98 and TA100 and RD 12942 evaluated the effect of the process impurity on bacterial strains of TA98, TA100 and WP2uvrAPM101 (Escherichia coli). The remainder of the studies evaluated the effect of the process impurities on bacterial strains of TA1535, TA1537, TA98, TA100 (Salmonella) and WP2uvrAPKM101 (Escherichia coli). Some of the impurities were tested with the 6-well plate incorporation method as described in Table 4.

Table 21 Gle Impurities Tested and Associated Results

Impurity[a]	Highest Concentration Tested	Result[b]	Report Number, Conducting Laboratory
(b) (4)	5000 µg/plate, +/- S9	Negative	R&D/14/0675, [22] (b) (4)
	5000 µg/plate, +/- S9	Negative	R&D/14/0675, [22]
	5000 µg/plate, +/- S9	Negative	R&D/14/0675, [22]
	1000 µg/well, +/- S9	Negative	R&D/15/0776, [26] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/15/0158, [25] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/15/0881, [27] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0245, [28] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0477, [29] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0477, [29] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0552, [30] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0561, [31] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0561, [31] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0561, [31] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0634, [32] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0634, [32] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0634, [32] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0634, [32] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0682, [33] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0682, [33] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0721, [34] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0822, [35] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0872, [36] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/0872, [36] AbbVie
	1000 µg/well, +/- S9	Negative	R&D/16/1006, [37] AbbVie
		Negative [38]	Literature Reference
	1000 µg/well, +/- S9	Positive	R&D/16/0561, [31] AbbVie
	1000 µg/plate, +/- S9	Positive	R&D/14/0063, [23] AbbVie
	1000 µg/plate, +/- S9	Positive	R&D/14/0416, [24] AbbVie
	1000 µg/plate, +/- S9	Positive	R&D/11/748, [39] AbbVie
	1000 µg/plate, +/- S9	Positive	R&D/14/0416, [24] AbbVie
	1000 µg/plate, +/- S9	Positive	R&D/12/942, [40] AbbVie

a. Although the toxicology report title and/or report for some of the impurities notes that the study was conducted for a compound other than either glecaprevir, the impurity evaluated is also a potential impurity in glecaprevir drug substance or the manufacturing process of glecaprevir drug substance

b. The Petri plate assay used the pre-incubation method, where the 6-well plate assays used the plate incorporation procedure. R&D/14/0063 and R&D/11/748 used the plate incorporation assay method and R&D/12/942 used both the plate incorporation and Petri assay method. The remainder of the studies utilized the 6-well plate incorporation method.

The (b) (4) exposures are acceptable.

Pibrentasvir

In the Pib drug substance and Gle/Pib drug product, there are seven specified impurities that are (b) (4) that exceed the ICH qualification thresholds. These impurities (table below) have the (b) (4) As such, this alert is qualified by the negative Ames test result (b) (4)). Based on each impurity's specification limit and the daily dose of pibrentasvir (120 mg), patients would receive less than (b) (4) mg/day of each of these drug substance or drug product impurities. With no mutagenicity concerns and a daily intake of less than (b) (4) mg/day, no genetic

toxicity tests need to be conducted for these individual impurities, as indicated by ICH M7 (Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk). Exposure to these impurities at the NOAEL in rodent and non-rodent studies also provided adequate coverage relative to the clinical dose of 120 mg.

Table 22 Toxicological Qualification for Impurities in Pib (that exceed the ICH qualification thresholds)

There are 69 mutagenic, potentially mutagenic, or carcinogenic impurities that may be present in the Pib drug substance process. Mutagenic and potentially mutagenic impurities are controlled to a daily intake of ≤ 20 µg/day based on a > 1 to 12 month treatment per ICH M7 (Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk).

There are two Class 1 impurities in the drug substance of Pib; (b) (4) . The levels of each will be below the specific permissible daily exposure limits for all three impurities as described below.

Based on available rodent carcinogenicity data, the compound specific limit for (b) (4) µg/day. This converts to (b) (4) µg/day for a drug given from >1 to 12 months. The value is less than the proposed controlled value of (b) (4) ppm in the DS.

The draft ICH M7 addendum lists a lifetime acceptable intake of (b) (4) µg/day. This converts to (b) (4) µg/day for a drug given from >1 to 12 months and is below the proposed controlled value of (b) (4) ppm in the DS.

(b) (4)

relevant species to which a patient is exposed. The (b) (4) degradation product was qualified in a four-week toxicity study in mice (see other toxicology studies below [ABT-530]).

The (b) (4) degradation product of Pib had structural alerts for mutagenicity in DEREK and CASEUltra. The (b) (4) degradation product, (b) (4), was therefore tested in the GLP Bacterial Reverse Mutation (Ames) Assay using Salmonella typhimurium tester strains TA98, TA100, TA1535 and TA1537 and Escherichia coli tester strain WP2uvrA in the presence and absence of Aroclor™ 1254-induced rat liver S9. 27 The mutagenicity assay via plate incorporation was used. Test item (b) (4) was concluded to be negative in the Bacterial Reverse Mutation Assay (RD 140229).

In addition to the impurities described above, the potential manufacturing impurities for Pib drug substance, including the (b) (4) have been subjected to mutagenicity assessment (i.e., DEREK analysis, CASEUltra analysis, literature review). The impurities that showed structural alerts for mutagenicity and were subsequently shown to be negative in a bacterial mutation (Ames) testing, and thus are not considered impurities of genotoxic concern. Mutagenic impurities or potentially mutagenic are either at an appropriate control point (b) (4) or have been shown, through process understanding (b) (4) experiments) to be reduced to appropriate levels by the process conditions (see table below- Pib Impurities Tested and Associated Results).

Table 23 Pib Impurities Tested and Associated Results

Impurity	Test [a] (GLP Status)	Bacterial Strains	Highest Tested Concentration	Result	Report No., Conducting Laboratory
(b) (4)	Mini Ames (non-GLP)	TA98, TA100,	180 µg/well, +/- S9	Positive	R&D/11/492, [28] (b) (4)
	Mini Ames (non-GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Positive	R&D/14/0045, [29] AbbVie
	Petri plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrA	5000 µg/plate, +/- S9	Negative	R&D/14/0675, [30] (b) (4)
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/14/1294, [31] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	28 µg/well, +/- S9, 1000 µg/well, +/- S9	Positive	R&D/14/1294, [31] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/15/0081, [32] AbbVie
	6-well bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/15/0423, [33] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/15/0497, [34] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/15/0602, [35] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/15/0879, [36] AbbVie

Impurity	Test [a] (GLP Status)	Bacterial Strains	Highest Tested Concentration	Result	Report No., Conducting Laboratory
(b) (4)	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/15/1234, [37] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/16/0165, [38] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/16/0344, [39] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/16/0366, [40] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/16/0688, [41] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/16/0736, [42] AbbVie
	6-well plate bacterial mutation test (GLP)	TA1535, TA1537, TA98, TA100, WP2uvrApKM101	1000 µg/well, +/- S9	Negative	R&D/16/1095, [43] AbbVie

a. The Petri plate assay used the pre-incubation method, where the 6-well plate assays (both GLP and non-GLP) used the plate incorporation procedure.

b. Although the toxicology report title and/or report for these impurities notes that the study was conducted for a compound other than pibrentasvir, the impurity evaluated is also a potential impurity in pibrentasvir drug substance or the manufacturing process of pibrentasvir drug substance.

The (b) (4) exposures are acceptable.

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/s/

ILONA G BEBENEK
05/15/2017

HANAN N GHANTOUS
05/15/2017

I concur with Dr. Ilona Bebenek that the submitted nonclinical data is sufficient to support marketing approval of Glecaprevir/Pibrentasvir.